Application of hurdle technology and 3D printing in the development of strawberry based functional food

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DOCTORAL DISSERTATION

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Supervisor:

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Sveučilište u Zagrebu

Prehrambeno-biotehnološki fakultet Sveučilišta u Zagrebu

Anica Bebek Markovinović

PRIMJENA TEHNOLOGIJE PREPREKAMA I 3D ISPISA U RAZVOJU FUNKCIONALNE HRANE NA BAZI JAGODE

DOKTORSKI RAD

Mentor:

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APPLICATION OF HURDLE TECHNOLOGY AND 3D PRINTING IN THE DEVELOPMENT OF STRAWBERRY-BASED FUNCTIONAL FOOD

Anica Bebek Markovinović, *MSc*

Thesis performed at the Faculty of Food Technology and Biotechnology in Laboratory for Chemistry and Technology of Fruits and Vegetables

Supervisors: PhD Danijela Bursać Kovačević, Full professor

Abstract: Strawberry (*Fragaria x Ananassa* Duch.) is valuable fruit for functional juices production, but its quality is affected by heat treatment. In this work, the effects of hurdle technology as an alternative to thermal treatment were investigated in combination of pulsed electric field and high-power ultrasound as non-thermal alternatives, with the aim of producing functional fruit juices of higher nutritional, biological and sensory quality. Juices processed in this way were used to develop functional fruit products with unique composition, geometry and extended shelf life using three-dimensional printing (3DP). Due to its physicochemical properties, strawberry is very challenging material for 3DP, therefore the addition of strawberry tree fruit (*Arbutus unedo* L.) as natural thickener provided a suitable consistency for 3DP, but also added an improved nutritional and biological value. Finally, the possibility of using sustainable processing that provides consumers with nutritionally and biologically valuable, healthy and attractive functional fruit product was explored.

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PRIMJENA TEHNOLOGIJE PREPREKAMA I 3D ISPISA U RAZVOJU FUNKCIONALNE HRANE NA BAZI JAGODE

Anica Bebek Markovinović, mag. ing. techn. aliment.

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Sažetak: Jagoda (*Fragaria x Ananassa* Duch.) je visokovrijedna sirovina za proizvodnju funkcionalnih sokova, no toplinskom obradom dolazi do narušavanja njihove kvalitete. U ovom radu su ispitani učinci tehnologije preprekama kao zamjena toplinskom tretmanu, u kombinaciji s pulsirajućim električnim poljem i ultrazvukom visoke snage kao netoplinskim alternativama, a s ciljem proizvodnje funkcionalnih voćnih sokova više nutritivne, biološke i senzorske kvalitete. Sokovi obrađeni na ovaj način korišteni su za razvoj funkcionalnih voćnih proizvoda jedinstvenog sastava i geometrije te produženog roka trajanja koristeći trodimenzionalni ispis (3DP). Zbog svojih fizikalno-kemijskih svojstava, jagoda je vrlo izazovan materijal za 3DP, stoga je dodatak plodova maginje (*Arbutus unedo* L.) kao prirodnog zgušnjivača omogućio prikladnu konzistenciju za 3DP, ali je također unaprijedio nutritivnu te biološku vrijednost ispisanih proizvoda. Tako je ispitana mogućnost primjene održivih procesa prerade koji potrošačima pružaju nutritivno i biološki vrijedne, zdravstveno ispravne i privlačne funkcionalne voćne proizvode.

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Extended abstract

Strawberries (*Fragaria x ananassa* Duch.) are recognized as functional foods due to their low-calorie content and rich nutritional profile, particularly their high level of polyphenolic compounds, which are known to have positive effects on human health. The demand for strawberry-based products, such as juices and functional foods, is steadily increasing due to these attractive properties. One objective of this dissertation was to explore the potential of strawberries as a raw material for producing functional fruit juices using cold pressing technology, with a focus on their physicochemical, biological, toxicological, and sensory properties. The findings revealed significant differences between strawberry juices and the raw strawberry fruit, with the highest concentration of bioactive compounds was found in the by-products and the lowest in the juice itself. Considering that traditional thermal processes used in juice production often lead to the degradation of bioactive compounds, this dissertation aimed to investigate the impact of non-thermal technologies, specifically, pulsed electric field (PEF) and high-power ultrasound (HPU), used in combination (i.e., hurdle technology) on the quality of the treated juices. Juice quality was primarily assessed based on biological potential, including the preservation of bioactive compounds during non-thermal treatments, as well as other parameters such as antioxidant potential, physicochemical and color measurements, hydroxymethylfurfural (HMF) formation, browning index, and safety during shelf-life.

Finally, based on the results obtained, the parameters of the hurdle technology were optimized to achieve juices with the highest content of bioactive compounds and antioxidant activity. The optimization of hurdle technology, specifically the combination of PEF and HPU, showed that a shorter PEF treatment time paired with a longer HPU treatment resulted in higher yields for the most of the studied bioactive compounds and antioxidant capacity. Conversely, a different trend was observed when HPU followed PEF, where shorter treatment times for both methods yielded higher concentrations of bioactive compounds.

The final part of this dissertation focused on the development of a 3D printed functional product based on strawberries, with the optimization of 3D printing parameters (type and content of added starch carriers and printing program) aimed at preserving biological activity during the printing process. Due to known challenges strawberries have as a raw material for 3D printing, the final 3D-printed product included strawberry tree fruit (*Arbutus unedo* L.) to reduce the starch carrier content, thereby enhancing the quality of rheological properties and improved biological potential. The results indicated that the starch carrier content significantly

influenced the yield of bioactive compounds and antioxidant capacity. By optimizing the 3D printing parameters, the highest yield of bioactive compounds was achieved in 3D printed products with the lowest starch content. The texture analysis showed that all 3D-printed products exhibited good textural properties, a high degree of stability and minimal geometric deviations.

In summary, the research demonstrates that strawberries are an excellent raw material for the production of functional fruit juices, owing that to their rich biological potential. The use of hurdle technology, combining PEF and HPU, has been shown to effectively preserve this potential. Moreover, the resulting juices can be further utilized in the production of 3D printed functional products. By optimizing 3D printing parameters, it is possible to create products that maintain their biological properties. This research significantly contributes to understanding of how sustainable non-thermal technologies like PEF and HPU, along with additive 3D printing, impact the quality and stability of strawberry-based functional products, particularly when combined with strawberry tree fruits.

Keywords: non-thermal technologies, pulsed electric field, high-power ultrasound, 3D printing, bioactive compounds, strawberry (*Fragaria x Ananassa* Duch.), strawberry tree fruit (*Arbutus unedo* L.)

Proširenisažetak

Jagode (*Fragaria x ananassa* Duch.) su prepoznate kao funkcionalna hrana zbog svog niskokaloričnog sadržaja i bogatog nutritivnog profila, osobito zbog visokog sadržaja polifenolnih spojeva koji su poznati po svojim pozitivnim učincima na zdravlje. Potražnja za proizvodima od jagoda, kao što su sokovi i funkcionalna hrana, stalno raste zbog ovih privlačnih svojstava. Jedan od ciljeva ove disertacije bio je istražiti potencijal jagoda kao sirovine za proizvodnju funkcionalnih voćnih sokova koristeći tehnologiju hladnog prešanja, s fokusom na njihova fizikalno-kemijska, biološka, toksikološka i senzorna svojstva. Rezultati su pokazali značajne razlike između sokova od jagoda i sirovine od koje su pripremljeni, pri čemu je najviši sadržaj bioaktivnih spojeva pronađen u nusproizvodima, a najniži u samom soku. S obzirom na to da tradicionalni toplinski procesi korišteni u proizvodnji sokova često dovode do degradacije bioaktivnih spojeva, ova disertacija imala je za cilj istražiti utjecaj netoplinskih tehnologija, konkretno pulsirajućeg električnog polja (PEF) i ultrazvuka visoke snage (HPU), korištenih u kombinaciji (eng. *hurdle concept*, tehnologija preprekama) na kvalitetu obrađenih sokova. Kvaliteta sokova procijenjena je prvenstveno na temelju biološkog potencijala, uključujući praćenje stabilnosti bioaktivnih spojeva tijekom netoplinskih tretmana, kao i antioksidacijskog potencijala, fizikalno-kemijskih i kolorimetrijskih svojstava, tvorbe hidroksimetilfurfurala (HMF), indeksa posmeđivanja te zdravstvene ispravnosti tijekom skladištenja.

Na kraju, na temelju dobivenih rezultata, procesni parametri tehnologije preprekama optimizirani su kako bi se prozveli sokovi s najvišim sadržajem bioaktivnih spojeva i najvišom antioksidacijskom aktivnošću. Optimizacija procesnih parametara tehnologije preprekama, konkretno u kombinaciji PEF + HPU, pokazala je da kraće vrijeme PEF tretmana u kombinaciji s dužim HPU tretmanom rezultira većim prinosima većine proučavanih bioaktivnih spojeva i antioksidacijskog kapaciteta. Nasuprot tome, opažen je drugačiji trend kada je HPU bio prethodio PEF-u, gdje su kraće vrijeme tretmana za obje tehnologije rezultirale višim koncentracijama bioaktivnih spojeva.

Zadnji dio ove disertacije fokusirao se na razvoj 3D ispisanog funkcionalnog proizvoda na bazi jagode, uz optimizaciju parametara 3D ispisa (vrsta i udio dodanih škrobnih nosača i program ispisa) s ciljem očuvanja biološke aktivnosti tijekom procesa ispisa. Zbog izazova koje jagode predstavljaju kao sirovina za 3D ispis, konačni 3D ispisani proizvod sadržavao je plodove maginje (*Arbutus unedo* L.) kako bi se smanjio dodatak škrobnih nosača, čime se poboljšala kvaliteta reoloških svojstava i biološki potencijal. Rezultati su pokazali da

je sadržaj škrobnog nosača značajno utjecao na prinos većine bioaktivnih spojeva i antioksidacijski kapacitet. Optimizacijom procesnih parametara 3D ispisa postignut je najveći prinos bioaktivnih spojeva u 3D ispisanim proizvodima s najmanjim sadržajem škroba. Teksturne analize pokazale su da su svi 3D ispisani proizvodi pokazali dobra teksturna svojstva, visok stupanj stabilnosti i minimalna geometrijska odstupanja.

Zaključno, rezultati istraživanja pokazuju da su jagode izvrsna sirovina za proizvodnju funkcionalnih voćnih sokova zbog svog bogatog biološkog potencijala. Primjena tehnologije preprekama temeljene na kombinaciji PEF i HPU uspješno je primjenjiva u očuvanju tog potencijala. Štoviše, ovakvi proizvodi mogu se dodatno koristiti u proizvodnji 3D ispisanih funkcionalnih proizvoda. Optimizacijom procesnih parametara 3D ispisa moguće je proizvesti funkcionalne proizvode očuvanih bioloških svojstava. Tako ovo istraživanje predstavlja značajan doprinos u segmentu razumijevanja utjecaja održivih netoplinskih tehnologija PEF-a i HPU-a te aditivne tehnologije 3D ispisa na kvalitetu i stabilnost funkcionalnih proizvoda na bazi jagode uz dodatak maginje.

Ključne riječi: netoplinske tehnologije, pulsirajuće električno polje, ultrazvuk visoke snage, 3D ispis, bioaktivni spojevi, jagoda (*Fragaria x Ananassa* Duch.), maginja (*Arbutus unedo* L.)

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Information about the supervisor

Full professor Danijela Bursać Kovačević graduated in 2002 from the Faculty of Food Technology and Biotechnology at the University of Zagreb (PBF), where she also completed her doctoral thesis in 2010. Since 2003, she has been a faculty member at the Laboratory for Fruit Chemistry and Technology within the Department of Food Engineering at PBF. She teaches both undergraduate and graduate courses, and has mentored over 30 students who have successfully defended their undergraduate and her guidance.

Dr. Bursać Kovačević is a distinguished Croatian researcher with significant international recognition in the field of Food Science and Technology. She has contributed to numerous national and international scientific research projects and is currently leading projects such as “Hurdle Technology and 3D Printing for Sustainable Fruit Juice Processing and Preservation” (IP-2019-04-2105) and “From Edible Sprouts to Healthy Food-FEED” (Prima Call 2022, Prima Section 2 – Multi Topic 2022, Topic 2.3.1 (RIA) Enabling the transition to healthy and sustainable dietary behavior, HORIZON 2020 Programme). Since 2019, she has coordinated the CEEPUS III project RS-1512-04-2324 “Improving Food Quality with Novel Food Processing Technologies”. Her dedicated work has earned her several national and international awards, including the National Science Award for 2019 (awarded in 2020 by the Ministry of Science and Education). Dr. Bursać Kovačević has been recognized as a Clarivate™ Highly Cited Researcher in 2021, 2022, and 2023, ranking in the top 1% by citations in agricultural sciences by Web of Science™. In 2020, she co-authored a prominent scientific publication in the Annual Review of Food Science and Technology with esteemed global scientists, marking a significant milestone in her career. She has participated in over a hundred international scientific conferences and has been involved in various organizational, executive, or scientific committees. She has delivered six invited and two plenary lectures at prestigious international conferences. Dr. Bursać Kovačević has co-authored more than a hundred scientific papers, with over 50% published in Q1 journals. She has edited notable scientific books, including "Agri-Food Industry Strategies for Healthy Diets and Sustainability" (2020, Academic Press) and "Sustainable Functional Food Processing" (2022, MDPI), and has co-authored 15 book chapters. In her editorial roles, she serves as Associate Editor for Food Reviews International Journal (Taylor and Francis) and is a member of the editorial boards for LWT-Food Science and Technology (Elsevier), Food Chemistry Advances (Elsevier), Frontiers in Food Science and Technology (Frontiers), and Discover Food (Springer).

Authors publications included in the doctoral dissertation:

Publication No.1

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Bebek Markovinović, A., Putnik, P., Duralija, B., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., Palac Bešlić, I., Pavlić, B., Manuel Lorenzo, J., Bursać Kovačević, D. (2022) Chemometric Valorization of Strawberry (*Fragaria x ananassa* Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. *Foods*, **11** (5), 640. doi: 10.3390/foods11050640.

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Table of Contents

General introduction

In relation to chronic, non-communicable diseases, increased consumption of fresh fruits and vegetables is considered essential for their prevention. This is supported by the rich micronutrient profiles of fruits and vegetables and the presence of numerous functional compounds that, when consumed in adequate amounts, provide significant health benefits (Bursać Kovačević et al., 2020). This has directed the food industry's interest in developing functional foods and integrating them into their product lines (Granato et al., 2020). Considering the fast-paced lifestyle and emerging dietary trends, fresh fruit juices are becoming increasingly popular in the market due to their convenience. Furthermore, consuming fruit juices can offer benefits equivalent to the recommended daily intake of fruit (Benton and Young, 2020). The selection of raw materials is influenced by their physicochemical, biological, nutritional, toxicological and sensory properties (Bebek Markovinović et al., 2022b). Consequently, strawberries are identified as a promising raw material for juice production and other functional products due to their high concentration of polyphenolic compounds, including anthocyanins, phenolic acids and tannins (Fierascu et al., 2020). The distinct flavor, low-calorie content and rich nutritional profile, particularly the high content of vitamin C and polyphenols, significantly enhance the attractiveness of strawberries for consumers (Giampieri et al., 2012). However, the thermal processing required for preserving fruit juices unfortunately reduces their biological value, leading to a decrease in bioactive compounds (BACs), including antioxidants. Specifically, polyphenols, which have antioxidant properties, have been shown to have beneficial and potentially therapeutic effects against the aforementioned health conditions (Giampieri et al., 2015).

To ensure maximum preservation of BACs and provide functional properties to the final product, non-thermal processing techniques are increasingly becoming the focus of research. These methods include pulsed electric fields, high hydrostatic pressure, cold plasma and ultrasound. The main goal of using these advanced food processing technologies is to produce a product that maintains a high level of biological value and safety in addition to its nutritional and sensory qualities. This approach aims to create a product comparable to fresh, untreated items, aligning which consumer expectations (Granato et al., 2017). Recently, combinations of thermal and/or non-thermal technologies, known as hurdle technologies, have been increasingly explored. The principle of hurdle technologies is that they are applied in a specific sequence, allowing each technology to achieve optimal results in terms of maintaining the quality and extending the shelf life of the product under non-invasive processing conditions (Putnik et al., 2020). The special feature of this concept lies in its ability to utilize the

synergistic interactions of several mechanisms to inhibit and/or deactivate certain microorganisms. Consequently, hurdle technology offers an advanced method for fruit juice processing with the potential to meet the stringent requirements of both consumers and producers (Putnik et al., 2020).

Pulsed electric field (PEF) technology is a promising method for food preservation, as it effectively deactivates pathogenic microorganisms and enzymes that affect food quality, with minimal impact on the nutritional content of the product (Koubaa et al., 2018). The core principle of this technique involves applying short high-voltage electrical pulses to food placed between two electrodes (Elez-Martínez et al., 2017). This process disrupts the membrane potential of the microorganisms, leading to damage to membrane integrity, pore formation (electroporation) and increased membrane permeability. Although the energy transfer raises the temperature of the sample during treatment, it usually remains below 40 °C, making this technology a viable alternative to conventional thermal food processing methods (Koubaa et al., 2018; Salehi, 2020a). Nevertheless, PEF technology is predominantly applied to liquid media, with ongoing research focusing on its use in the preservation of fruit and vegetable juices (Gabrić et al., 2017; Elez-Martínez et al., 2017). In addition to ensuring microbiological safety, the mechanism of membrane rupture can be used to extract specific BACs from cellular compartments, thereby improving the nutritional quality of fruit juices using this innovative method (Elez-Martínez et al., 2017; Koubaa et al., 2018).

High Power Ultrasound (HPU) uses ultrasound waves with high intensity (10–1000 W cm^{-2}) and low frequencies (20–100 kHz). This technology is based on cavitation, i.e. the formation and growth of small air bubbles and their subsequent implosion when ultrasound waves penetrate a liquid (Soltani Firouz et al., 2019). The change in pressure gradient caused by the collapse of the cavitation bubbles in or around microbial cells leads to mechanical destabilization of their cell membranes. The effectiveness of HPU treatment depends on several parameters, including power, frequency, probe diameter, amplitude, treatment duration and temperature (Soltani Firouz et al., 2019). Although previous research indicates that hurdle technologies combining PEF and HPU can contribute to a comprehensive improvement in juice quality, this approach has not yet been sufficiently explored (Putnik et al., 2020).

Three-dimensional (3D) food printing is an innovative, digitally controlled food manufacturing process that uses additive technology to create three-dimensional shapes of food by layering materials. This technology offers a solution for producing personalized food

products tailored to consumer needs, while using alternative sources of raw materials and reducing energy consumption (Gaikwad et al., 2018). Furthermore, 3D printing enhances the stability of BACs and antioxidant capacity in 3D printed fruit-based products (Azam et al., 2018). When selecting fruit raw materials for 3D printing, it is crucial to consider the viscosity of the mixture. To achieve an appropriate consistency for 3D printing of functional fruit products, thickening agents are required. Strawberries, with their low dry matter content, pose a challenge as a raw material for 3D printing. In contrast, strawberry tree fruit (*Arbutus unedo* L.) is characterized by its high fiber content, making it a promising natural complement to strawberries for 3D printing applications. Additionally, strawberry tree fruits are known for their high nutritional and biological potential (Bebek Markovinović et al., 2022a), and are believed to have antioxidant (Zitouni et al., 2020a), anti-inflammatory (Morgado et al., 2018), and anticancer properties (Guimarães et al., 2013b). Although they are usually used for the production of alcoholic beverages, strawberry tree fruits are rarely processed into other foods. Due to their properties, they could be an excellent complementary raw material alongside strawberries for the production of innovative 3D printed functional products.

The objectives of this research, presented in this dissertation based on published papers and a comprehensive final evaluation, were to study the quality of strawberry fruit and to evaluate the effects of processing on the juices produced. In addition, the produced juices were treated individually and in combination with technologies (hurdle technologies) related to the PEF and HPU to study their effects on the quality of the strawberry juices. Based on the results obtained, the process parameters for the hurdle technologies were optimized with the aim of preparing the juice as a raw material for further processing, i.e. 3D printing, thus developing a functional product with a unique composition, geometry and extended shelf life. Considering that strawberries are a major challenge for 3D printing due to their physicochemical properties, the study investigated the possibility of combining them with strawberry tree fruit (*Arbutus unedo* L.) as a natural thickening agent to create a fruit matrix of suitable consistency for 3D printing. Furthermore, the effects of adding different starch carriers (wheat *vs*. corn) in different concentrations for each raw material (strawberries and strawberry tree fruits) as well as the effects of different printing programs on the quality of the 3D printed samples were investigated. After statistically analyzing the results, optimal 3D printing parameters were selected that led to the production of functional products from strawberries and strawberry tree fruits.

Theoretical background

- **Strawberry (*Fragaria x ananassa* Duch.)**
- **Bioactive potential of the strawberry**
- **Hurdle technology – Principles of the food processing and preservation**
- **3D printing of food**
- **The potential of strawberry tree fruit as a functional food additive**
- **Objectives, hypotheses and expected contribution**

1. Strawberry (*Fragaria x ananassa* Duch.)

Originally from Europe (France) but now cultivated worldwide, the garden strawberry (lat. *Fragaria x ananassa* Duch.) was developed in the 18th century by crossing the Virginian strawberry (lat. *Fragaria virginiana*) and the Chilean strawberry (lat. *Fragaria chiloensis*). It is the most significant representative of the berry group. Within the genus Fragaria, a large number of genotypically and phenotypically diverse species have been cultivated in different parts of the world throughout history. Of the 47 known strawberry species, ranging from diploid to decaploid species, the wild strawberry (lat. *Fragaria vesca* L.) and the Chilean strawberry are particularly notable from a commercial perspective (Gambardella and Sánchez, 2016). Morphological differences between species, or even within the same species, are evident in traits such as the color and shape of achenes, and the morphology of bracts and leaves (Liston et al., 2014). The current economic importance of all other species within the genus *Fragaria* is negligible compared to *Fragaria x ananassa* Duch. which is the most important berry species globally. This is underscored by FAO data, which shows that global strawberry production in 2021 exceeds 9 million tons, while the total production in the Republic of Croatia for the same year is 1,960 tons (FAO, 2022).

Regardless of the species, strawberries are perennial herbaceous plants with trifoliate leaves and flowers consisting of five symmetrical white petals. About 4 to 5 weeks after flowering, the strawberry fruits are ready for harvesting and should have at least 7% total soluble solids (TSS) and a total acidity of 0.8% (Kader, 1999). The key differences between the species pertain to the color and shape of the fruit, leading to variations in chemical composition and numerous possibilities for commercial use (Fierascu et al., 2020). Strawberries are not only consumed fresh but also serve as an essential raw material for the production of jellies and fruit juices. Their appealing aroma, low calorie content and rich nutritional profile, particularly high in vitamin C and polyphenols, are the main factors contributing to their popularity among consumers (Fierascu et al., 2020; Milosavljević et al., 2020; Giampieri et al., 2013).

Strawberries have a high-water content (90%), which is the primary reason for their low energy value of just 30 kcal per 100 g of fruit. Among the macronutrients, carbohydrates (7%) are the most significant, with the predominant reducing sugars being fructose and glucose (80-90%) in a 1:1 ratio, with the remainder being sucrose. Strawberry consumption does not significantly contribute to protein intake, as strawberries contain only 0.67 g of protein per 100

g. The oil from strawberry seeds is a source of essential fatty acids, 72% of which are polyunsaturated. However, due to the very low mass of the seeds, the total fat and protein content in strawberries is almost negligible. Among the organic acids, citric acid (80%) is the most prevalent, though its concentration does not vary with fruit ripeness. Citric acid plays a crucial role in influencing pH levels, color stability, and the activity of oxidation enzymes (Sood and Dogra Bandral, 2019). The balance of sugars and acids is particularly important for the perception of strawberry flavor, with a TSS-to-acid ratio of 8.52/13.79 contributing to a full strawberry flavor. Strawberries with a lower pH (3.27 – 3.86) have a more vibrant red color, while those with higher TSS (8.0 – 11.5%) are best suited for processing (Sood and Dogra Bandral, 2019). Strawberries are also renowned for their micronutrient content, particularly vitamin C, with recorded values exceeding 50 mg per 100 g of fruit, providing over 50% of the recommended daily intake for an adult (Giampieri et al., 2013).

2. Bioactive potential of the strawberry

Due to the presence of a large number of BACs with diverse health effects, strawberries and strawberry-based products are considered functional foods. Although there is no uniform definition of this term, foods can be considered functional if, in addition to providing basic nutritional benefits, they also show potential positive effects on human health when consumed regularly as part of a varied diet (Granato et al., 2017). The term “functional food” can refer to both industrially processed foods and natural foods in their original, raw form. The effects of BACs on human health is continuously being studied, and epidemiological data suggest that a high intake of natural functional foods rich in BACs, such as certain fruits and vegetables, is associated with a lower risk of chronic diseases including cardiovascular disease, certain cancers, metabolic syndrome, type II diabetes and obesity (Karasawa and Mohan, 2018; Giampieri et al., 2015). And as previously mentioned, due to the unique combination and synergistic effect of numerous BACs, strawberries are recognized as a functional food. The health benefits and potential prevention of chronic diseases associated with their frequent consumption are supported by numerous studies showing that the BACs in strawberries have a wide range of biological activities (Afrin et al., 2016). These health benefits include a potential role in preventing cardiovascular and heart diseases, certain cancers, diabetes, obesity, and neurodegenerative diseases (Giampieri et al., 2013). Examples of strawberry-based functional products, often combined with other functional ingredients, include juices,

fermented drinks, smoothies, yogurts, jams and other jellied products (Ali et al., 2021; Cervera-Chiner et al., 2021; Zhao et al., 2021; Kowaleski et al., 2020; Barbosa et al., 2017).

Strawberries are characterized by a high content of polyphenolic compounds. The basic chemical structure of these compounds includes an aromatic ring with at least one hydroxyl group, ranging from simple molecules to complex polymers. Based on their chemical structure, polyphenolic compounds are broadly classified into two main groups: flavonoids and non-flavonoid polyphenols (Durazzo et al., 2019). Flavonoids, the largest group of polyphenols, are further divided into six subclasses (Forbes-Hernández et al., 2014). The majority of phenolic compounds in strawberries are polyphenols from the flavonoid group, including flavonols, flavanols and anthocyanins, with the latter being the most prominent. Flavonols, such as kaempferol and quercetin, are present in small amounts in strawberries (Newerli-Guz et al., 2023). Flavanols, particularly flavan-3-ols, are the second most important phenolic compounds in strawberries after anthocyanins and are found in both monomeric (catechins) and polymeric forms known as condensed tannins or procyanidins (Alvarez-Suarez et al., 2014; Aaby et al., 2012). Alongside anthocyanins and flavan-3-ols, ellagitannins, which belong to the group of hydrolyzed tannins, are also abundant in strawberries. Additionally, strawberries contain various phenolic acids, primarily as derivatives of hydroxycinnamic acid and hydroxybenzoic acid, with *p*-coumaric and ellagic acids being the most prevalent (Aaby et al., 2012; Skupien and Oszmianski, 2004). The total antioxidant capacity of strawberries serves as an indicator of the beneficial BACs present and is closely linked to health benefits. The antioxidant capacity significantly enhances the nutritional value of strawberries due to the presence of compounds like vitamin C and polyphenolic compounds, which neutralize the harmful effects of free radicals (Giampieri et al., 2012). The content of polyphenolic compounds in strawberries varies depending on factors such as genotype, growing conditions, ripeness and post-harvest storage conditions (Giampieri et al., 2012).

3. Hurdle technologies - Principles of the food processing and preservation

The quality of fruit juices is determined by their physical, sensory and microbiological properties, as well as their enzymatic activity. Enzymatic activity, microbial growth, and oxidative reactions can reduce the shelf life of the product. Traditionally, thermal processing is used to inactivate enzymes and ensure the microbiological safety of fruit juices (Iqbal et al.,

2019). However, elevated temperatures negatively affect the BACs, color parameters, and sensory properties of fruit juices. Consequently, interest in non-thermal preservation methods has grown in recent years (Putnik and Bursać Kovačević, 2021). Non-thermal processing techniques include high-pressure processing (HPP), inert gas treatment, membrane processes, cold atmospheric plasma, irradiation, pulsed electric fields (PEF), high-power ultrasound (HPU) and others. Although these methods are generally considered less invasive compared to thermal treatments, their effectiveness depends on the specific food matrix being treated (Alves Filho et al., 2016). Therefore, selecting the most suitable non-thermal method with validated processing parameters is crucial to preserving nutrients and maintaining the original sensory properties (Koutchma et al., 2016).

Consumers are increasingly interested in minimally processed products without additives that offer greater safety, extended shelf life, and preserved biological and functional properties (Granato et al., 2020). In response, the food industry is integrating various processing technologies and employing the hurdle technology approach. This strategy involves applying a well-defined sequence of process barriers that inhibit microbial growth. The aim of hurdle technology is to enhance nutritional value and sensory properties while ensuring food safety. The main advantage of this concept lies in the synergistic effects resulting from the combination of different technologies, which together provide a more effective preservation outcome (Rahman, 2015).

The combined application of various novel technologies, such as PEF, high hydrostatic pressure (HHP), HPU and cold plasma, is increasingly being explored in the field of fruit juice processing. For instance, the synergistic effect of PEF (20 kV cm^{-1}, 1 kHz, 600 μs) and HPU (600 W, 28 kHz, 30 minutes) on BACs in grapefruit juice has demonstrated a remarkable increase in total anthocyanins compared to the untreated sample (Aadil et al., 2017). The highest concentration of anthocyanins was observed when both treatments were applied simultaneously. Similarly, the combination of HPU (40 kHz, 200 W) and PEF (9 kV cm^{-1}, 1 kHz, 60 mL min^{-1}) in spinach juice resulted in a greater yield of anthocyanins and other BACs than when each technology was applied separately (Faisal Manzoor et al., 2021).

Despite the promising results with other fruit juices, the application of hurdle technology involving the combination of PEF and HPU in strawberry juice has been minimally explored. Over the past seven years, only a limited number of studies have investigated the effects of hurdle technology on strawberry juice quality. For example, one study explored the influence of ultrasound (40 kHz; 180 W; 0, 15 and 30 minutes) combined with natural

antimicrobial additives such as geraniol and pomegranate extract on the microbial, sensory and nutritional quality of strawberry juice (Tomadoni et al., 2019). Another study examined the effects of ultrasound and antimicrobial ZnO nanostructure packaging on the quality of fresh strawberry juice during 35 days of refrigerated storage (Emamifar and Mohamadizadeh, 2020). However, to date, the combined use of PEF and HPU technologies has not been applied to strawberry juice.

PEF is a non-thermal method of food processing known for its effectiveness in inactivating microorganisms and enzymes, making it a viable alternative to traditional thermal pasteurization (García-García et al., 2015). Also, PEF is utilized for extracting BACs (Koubaa et al., 2018), and it is commonly applied to liquid products, including fruit juices, liquid eggs, alcoholic beverages, and dairy products (Dziadek et al., 2019). The effectiveness of PEF depends on process parameters such as electric field strength and pulse frequency, allowing for diverse applications (Gabrić et al., 2017; Yu et al., 2015). During PEF treatment, food is placed between two electrodes and subjected to short high-voltage electrical pulses (between 0.1 and 50 kV cm^{-1}). The primary mechanism involves electroporation and electropermeabilization of cell membranes, leading to their disruption (Salehi, 2020a). Studies suggest that PEF treatment in juice production can significantly increase the content of vitamins and polyphenolic compounds compared to conventional processing methods (Nowosad et al., 2020). PEF offers several advantages over traditional thermal processing, such as shorter processing times, lower operating temperatures, and improved extraction of polyphenols (Salehi, 2020a). Strawberry juice treated with High-Intensity Pulsed Electric Field (HIPEF) treatment retains 75-100% of its antioxidant capacity, compared to untreated juice, which retains only 38.5% as measured by the DPPH method (Odriozola-Serrano et al., 2009). PEF treatment not only ensures microbiological safety and stability of the juices, potentially outperforming thermal treatments, but also increases the concentration of antioxidant compounds in the final product (Narendar et al., 2018). In addition, HIPEF helps to preserve the original color of the strawberry juice and reduce browning during storage compared to conventional methods (Aguiló-Aguayo et al., 2009). However, PEF primarily targets vegetative microbial cells and is ineffective against spores (Kempkes, 2017). To ensure sufficient product safety, PEF can be combined with additional preservation techniques, such as mild heat treatment, antimicrobial agents, bacteriocins, ultrasound, UV radiation, and more (Putnik et al., 2020).

HPU is an advanced non-thermal food processing technology recognized for its minimal ecological impact, making it environmentally friendly. HPU operates based on the

phenomenon of cavitation, which induces both physical and chemical changes in the material. Specifically, HPU generates a longitudinal wave in a liquid medium, leading to alternating phases of compression and expansion, which trigger cavitation. During cavitation, gas bubbles form in the liquid and gradually increase in size until they reach a critical point, at which they collapse, causing rapid molecular collisions that generate shock waves. These shock waves produce extremely high temperatures (up to 5500 K) and pressures (up to 100 MPa). The effectiveness of cavitation is influenced by factors such as ultrasound parameters (frequency, intensity), the properties of the product (viscosity, density, surface tension) and the environmental conditions (temperature, pressure and humidity) (Bosiljkov et al., 2011; Cheng et al., 2019). HPU effectively reduces microorganisms by disrupting cell structures, locally heating the material, and generating free radicals. In addition, ultrasound treatment can enhance juice consistency (apparent viscosity), color, sensory acceptance and the stability of BACs (Rojas et al., 2017). Ultrasonic treatment shows significant potential for fruit juice processing by inactivating enzymes such as polygalacturonase and polyphenol oxidase, which helps extend shelf life and improve product quality (Salehi, 2020b). Studies on strawberry juice have demonstrated that HPU treatment does not alter the physicochemical properties or degrade antioxidant compounds in the juice (Tomadoni et al., 2017). Furthermore, studies indicate that HPU treatment (40 kHz, 250 W, 4 °C, 20 min) does not affect the color parameters of fruits and vegetables, but can help to minimize color loss during storage (Lafarga et al., 2019).

Ultrasound has been shown to achieve a 5-log reduction in microbial counts for fruit juices, meeting FDA standards and positioning it as a potential alternative to pasteurization (Patil et al., 2009). However, ultrasound treatment can also negatively impact juice quality, which is why recent studies recommend combining it with other technologies (Mahmoud et al., 2022). Traditional preservation techniques, such as high temperatures and pressures combined with antimicrobial additives, can be integrated with non-thermal methods. The use of advanced technologies like ultraviolet light, pulsed light, PEF and HPP is becoming increasingly common (Granato et al., 2020).

4. 3D printing of food

Three-dimensional printing, first introduced in 1986, is defined as a process that creates a digitally designed 3D object by adding layers of material. This method, also known as "additive manufacturing", allows for the production of complex 3D models with minimal

material waste and eliminates the need for molds, dies, supports and cutting equipment used in conventional manufacturing (Escalante-Aburto et al., 2021; Es-Said et al., 2018; Feng et al., 2018).

3D printing is an advanced food manufacturing technique that offers numerous benefits. It allows for the creation of food with customized compositions to meet specific dietary requirements, the enrichment of meals with targeted nutrients, and the production of intricate textures and shapes. The integration of digital technology into the printing process facilitates continuous monitoring and real-time design adjustments. This method helps reducing waste, carbon dioxide emissions and energy consumption, while also lowering the costs associated with preparation and transportation. Additionally, it presents significant opportunities for innovation in food production (Dick et al., 2019; Jiang et al., 2018; Portanguen et al., 2019; Piatti et al., 2019; Sun et al., 2015).

The process of 3D food printing can be divided into three different stages. The first stage involves the pre-processing and preparation of food materials to make them suitable for printing. Ingredients, which may be in forms such as powders, pastes, liquids or doughs, require preparatory steps like grinding, cooking or mixing (Escalante-Aburto et al., 2021). Additionally, additives such as hydrocolloids may be needed to achieve optimal rheological properties (Manstan and McSweeney, 2019). The second stage is the actual 3D printing of food, facilitated by various techniques. The predominant method is extrusion, where the prepared material is ejected layer by layer through a print head (extruder) onto a substrate, forming a predetermined 3D shape. By adjusting the material's temperature and the distance between the print head and substrate, the consistency, degree of cross-linking and thermal processing of the product can be controlled (Escalante-Aburto et al., 2021; Sun et al., 2015). The third, optional stage involves additional processes to prepare the product for consumption or to achieve the desired final texture. Common techniques in this phase include freezing, baking and roasting (Manstan and McSweeney, 2019).

Materials for 3D food printing can be categorized based on their printability, primary nutritional and health-related components, such as proteins, starches, fibers and functional compounds like vitamins and antioxidants, and their origin, including dairy-, meat-, fruit- and vegetable-based materials. "Naturally extrudable materials" possess the necessary rheological and mechanical properties for direct extrusion without the need for additional additives like gums (Voon et al., 2019). Common examples include chocolate and mashed potatoes. In

contrast, traditional foods that lack the required properties for direct extrusion (e.g. fruit, vegetables, rice and meat) often need additives to adjust their rheological and mechanical properties as well as their nutritional content. For example, the nutritional profile of chocolate can be enhanced by adding vitamin C, cranberry powder or plant sterol powder (Hao et al., 2019; Mantihal et al., 2019). Additionally, a 3D printed material was developed using fish collagen combined with a mixture of fruits and vegetables (carrot, kiwi, broccoli, avocado, and pear) (Severini et al., 2018). Similarly, 3D printed materials were created from canned tuna, beetroot, and butternut squash (Kouzani et al., 2017). The 3D printing did not alter the phenolic content, sensory properties and antioxidant properties of the materials. Moreover, 3D food printing is increasingly utilizing alternative ingredients that can replace conventional food sources. Functional components, such as proteins and fibers, can be sourced from insects (Severini and Derossi, 2016), algae, seaweed or mushrooms (Sun et al., 2015). These alternative ingredients, available in paste or powder form, can be combined with conventional ingredients to produce customized foods (Escalante-Aburto et al., 2021).

The practical application of 3D food printing is particularly relevant for producing foods that are tailored to individual health and nutritional needs. Recent advancements have greatly impacted personalized medicine, nutritional management, and therapeutic approaches, as well as developments in sustainable food engineering and processing technologies (Tomašević et al., 2021). An overview of 3D food printing techniques and devices, an assessment of the advantages and limitations of this technology, its potential for the development of functional foods, and its impact on textural and rheological properties, are described in *Publication No.1*.

Publication No.1: 3D printing as novel tool for fruit-based functional food production

Publication No.1

Tomašević, I., Putnik, P., Valjak, F., Pavlić, B., Šojić, B., **Bebek Markovinović, A.**, Bursać Kovačević, D. (2021) 3D printing as novel tool for fruit-based functional food production. *Current Opinion in Food Science*, **41**, 138–145. doi: 10.1016/j.cofs.2021.03.015.

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5. The potential of strawberry tree fruit as a functional food

The strawberry tree (*Arbutus unedo* L.) is a wild fruit species that thrives in the moderately warm regions of the Mediterranean and Western Europe (Caudullo et al., 2017). In the Republic of Croatia, the strawberry tree is an integral part of the maquis and Mediterranean forests along the entire Adriatic coast, from Istria to Dubrovnik-Neretva County, including the islands. However, its cultivation and potential uses in Croatia remain underdeveloped, as the tree is undervalued and relatively unknown (Skendrović Babojelić et al., 2020).

The strawberry tree is notable for the simultaneous presence of flowers and fruit at various stages of ripeness. The edible fruit is a berry measuring 1.5-2.5 cm in diameter and ranges in color from green to yellow-orange to dark red, depending on its ripeness. The fruit's exterior is rough, while the inside is soft, yellow and contains seeds. It takes 12 months for the fruit to ripen, resulting in staggered ripening periods from October to January. Ripe fruits are sweet and are a traditional part of the Mediterranean diet, often processed into jams, preserves and strong alcoholic beverages (Tardío et al., 2006; Tardío et al., 2016).

Numerous studies have explored the chemical composition of the strawberry tree fruit, highlighting that its nutritional properties stem from the variety and ratio of its chemical constituents. The total dry matter content is an important measure for assessing the fruits' quality for technological applications and processing, with a higher dry matter content being preferable. On average, the water content of strawberry tree fruit is about 50% (Skendrović Babojelić et al., 2020). The total soluble solids content varies between 16.87% and 19.4% (Zitouni et al., 2020b; Serçe et al., 2010). Analysis has identified the presence of various carbohydrates, including fructose, glucose, sucrose, cellulose and starch (Özcan and Hacıseferoğulları, 2007). Carbohydrates make up approximately 50% of the fruit's dry matter, significantly enhancing its energy value and nutritional potential. The specific carbohydrate content is influenced by the fruit's ripeness; sucrose predominates in unripe fruit, while fructose is the main carbohydrate in ripe fruit (Alarcão-E-Silva et al., 2001). Studies consistently show that fructose is the predominant sugar in ripe druit, followed by glucose (Ruiz-Rodríguez et al., 2011). This composition contributes to the fruits' the sweetness and its susceptibility to alcoholic fermentation in the presence of yeast.

The strawberry tree fruit is not only characterized by its sugar content, but also by its richness in minerals, fiber, vitamins (particularly vitamin C), amino acids, and a variety of other BACs (Miguel et al., 2014). Compared to other berries such as strawberries, blackberries,

raspberries and blueberries, the strawberry tree fruit stands out for its high fiber content (Jurica and Brčić Karačonji, 2016). In the literature, it is often described as an exceptional source of fibers (Sagbas et al., 2020; Colak, 2019). Studies have shown that consuming 100 grams of this fruit can provide up to 40% of the recommended daily fiber intake (Trumbo et al., 2002). Additionally, the fruit contains proteins (Boussalah et al., 2018) and organic acids such as fumaric, malic, lactic, suberic and citric acids (Zitouni et al., 2020b; Ayaz et al., 2000). Researchers have identified fatty acids in the fruit, with a significant presence of unsaturated fatty acids such as linolenic, linoleic and oleic acids. Among the saturated fatty acids, palmitic acid is the most abundant, followed by stearic acid (Boussalah et al., 2018; Barros et al., 2010).

Phenolic compounds, which naturally form in fruits in response to environmental stress, enhance the fruit's bioactive properties. These compounds are secondary plant metabolites characterized by the presence of two or more hydroxyl groups attached directly to a benzene or aromatic ring (Dai and Mumper, 2010). Studies indicate that the average total phenolic content in strawberry tree fruit is around 1000 mg gallic acid equivalent 100 g^{-1} fresh sample. Variations in phenolic content are influenced by environmental conditions, such as air temperature and rainfall. Specifically, fruits from regions with higher temperatures and lower precipitation tend to have a higher total phenolic content, as these compounds act as a defense mechanism against environmental stress. Notably, the strawberry tree fruit has a higher concentration of phenolic compounds compared to hawthorn and blackberry (Barros et al., 2010; Fortalezas et al., 2010). Research has identified gallic acid as the most abundant phenolic compound in strawberry tree fruit (Gündoğdu et al., 2018; Sagbas et al., 2020). However, other studies highlight that galocatechin is the dominant phenolic component, followed by derivatives of catechic acid and gallic acid (Zitouni et al., 2020a; Zitouni et al., 2020b). Among anthocyanins present in the strawberry tree fruit, cyanidin-3-galactoside is quantitatively the most prevalent, followed by cyanidin-3-glucoside, along with delphinidin-3-galactoside and cyanidin-3-arabinoside (Miguel et al., 2014). Additionally, carotenoids, especially β-carotene, play a protective role as photoprotective agents of the fruit (Maoka, 2019). Collectively, these BACs contribute to the fruit's remarkable antioxidant capacity, with the strawberry tree fruit outperforming red and green grapes, pomegranate, blueberries and apple juice in terms of antioxidant activity (Maragò et al., 2015; Liu et al., 2018; Gil et al., 2000).

Phenolic compounds are known for their diverse biological effects, including antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, anticancer and vasodilatory properties, which are primarily due to their antioxidant abilities

(Soobrattee et al., 2005). For example, ethanol extracts from the strawberry tree fruit have been shown to protect DNA from peroxidative damage (Schaffer et al., 2005). These extracts have also been found to inhibit cancer cells proliferation and reduce their viability (Fortalezas et al., 2010; Guimarães et al., 2013a). Additionally, strawberry tree fruits exhibit antimicrobial activity (Salem et al., 2018). Research on the leaves and roots of the strawberry tree has revealed various therapeutic effects, including antihypersensitizing, anticancer, anti-inflammatory, vasodilatory, antiparasitic, antiplatelet and antimicrobial activities (Morgado et al., 2018).

Due to its extensive biological potential, rich nutritional profile, particularly its high crude fiber content, and desirable organoleptic properties, the strawberry tree fruit could serve as an excellent raw material for the development and/or enrichment of 3D-printed functional products. Full details on the fruits and leaves of the strawberry tree, including aspects of cultivation, nutritional value, biological potential and economic properties, can be found in the accompanying *Publication No2*.

Publication No.2: Strawberry Tree Fruits and Leaves (*Arbutus unedo* L.) as Raw Material for Sustainable Functional Food Processing: A Review

Publication No.2

Bebek Markovinović, A., Brčić Karačonji, I., Jurica, K., Lasić, D., Skendrović Babojelić, M., Duralija, B., Šic Žlabur, J., Putnik, P., Bursać Kovačević, D. (2022) Strawberry Tree Fruits and Leaves (*Arbutus unedo* L.) as Raw Material for Sustainable Functional Food Processing: A Review. *Horticulturae*, **8**, 881. doi: 10.3390/horticulturae8100881.

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Review

Strawberry Tree Fruits and Leaves (*Arbutus unedo* L.) as Raw Material for Sustainable Functional Food Processing: A Review

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Abstract: The strawberry tree (*Arbutus unedo* L.) is a Mediterranean plant known for the traditional use of its fruits and leaves due to their health benefits. Thus, it has been used for years in folk medicine to relieve various health conditions such as urological and kidney problems, dermatological, cardiovascular and gastrointestinal diseases. The fruits are traditionally used for making jams, jellies, and strong alcoholic beverages, while the leaves are mostly used for preparing tea. Since the leaves were more researched, previous results indicated that they have important biological effects, so further research should focus on the fruits. Due to its chemical composition, rich polyphenolic profile and the biological potential derived from it, the plant has great prospects for the production of functional foods and nutraceuticals. However, the plant's potential is underutilized in terms of processing. Therefore, this review summarizes the properties and the potential of the fruits and leaves of *A. unedo* and their possible benefits for processing with respect to agricultural, nutritive, biological and economic values.

Keywords: *Arbutus unedo* L.; agriculture; biological potential; nutritive value; bioactive compounds

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1. Introduction

The fruits of the strawberry tree (*Arbutus unedo* L.) are noticeable, globular, green, and orange to red in color (Figure 1). The fruits ripen several times during the year, in late September/mid-October to early December. The leaves of the strawberry tree are simple, alternately arranged, with serrated margins, leathery, dark green in color, and short-stalked. Since the flowering of the strawberry tree takes 12 months to flourish, the tree sometimes bears the ripe fruit and white-pink flowers at the same time, which creates a beautiful decorative atmosphere in the environment during the winter months [1]. Carl Linnaeus described and named the strawberry tree in volume one of his seminal 1753 work "Species Plantarum" with the Latin name *Arbutus unedo*, which is still used today [2]. The strawberry tree (*Arbutus unedo* L.) has long been used by people in the Mediterranean region mainly as fresh food or processed in various products, as medicine or as wood fuel for heating and cooking [3]. The strawberry tree belongs to the Ericaceae family and forms specific arbutoid mycorrhizae with some fungi [4]. This plant has been studied not only for its nutrient-rich

fruits, but also for its biological properties. All parts of *A. unedo* have roles in Mediterranean folk medicine due to the high content of polyphenols and other phytochemicals [5].

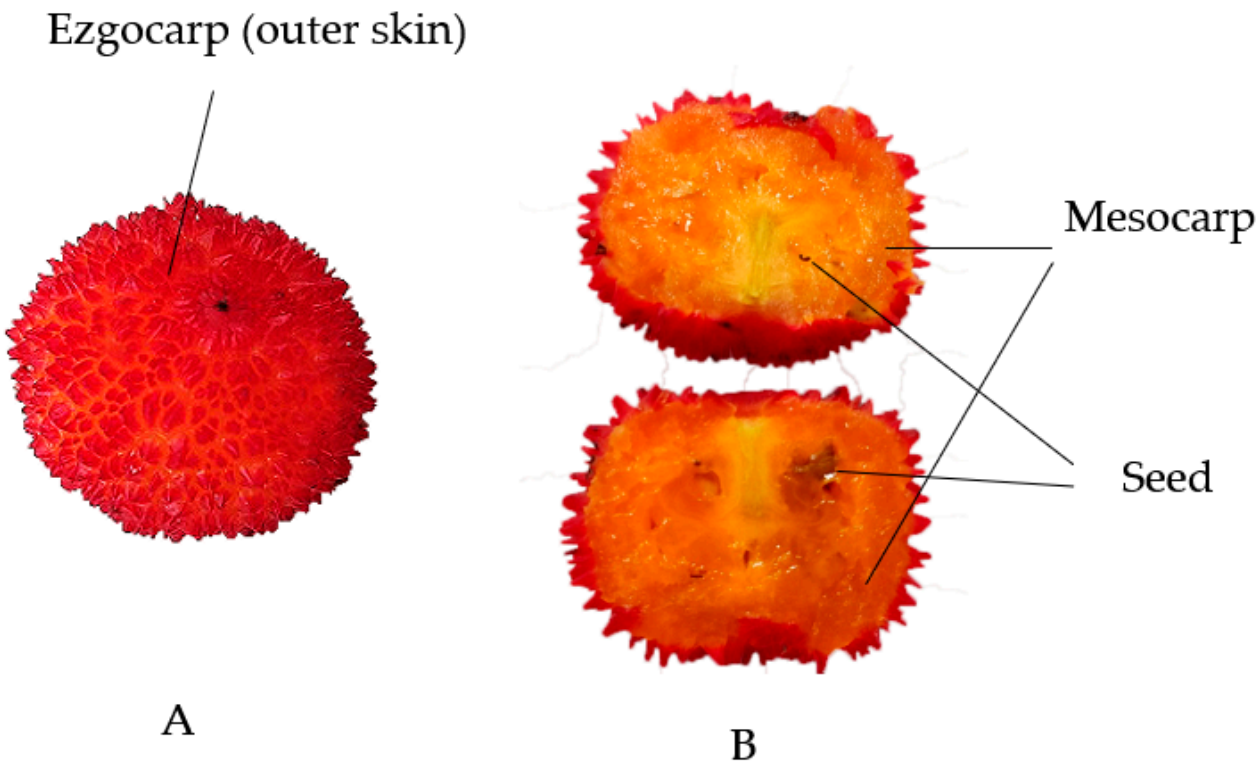

Figure 1. Strawberry tree (*Arbutus unedo* L.) fruit: (**A**) the whole fruit, and (**B**) cross section.

These phytochemicals have been studied for numerous biological activities, including antimicrobial, antioxidant, anti-inflammatory, anti-proliferative and anti-diabetic effects [5,6]. Additionally, polyphenols have the possibility to stimulate cellular defense and the enzymatic systems responsible for detoxification [1]. Strawberry tree leaf extracts have remarkable uroantiseptic, diuretic, astringent and antidiabetic properties [6–9]. The fruits of the strawberry tree play a role in folk medicine in the treatment of gastrointestinal, urological, cardiovascular and dermatological problems, due to their diuretic, antiseptic and laxative properties [5,9]. Decocts of the bark and roots of the strawberry tree are used in folk medicine for gastrointestinal, urological, cardiovascular and dermatological problems [10]. Strawberry tree root is used to relieve abdominal pain, lower cholesterol, treat bladder and kidney diseases, diabetes (by inhibiting glucose absorption in the intestine), treat hypertension and heart disease [10]. It is also used as a diuretic, anti-inflammatory and anti-diarrheal agent [10–12].

The most complete phenolic profile ("fingerprint") of strawberry tree leaves and fruits was recently established in Croatia. A strong correlation between total phenolic content and radical scavenging activity indicated that phenolic compounds are responsible for the antioxidant properties of *A. unedo* leaves and fruits [5].

The fruits of the strawberry tree are the most commonly consumed in the form of jams, marmalades or alcoholic distillates [10]. The honey of *A. unedo*, also known as "bitter honey," has a strong and astringent taste and a very high content of phenols [13]. The production of jams, marmalades or liqueurs from *A. unedo* fruit is often an important additional source of income, especially in rural areas, and its production confirms the economic potential of this shrubby tree.

Recently, consumers have been asking for foods that have a positive impact on their health, such as functional foods [14]. Since the strawberry tree is a nutritionally valuable food with strong biological potential that could be used to produce new products with

positive effects on human health, this article aims to provide an overview of the agricultural, nutritional and biological potential of the fruits and leaves of the strawberry tree, and their possible processing for the production of functional foods.

2. Agriculture Perspective of Strawberry Tree Cultivation

Geographic Distribution of A. unedo L.

The fruits of the strawberry tree are traditionally used for human consumption in Mediterranean regions. The strawberry tree is an evergreen fruit species of the Ericaceae family with natural populations in: the Atlantic region of western Europe (including Ireland); European countries around the Mediterranean Sea; northeastern Africa (including Egypt and Libya); and the Canary Islands and western Asia (Figure 2), where frost is not very frequent and dry summer air is not very intense [15].

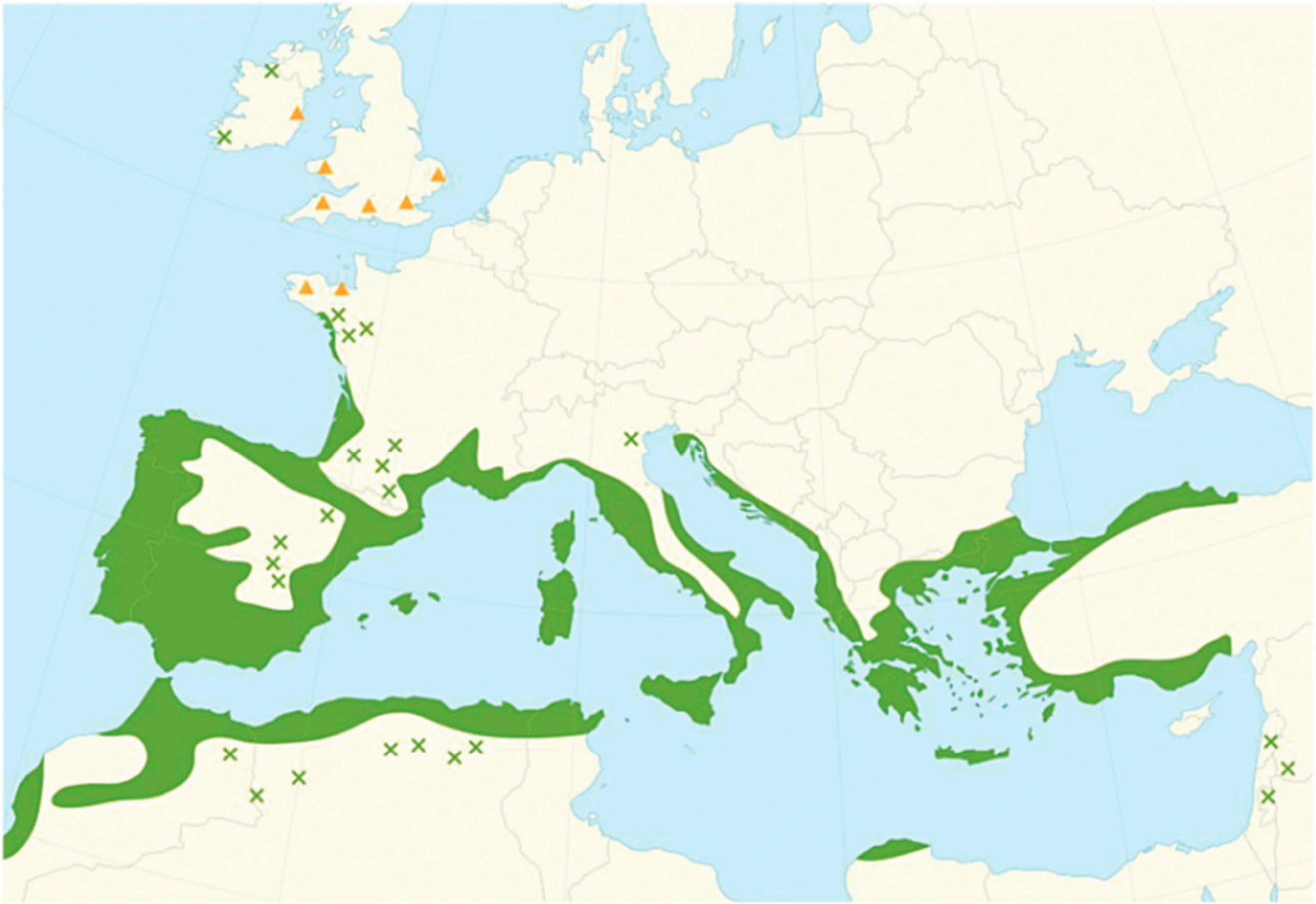

Figure 2. Geographic distribution of *Arbutus unedo* L. (source: Caudullo et al. [16], according to Skendrović Babojelić et al. [17]). Legend: natural habitat; × isolated populations; ▲ introduced and naturalized populations.

In Croatia, it is widely distributed as a natural wild plant along the Adriatic coast, from Istria to Dalmatia and the islands (Figure 3), and is an important component of the maquis vegetation and forests *Quercus ilex* L. [18].

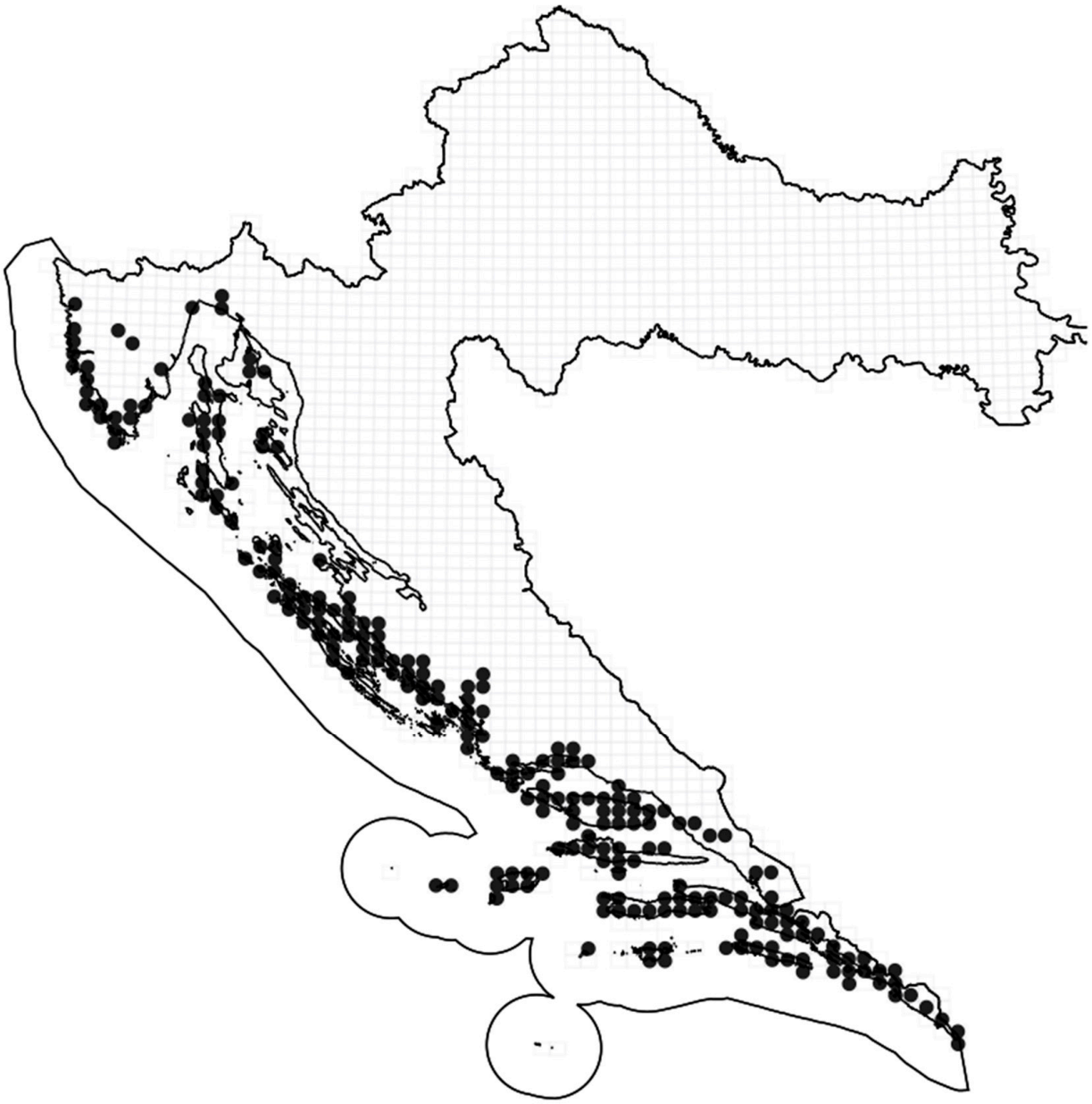

Figure 3. Distribution of wild plants of *Arbutus unedo* L. in Croatia according to the Flora Croatica Database [19].

In some countries, interest in strawberry trees is increasing and new selection studies are focusing on selecting highly productive cultivars with larger fruits. In Turkey, strawberry tree fruits have developed from a small local production to a niche product that fetches considerable prices [20].

In the last century, the cultivation of the strawberry tree has not yet been able to gain acceptance due to the lack of cultivars for higher production and better fruit quality, as well as quality information for cultivation techniques on a larger scale. According to Celikel et al. [21], selection was mainly done in China with the cultivars 'Zaose' [22], 'Dongkui' [23], 'Daliziyangme', 'Baiyangmei', 'Zaohongmei' and 'Dahuamei' [24]. On the market, there are several cultivars such as: 'Compacta', a smaller shrub, from 1.8 to 3 m tall and wide; 'Elfin King', which has a bordered, dwarf form, and flowers and fruits throughout the year; and 'Rubra' has deep pink flowers [25], which are mainly planted in backyards for ornamental purposes.

Some countries have implemented selection programs aimed at selecting strawberry tree genotypes with high fruit quality, promoting extensive cultivation, and preventing deforestation and excessive harvesting [21]. Given the growing interest of farmers, the selected cultivars need to be multiplied on a large scale using appropriate propagation techniques [3]. In Portugal, adult plants were selected and micro propagated to optimize fruit production and quality. It has been shown that it is possible to produce promising genotypes on a large scale and distribute them to farmers interested in this crop [26]. Nowadays, there are some plantations in Portugal and some research is being conducted [27]. In Italy (Sardinia), the fruits of 20 different genotypes have been characterized [28]. Additionally, in Turkey, the phenological and pomological characteristics of different genotypes of the common strawberry tree and species of Greek strawberry tree have been determined [29–32] according to Celikel et al. [21]. Strawberry trees are characterized by high genetic, morphological and phenological variability [15].

As with some other fruits, the quality of strawberry tree fruit is probably subject to various influences, such as: location; selection of plant material; growing conditions; cultivation techniques; and fruit ripening stage, etc. [33,34].

In areas where the average temperature in January is above 4 °C, growth and fertility are limited, and temperatures of −10 °C and below can cause plant death, which largely depends on the duration of low temperatures [17]. It is important to choose areas for growing strawberry trees that are not exposed to frequent occurrence of stress factors such as frost, hail, strong winds and prolonged drought. Naturally, strawberry trees grow in different soils, the pH of which ranges from 5.0 to 7.2. In addition to soil pH, soil texture and organic matter content must also be considered to get a better estimate. It is quite adaptable to different conditions and soil types [15,25]. The microsite is determined by longitude, altitude, slope, solar radiation, air and water drainage, species diversity and other factors. The strawberry tree usually grows between 20 m and 1000 m above sea level [35], but it can grow up to 1200 m high [36]. It grows best and bears fruit when the entire plant is in full or partial sun [15,25]. The reproductive cycle of strawberry trees is much longer than that of other fruit tree species; from flowering to fruit ripening it lasts the whole year, which should be taken into account.

In nature, the strawberry tree is propagated by seeds, which leads to a greater diversity of genetic and morphological characteristics of wild plants. For the establishment of a modern plantation, it is necessary to use plant material with high yield potential and high-quality fruit. Fertilization before planting is the most important basis for successful planting and obtaining a good yield and fruit quality, because the plants are perennial and remain in the same place for a long time [37]. The establishment of some first strawberry tree plantations shows that clonal plants had significantly higher fruit production when fertilized, while the lowest values were observed in seedlings without fertilization [27]. Row orientation (north-south preferred) and plant spacing in and between rows are also important.

To achieve excellent fruit quality, regular implementation of agro- and pomotechnical measures must be taken into account when growing strawberry trees [17]. Since the strawberry tree is a species that can have very lush vegetation under good growing conditions, several maintenance measures are required in strawberry tree cultivation to be successful: tree pruning and training system; irrigation; orchard maintenance; fertilization; pollination; pest, disease and weed control; and fruit harvesting, etc.

According to the International Center for Underutilized Crops and the Global Facilitation Unit for Underutilized Species [38], the strawberry tree belongs to the category of neglected or underutilized species. The strawberry tree belongs to a species that has been traditionally collected throughout the Mediterranean region since ancient times for its valuable medicinal and aromatic properties [26]. It is considered an underappreciated fruit species with various commercial uses, from the production of fresh fruit and processed products, to its use in the food, pharmaceutical and chemical industries, beekeeping, reforestation, as an ornamental plant and for other purposes. The strawberry

tree has extraordinary ecological importance as it prevents soil erosion and regenerates quickly after fire [15]. It is also important for biodiversity as it forms different plant associations and provides shelter and food for various organisms such as insects, fungi, birds and mammals [17].

The introduction of these wild fruit species into cultivation could exploit their economic and environmental potential and contribute to the sustainability of horticultural production.

3. Nutritive Value of Strawberry Tree

3.1. Fruits

The components of the nutritional and chemical composition of strawberry tree fruits, through their content and mutual interactions, determine the sensory, nutritional and biological properties of the raw material, as well as the final product. The fruits contain a variety of compounds with excellent nutritional quality, including sugars, unsaturated fatty acids, organic and phenolic acids, fibers, vitamins, proteins and carotenoids [39]. Given the basic nutritional and chemical composition, strawberry tree fruits are characterized by a high sugar content, mainly fructose (20–30%), and glucose (about 20%), followed by sucrose (1.5–3%) and maltose (1–2%) [10]. The chemical composition of strawberry tree fruits depends on climatic conditions, soil and seasonal harvest [10]. Given the specific ripening stages of strawberry tree fruits (not all ripen at the same time), the carbohydrate contents vary greatly. For example, sucrose content may be even lower at the fully ripe stage due to hydrolysis on glucose and fructose during ripening. Because of the high carbohydrate content, the fruits of the strawberry tree also have a high energy value. However, care should be taken when eating them, as the slight fermentation of the fruit can cause digestive problems. In addition to sugar, the fruits are also rich in fiber, both soluble and insoluble, with pectin being the most abundant, which also distinguishes this species from a health point of view.

With regards to mineral composition, strawberry tree fruits are a very good source of potassium, calcium, phosphorus, magnesium and sodium [39,40]. In addition, the fruits are rich in vitamins, and the high content of vitamins C and E is particularly noteworthy. Some researchers indicate that fresh strawberry tree fruit may contain between 200 and 300 mg 100 g^{-1} fresh weight (FW) of vitamin C, while vitamin E in unripe fruit may be as high as 1369 mg kg^{-1} FW [34]. In a study conducted in Croatia, the highest vitamin C content was found in wild varieties with 402.41 mg 100 g^{-1} FW [37], which proves that this species can be considered a very good source of vitamin C, even several times higher than certain fruits and vegetables known for their high content of this vitamin, such as citrus fruits, kiwi, peppers, parsley and others. Moreover, fatty acids with a favorable ratio of ω3/ω6-fatty acids have been detected in the fruit, which is due to the linolenic acid, which accounts for 58% of the total percentage of fatty acids [10].

Strawberry tree fruits are also characterized by a high content of polyphenolic compounds, including phenolic acids, flavonoids, anthocyanins, catechuic tannins, gallic tannins, coumarins, quinones and anthraquinones, which makes them a plant material with an extremely high antioxidant potential [15,39]. The polyphenolic contents and polyphenolic profiles of strawberry tree fruits differ greatly in different studies, which is primarily a consequence of the specific environmental factors (e.g., climatic conditions) of the particular site where the fruits were collected. For example: El Cadi et al. [41] reported the total polyphenolic contents of fruits collected in northern Morocco ranged from 34.8 to 51.61 mg GAE g^{-1} dry weight (DW); Ruiz-Rodríguez et al. [42] as 9.51 to 19.73 mg GAE g^{-1} DW in fruits from Spain; Mendes et al. [43] found an average of 16.7 mg GAE g^{-1} DW in fruits collected in Portugal; Barros et al. [44] found an average value of 126.83 mg GAE g^{-1} DW in fruits from the northeastern regions of Portugal; while Colak [45] reported an average value of 557 mg GAE 100 g^{-1} FW from fruits collected in the eastern region of Turkey. Šic Žlabur et al. [37] studied strawberry tree fruits from different locations on the Croatian Adriatic coast (from northern parts to the southern parts, including the islands) and determined a total phenolic contents ranged from 14.29 (Hvar island) to 18.94 to 18.94 mg GAE g^{-1} DW (Cres island) [37], proving that

not only the location but also the specific microsite has a strong influence on the content of polyphenolic compounds.

In addition to the total phenolic content, the fruits of the strawberry tree are also rich in flavonoids and anthocyanins, as shown by various studies. The total anthocyanin contents varied considerably depending on the analyst or location, but also on the ripening stage of the fruits, with the following values found: from 0.13 to 1.42 mg pelargonidin-3-glucoside g^{-1} DW [41], 762.6 mg cyanidin-3-glucoside kg^{-1} DW [46]; from 1.23 to 21.73 mg kg^{-1} FW [37]. Alarcão-e-Silva et al. [47] found that the total anthocyanin contents in strawberry tree fruits varied according to the ripening stage, with the total anthocyanin content increasing during ripening from 0.25 g kg^{-1} DW in unripe fruits to 1.01 g kg^{-1} DW in red fruits (fully ripe).

Among anthocyanins, delphinidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside and cyanidin-3-galactoside were determined [46,48,49]. As described in the literature [48], the most abundant anthocyanin detected in strawberry tree fruits is cyanidin-3-glucoside (average 3.9 mg kg^{-1} FW), while other authors [49] have managed to distinguish two anthocyanin isomers differing only by the saccharide contained in anthocyanidin, in this case the glucoside and galactoside of cyanidin, and suggested that the most abundant anthocyanin is cyanidin-3-galactoside, with the other isomer containing an average of 28.4 mg kg^{-1} FW. Similar results were obtained in a study on fruits of wild variety from southern Italy (Pisa region). The most abundant anthocyanins were cyanidin-3-*O*-glucose, cyanidin-3-*O*-arabinoside and delphinidin-3-*O*-galactoside [48].

As mentioned earlier, it is important to emphasize that the polyphenolic profile of individual compounds varies greatly depending on the location and also on the stage of ripeness of the fruit [15]. Accordingly, El Cadi et al. [41] reported values for total flavonoid contents ranging from 37.43 to 41.51 mg quercetin g^{-1} DW, Barros et al. [44] reported an average value of 34.99 mg g^{-1} extract, while Šic Žlabur et al. [37] found values ranging from 7 to 15.58 mg catechin g^{-1} DW. Regarding the phenolic acids, quinic, protocatechuic, gallic, caffeic, ferulic, cinnamic, ellagic, syringic, hydroxycoumarin and vanillic acids were strongly represented [41,50,51]. Considering the flavones, dihydroxyflavone is the most abundant [41]; of the flavan-3-ols, catechins, epicatechins, procyanidin dimer with corresponding gallate and prodelphinidin; and of the flavonols, the hexoside of isorhamnetin, myricetin, quercetin, kaempferol and apigenin are the most abundant [41]. As suggested by some studies [50], the most abundant compound from the group of polyphenols detected in the fruits of strawberry tree collected in Turkey was gallic acid, followed by gentisic, protocatechuic, *p*-hydroxybenzoic, vanillic and *m*-anisic acids. Different authors from Spain [52] quantified the main important polyphenols (mg 100 g^{-1} DW) as follows: catechins (313.4); hydroxybenzoic acids (112.2); hydroxycinnamic acids (1.0); flavonols (3.6); ellagic acid (6.9); anthocyanins (5.8); and procyanidins (474.1).

Differences were also found in other polyphenols, for example, in flavonol content between fruits collected in Portugal and Spain. Myricetin-3-*O*-xyloside and quercetin-3-*O*-xyloside were not detected in fruits from Portugal, whereas this was the case in Spanish samples, while quercetin-3-*O*-rutinoside and quercetin-3-*O*-rhamnoside were present in both Portuguese and Spanish wild samples. Moreover, in wild fruits from northeastern Portugal, the main phenolic compounds were flavan-3-ols and galloyl derivatives (60.93 mg 100 g^{-1}), followed by anthocyanins (13.77 mg 100 g^{-1}) and flavonols (10.86 mg 100 g^{-1}) [53]. Within the group of flavan-3-ols and galloyl derivatives, in a study conducted in Spain Pallauf et al. indicated gallocatechin, gallocatechin-4,8-catechin, the proanthocyanidin dimers and epicatechin as the most abundant [49].

The identification of volatile compounds in the fruits of the strawberry tree led to the determination of 41 compounds, which are divided into several subclasses: alcohols are the most abundant volatile compounds; followed by aldehydes and esters. It should be noted that the contents of the listed compounds decrease sharply during ripening. Norisoprenoid derivatives, sesquiterpenes and monoterpenes are other volatile compounds found in strawberry tree fruit, but in very small amounts. The amount of these compounds also varies greatly with the progress of fruit ripening. The content of monoterpenes decreases

from unripe to mid-ripe and is highest at the ripe stage. The content of sesquiterpenes increases from unripe to mid-ripeness, after which it is lower. The content of norisoprenoid derivatives decreases with ripeness, which also confirms the fact that the content of volatile compounds strongly depends on the ripening stage of strawberry tree fruit [51].

3.2. Leaves

In addition to the fruit, the leaf of the strawberry tree is also an important raw material for both nutritional and medicinal purposes. Strawberry tree leaves have a high dry matter content (51–92%), total acidity ranging from 0.7–1.9%, higher acidity, and pH ranging from 3.89 to 5.35, which depends on the location and climate [37]. They also contain various types of phytochemical compounds such as phenolic compounds, vitamins, terpenoids and essential oils [51]. In general, according to the numerous studies conducted, the leaves of the strawberry tree contain a significantly higher content of polyphenolic compounds than the fruits, so the leaf can be considered as a valuable material, especially in terms of human health. Oliveira et al. [54] determined the total phenolic content of strawberry tree leaf extract to average 192.66 mg GAE g^{-1}; Mendes et al. [43] reported values of total phenolic content in leaves to average 170.3 mg GAE g^{-1}; Bouyahya et al. [55] obtained results for total phenolic content ranging from 94.51 and 141.726 GAE mg g^{-1} extract depending on the solvent type; Šic Žlabur et al. [37] between 18.69 and 26.94 mg GAE g^{-1} FW; Martins et al. [56] reported values for total phenolic content between 254.96 and 495.24 mg g^{-1} leaf FW; and Brčić Karačonji et al. between 67.07 and 104.74 mg GAE g^{-1} DW [5].

Regarding polyphenols, here several compounds have been determined, such as: tannins; flavonoids (catechin gallate, myricetin, rutin, afzelin, juglanin, avicularin); phenolic glycosides (quercitrin, isoquercitrin, hyperoside); and iridoid glucosides [57–60], of which arbutin (62.7 mg 100 g^{-1} FW), ethyl gallate (44.00 mg 100 g^{-1} FW) and catechin (54.6 mg 100 g^{-1} FW) were the most abundant [61]. Hydroquinone, a bioactive metabolite of arbutin, was not detected in any leaf of *A. unedo* [62]. Thanks to advances in analytical techniques, the detailed profiles of leaf phenolics have been established in recent years. Using an ultra-high-performance liquid chromatograph (UHPLC) coupled with a hybrid mass spectrometer-(LTQ OrbiTrap MS), a total of 60 phenols have been identified in the aqueous and methanolic leaf extracts. Flavonoid aglycones (morin, naringenin, myricetin and kaempferol), phenolic acids (protocatechuic acid and chlorogenic acid), and arbutin and its derivatives were detected in leaves, but not in fruits [5]. Using the same technique, Maldini et al. [11] detected 19 phenols in ethanolic leaf extracts from Sardinia. The main phenols detected were flavonoids, mainly quercetin, kaempferol and myricetin derivatives. With a liquid chromatograph coupled with a quadrupole time-of-flight mass spectrometer (LC-QTOF-MS), a total of 37 phytochemicals were detected, and the main constituents in the leaf extracts being phenolic acids, iridoids, proanthocyanidins and flavonoids [8]. The levels of total flavonoids (expressed as % of quercetin) measured in the leaves ranged from 0.52 to 2.14% [7]. In addition, Jurica et al. [7] determined for the first time the total phenolic acid content in the leaf extracts and it was 1.48%, expressed as % of rosemarinic acid.

When observing terpenoids, amyrin acetate, betulinic acid and lupeol were strongly represented in the leaves [44]. Among vitamins, α-tocopherol and vitamin C stand out as highly contained. Further, authors from Croatia determined that the vitamin C contents in the leaves of wild strawberry trees collected from different locations on the Adriatic coast ranged from 61.61 to even 333.83 mg 100 g^{-1} FW [37].

Among the macroelements in the leaves of *A. unedo*, potassium (1743 mg 100 g^{-1} DW) and calcium (1299 mg 100 g^{-1} DW) were the most abundant, while iron (26.8 mg 100 g^{-1} DW) was the most abundant of the microelements [63], which was similar to the mineral profile in the fruits. According to Asmaa et al. [63] the most abundant volatiles in *A. unedo* leaves were: camphor (43.5%); α-fenchone (17.5%); bornyl acetate (16.0%); eucarvone (3.16%); and myrtenyl acetate (3.16%). Kivack et al. [64] reported that (E)-2-decenal (12.0%); α-terpineol (8.8%); hexadecanoic acid (5.1%); and (E)-2-undecenal (4.8%) were the most abundant. These compositional differences may be the result of differences in cultivation area or

extraction procedure [65]. Among the fatty acids, according to Koukos et al. [66], linolenic acid was the most abundant (44.2%), followed by palmitic acid (25.5%) and linoleic acid (7.9%), while according to Dib et al. [67], palmitic acid was the most abundant (38.5%), followed by oleic acid (10.6%), linolenic acid (9.3%) and linoleic fatty acid (5.5%).

Total carotenoid contents ranged from 0.06 to 0.27 mg g^{-1}, and chlorophyll concentrations from 0.19 to 2.37 mg g^{-1}, again which could be correlated with the climate and geolocation [37]. According to Kachoul et al. [68] the anthocyanin contents in strawberry tree leaves ranged from 0.33 to 0.8 mg of cyanidin-3-glucoside per gram of extract, depending on the type of solvent and the extraction procedure.

Since the fruits and leaves of the strawberry tree are a rich source of nutrients and bioactive compounds (Table 1) attributed with various biological activities, they represent a perspective raw material to be considered for the development and formulation of functional foods and nutraceuticals.

Table 1. Individual bioactive compounds found in *A. unedo* fruits and leaves.

Plant Source	Analytics	Bioactive Compound	Concentration	References
Fruit	HPLC	Gallic acid	4.56–36.93 mg 100 g^{-1} DW	[69]
		Protocatechuic	1.84–5.90 mg 100 g^{-1} DW	
		Gallocatechin	16.15–65.31 mg 100 g^{-1} DW	
		Catechin	22.09–49.36 mg 100 g^{-1} DW	
		Chlorogenic acid	5.55–27.42 mg 100 g^{-1} DW	
		Syringic acid	4.27–7.94 mg 100 g^{-1} DW	
		Ellagic acid	8.42–33.73 mg 100 g^{-1} DW	
		Quercetin-3-xyloside	1.43–4.09 mg 100 g^{-1} DW	
		Rutin	0.90–1.26 mg 100 g^{-1} DW	
		Quercetin-3-galactoside	1.66–3.46 mg 100 g^{-1} DW	
		Quercetin-3-glucoside	2.11–2.89 mg 100 g^{-1} DW	
		Cyanidin-3-glucoside	0.43–7.21 mg 100 g^{-1} DW	
		Cyanidin-3-arabinoside	0.36–1.64 mg 100 g^{-1} DW	
Fruit	HPLC-TQ-MS/MS	4-hydroxybenzoic acid	0.34–0.50 mg 100 g^{-1} DW	[70]
		Gallic acid	1.4–4.7 mg 100 g^{-1} DW	
		Syringic acid	0.63 mg 100 g^{-1} DW	
		Chlorogenic acid	0.676 mg 100 g^{-1} DW	
		Quercetin	0.79–0.84 mg 100 g^{-1} DW	
		Quercetin 3-β-glucoside	1.7–2.6 mg 100 g^{-1} DW	
		Rutin	0.43–0.57 mg 100 g^{-1} DW	
		Kaempherol	0.39–0.74 mg 100 g^{-1} DW	
		Catequin	28–149 mg 100 g^{-1} DW	
		Epigallocatechin	10–26 mg 100 g^{-1} DW	
		Naringin	0.35 mg 100 g^{-1} DW	
Fruit	MS/MS	Arbutin	NQ	[11]
		Myricetin pentoside	NQ	
		Myricetin rhamnoside	NQ	
		Kaempferol-rhamnoside (afzelin)	NQ	
Fruit	HPLC	Protocatechuic acid	0.11–0.61 mg 100 g^{-1} FW	[71]
		Vanillic acid	0.10–1.17 mg 100 g^{-1} FW	
		Ellagic acid	1.11–2.13 mg 100 g^{-1} FW	
		Rutin	0.15–0.95 mg 100 g^{-1} FW	
		Quercetin	0.12–0.31 mg 100 g^{-1} FW	
		Gallic acid	1.62–7.29 mg 100 g^{-1} FW	
		Catechin	1.16–5.75 mg 100 g^{-1} FW	
Leaves	UHPLC-LTQ Orbitrap MS	Gallocatechin	64.21–211.60 mg kg^{-1} DW	[5]

Table 1. *Cont.*

Plant Source	Analytics	Bioactive Compound	Concentration	References
		Protocatechuic acid	1.27–2.47 mg kg^{-1} DW	
		Aesculin	1.95–5.88 mg kg^{-1} DW	
		Chlorogenic acid	ND–1.95 mg kg^{-1} DW	
		Catechin	47.73–102.95 mg kg^{-1} DW	
		p-Hydroxybenzoic acid	16.21–27.08 mg kg^{-1} DW	
		Caffeic acid	2.61–5.75 mg kg^{-1} DW	
		Syringic acid	0.66–2.67 mg kg^{-1} DW	
		Vanillic acid	3.71–7.96 mg kg^{-1} DW	
		Rutin	29.93–106.03 mg kg^{-1} DW	
		p-Hydroxyphenylacetic acid	4.35–6.57 mg kg^{-1} DW	
		Hyperoside	635.10–1512.94 mg kg^{-1} DW	
		p-Coumaric acid	10.11–32.83 mg kg^{-1} DW	
		Catechin gallate	34.48–73.70 mg kg^{-1} DW	
		Ferulic acid	2.55–4.85 mg kg^{-1} DW	
		Myricetin	ND–1.78 mg kg^{-1} DW	
		Quercetin	41.28–124.91 mg kg^{-1} DW	
		Naringenin	ND–4.39 mg kg^{-1} DW	
		Kaempferol	10.63–35.50 mg kg^{-1} DW	
Leaves	HPTLC	Quercitrin	1.21–2.20 mg g^{-1} DW	[72]
		Isoquercitrin	ND–0.33 mg g^{-1} DW	
		Hyperoside	ND–0.35 mg g^{-1} DW	
		Chlorogenic acid	0.61–1.46 mg g^{-1} DW	
Leaves	HPLC-PDA	Chlorogenic acid	0.8–6.5 mg g^{-1} DW	[73]
		Caffeic acid	0.6–1.0 mg g^{-1} DW	
		p-Coumaric acid	0.2–6.6 mg g^{-1} DW	
		Quercetin	0.5–10.7 mg g^{-1} DW	
Leaves	GC-MS	Arbutin	2.75–6.82 mg g^{-1} DW	[74]
Leaves	HPLC-PDA	Arbutin	12.1 mg g^{-1} DW	[75]

UHPLC-LTQ Orbitrap MS—ultra-high performance liquid chromatography- linear ion trap-Orbitrap hybrid mass spectrometry; DW—Dry weight; FW—Fresh weight; ND—not detected; HPTLC—high-performance thin-layer chromatography; HPLC-PDA—high-performance liquid chromatography-photo diode array detection; GC-MS—gas chromatography-mass spectrometry.

4. Biological Potential of Strawberry Tree

Because polyphenols are potent antioxidants against oxidative stress caused by oxygenic metabolites, the total concentration of phenols is critical to understanding the health-promoting properties of these plants. The determination of total phenols, phenolic acids, tannins, flavonoids and vitamin E (tocopherols in seeds) as known antioxidants has been carried out in numerous studies [5,51,76].

The phytochemical profiling of fruits and leaves revealed the presence of flavonoids, iridoids, anthocyanins carotenoids, terpenoids and fatty acids as major classes of bioactive constituents [8]. However, based on the literature search, it seems that the leaves of the strawberry tree have been researched in much more extensive way than the fruits.

4.1. Fruits

Considering the favorable nutritional composition, especially the extremely high contents of numerous phytochemicals, particularly polyphenolic compounds, vitamins and dietary fibers, it is not surprising that the nutritional and medicinal values of these delightful fruits were known since ancient Greece and are used today. They are mainly used in Mediterranean countries, for traditional, industrial, chemical and pharmaceutical purposes [42]. The fruits of the plant are traditionally used as antiseptic, diuretic and laxative, carminative, digestive, odontalgic and cardiotonic [77–79]. Scientific studies suggested that strawberry tree fruits also have high pharmacological potential due to their in vitro and preclinical antibacterial, anti-inflammatory, antitumor and antioxidant

properties [6,8]. For example, Salem et al. [80] studied the antimicrobial activity of ethanolic extract of strawberry tree fruits and concluded that they have strong antibacterial effects on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, and a moderate effect on *Salmonella typhimurium*, *Enterococcus feacium*, *Escherichia coli* and *Candida albicans*. It should be noted that the antimicrobial effects were influenced by the choice of extraction solvents and the extraction procedures [81,82].

Since the fruits are rich in polyphenolic compounds (mainly phenolic acids, flavonoids and anthocyanins), vitamins (especially C and E) and other bioactive compounds, their antioxidant and free radical scavenging activity is very pronounced. Many literature data emphasized high antioxidant activity of strawberry tree fruits [15,37,83,84] which consider this species very important for the prevention of numerous diseases, especially neurodegenerative [46], cardiovascular [85] and diabetes/hypoglycemia [8], while some of the polyphenols identified in strawberry tree fruits have a strong anticancer effect [86]. Possibly, the anticancer effects tested on different tumor cell lines were likely related to the gallic acid derivatives that are dominant in the fruits [87].

Moreover, since fruits are rich in flavonoids, it is important to highlight that flavonoids are highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which may be involved in DNA damages and promotion of tumors [88]. The fruits can be successfully used in the treatment of various urological [89], dermatological [90] and gastrointestinal problems [91]. The anti-radical activities of strawberry fruits were investigated in the study by Mendes et al. [43], using experimental human cell line models. This was one of the first studies to evaluate the antioxidant activity of *A. unedo* on human biological membranes. In general, the results of the study suggested that both the fruits and leaves were promising sources of natural antioxidants that can be used in free radical-induced diseases. Figure 4 shows the biological and functional properties of *A. unedo* plant [6,12,55,58,68,83,85,86,92–94].

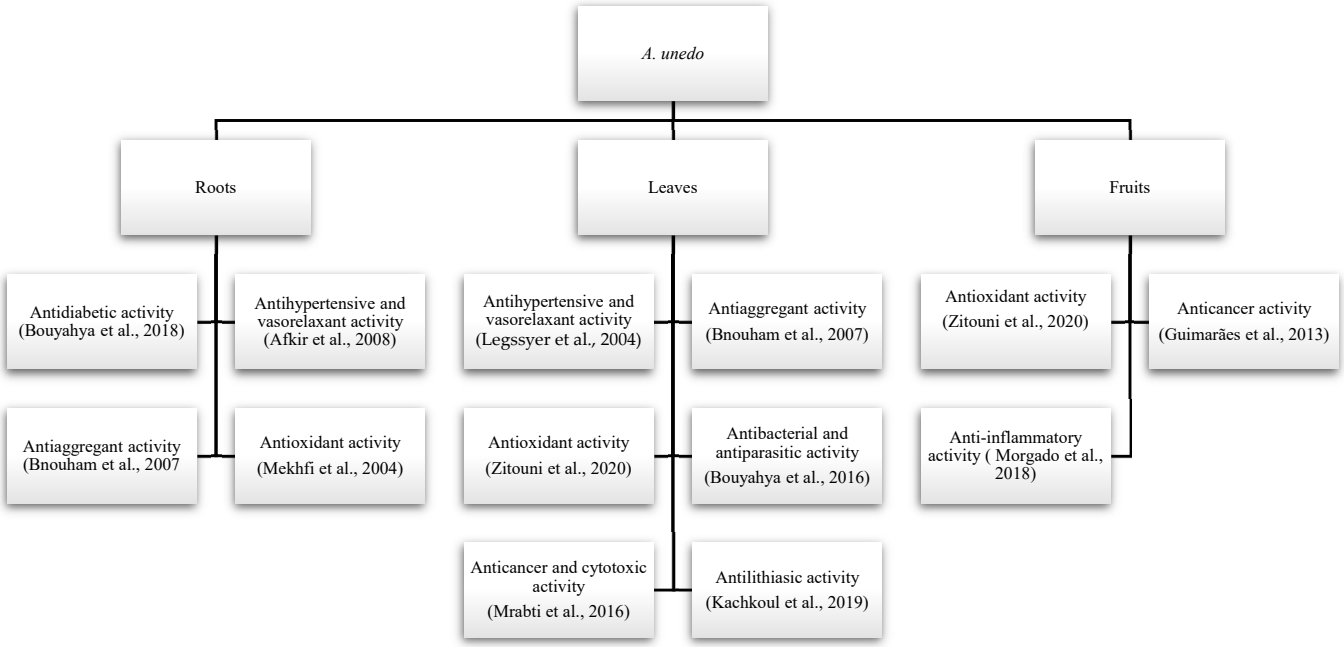

Figure 4. Biological and functional properties of *Arbutus unedo* L. plant [6,12,55,58,68,83,85,86,92–94].

4.2. Leaves

Since polyphenols are strong antioxidants that protect the organism from oxidative stress caused by reactive oxygen species, the determination of total phenols and groups of phenolic compounds (tannins, flavonoids and phenolic acids) as well as individual phenols, could lead to an understanding of the biological potential of *A. unedo* [1]. The wide range of values for total phenolic contents obtained in different studies was the result of differences

in the climate in which *A. unedo* grows, as well as different extraction methods and the types of solvents used to extract the active compounds from the leaves [5,8,54,95].

Jurica et al. [7] reported that tannins accounted for 83% of the total phenols in the leaves. Therefore, the strong antioxidant activity of leaves can be attributed, among other things, to the higher content of tannins which have a strong antioxidant effect due to the large number of hydroxyl and galoyl groups.

The importance of flavonoids lies in the fact that some of them (e.g., the flavonols myricetin, rutin, quercetin and querictrin) have high free radical scavenging activities, and some (catechins) have the ability to chelate metals and thus prevent the formation of free radicals [96]. Phenolic acids show different radical scavenging activities depending on the number and the position of hydroxyl groups and methoxy substitutions in the molecules [96].

The antioxidant properties of *A. unedo* leaves were studied by different spectrophotometric methods. Ferric reducing antioxidant power (FRAP) method showed better activity of the methanolic extracts (1.896 mmol $FeSO_4$ g^{-1}) than the aqueous counterparts (1.187 mmol $FeSO_4$ g^{-1}). The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay also favored the methanolic extracts (165.510 mg TE g^{-1}), as compared to the aqueous alternative (130.172 mg TE g^{-1}) [7]. The superiority of alcoholic over aqueous extraction of leaf phenolics was also reported in the study by Kachkoul et al. [68] who found that the hydroalcoholic extracts exerted higher antioxidant capacities than the aqueous extracts, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (IC_{50} 76.14 μg mL^{-1} versus 202.64 μg mL^{-1} and the FRAP assay (53.77 μg mL^{-1} versus 236.86 μg mL^{-1}). Several studies have investigated the radical scavenging activity of *A. unedo* leaf samples by the DPPH method, indicating a large antiradical activity (IC_{50} 23–95 mg L^{-1}), more efficient phenolic extraction with methanol or ethanol than with water, and a strong correlation between the phenolic content and antioxidant activity [5,7,11]. In addition to the high content of the phenolic glycoside arbutin, the high antioxidant activity measured in the ABTS assay could be due to the high content of quinic acid, which belongs to the polyols, so there might be another class of compounds, hence different from phenolics, responsible for antioxidative activity of the leaves [62].

The extracts from the leaves were able to reduce platelet adhesion (coagulation), which is an important factor in the pathogenesis of inflammatory diseases. The treatment of human platelets with an increasing concentration of crude water leaf extracts (0.015–1.5 mg mL^{-1}) reduced thrombin-induced platelet aggregation in a concentration-dependent manner. This activity was probably related to the presence of tannins from leaves [97]. In an ex vivo study on isolated rat aorta, the extract from the leaves of *A. unedo* (0.01 g L^{-1}) showed potent vasodilatory properties and improvement in cardiovascular health that correlated with the presence of condensed tannins and catechin gallates [58]. This was additional to inhibition of enzymes related to rheumatoid arthritis, tumor cell proliferation, and metastases with the existence of gallic acid derivatives [98].

Arbutin showed antibacterial activities especially against *Enteroccocus* species [7,99]. The mechanism of action was the same as in bearberry leaves (*Arctostaphylos uva-ursi* L.), which contained higher amounts of arbutin than the strawberry tree. The antimicrobial effects of arbutin was strongly dependent on the extracellular activity of beta-glucosidase, an enzyme responsible for the conversion of arbutin to free hydroquinone, which was responsible for antimicrobial activity [7]. Leaf extracts also inhibited the growth of *Candida tropicalis*, and *Crataegus lucitaniae* [100], various mycobacteria and *Leishmania* parasites [39].

The extracts of fresh leaves obtained by ethanolic ultrasonic extraction (IC_{50} 19.56 mg L^{-1}) and hydroethanolic maceration (IC_{50} 19.56 mg L^{-1}) showed hypoglicemic activities by inhibiting beta-glucosidase, digestive enzyme responsible for carbohydrate absorption [8], while the leaf infusion showed litholytic activity against calcium oxalate stones in vitro [68].

The cytotoxic effects of the leaf extracts were tested on different tumor cell lines, whereas the cytoprotective effect were tested only on isolated human lymphocytes. The hydromethanolic leaf extract caused a blockade of the cell cycle G2/N phase in human os-

teosarcoma cells U2OS and did not induce apoptotic cell death, indicating cytostatic rather than cytotoxic effects. In contrast, cytotoxicity against human umbilical vein endothelial cells (HUVEC) was reported [9]. An extract of leaf protein of *A. unedo* showed an inhibitory effect on in HT29 colon cancer cells [101].

Jurica et al. [102] performed an in vitro safety assessment of 24 h exposure of lymphocyte to aqueous leaf extracts and reported absence of cytotoxic effects at a concentrations equivalent to the maximum allowable daily intake of arbutin, with negligible potential to cause primary DNA damages, while preventing micronuclei formations in lymphocytes.

As described above, the biological activities of leaves have been extensively investigated only in vitro, while there are only few important in vivo studies despite their well-documented health-promoting properties. Further, Jurica et al. [103–105] evaluated the in vivo safety of an aqueous leaf extracts administered *per os* to rats at a dose of 200 mg kg^{-1} body weight day^{-1} for 14 and 28 days. Following exposure to the extract, low DNA damages in white blood cells and no significant changes in the hematological parameters were observed [103]. The leaf extracts showed high biocompatibility with liver and kidney tissues by preserving organ function and DNA integrity in rat organ cells [105,106]. Table 2 shows a summary of some biological potentials of the fruits and leaves of the strawberry tree recently reported.

Table 2. Biological potential of *Arbutus unedo* L. fruits and leaves.

Part of Plant	Type of Study	Biological Potential	References
Leaves	Determination of growth inhibition zones by radial diffusion	Antibacterial and antifungal potential	[95]
Leaves	Determination of growth inhibition zones by disc diffusion	Antibacterial and antifungal potential	[106]
Fruits	Determination of MIC by dilution on broth media	Antibacterial potential	[107]
Leaves	In vitro platelet aggregation	Antiaggregant potential	[97]
Leaves	In vitro platelet aggregation	Antiaggregant potential	[108]
Fruits	In vitro, BrdU assay	Antitumoral potential	[84]
Fruits	DPPH assay, scavenging activity, β-carotene bleaching activity	Antioxidant potential	[109]
Leaves	DPPH assay	Antioxidant potential	[110]
Leaves and fruits	ORAC assay, MMP-9 inhibitory activity assay	Antioxidant potential	[98]
Leaves	FRAP, Lipid peroxidation, DPPH assay	Antioxidant potential	[75]
Fruits	DPPH assay, DNA damage	Antioxidant potential	[84]
Leaves	Inflammatory activation, In vitro inhibition of STAT1 activation	Anti-inflammatory potential	[111,112]

MIC—Minimal inhibitory concentration; BrdU—5-Bromo-2-deoxyuridine; DPPH—1,1-Diphenyl-2-picrylhydrazyl; ORAC—oxygen radical absorbance capacity; MMP-9—matrix metalloproteinase-9; FRAP—ferric reducing antioxidant power; STAT1—signal transducer and activator of transcription 1.

5. Economic Properties of Strawberry Tree

Aware of the fact that an insufficient, and unbalanced diet has negative impacts on their health, consumers have recently been demanding minimally processed products with preserved nutritional properties, preferably without any additives or only with natural origin. For the above reasons, there is a growing demand for functional foods that are expected to have a positive effect on consumers' health [113]. Due to various needs, lactose intolerance, allergies or simply the need for a healthier diet, consumers are increasingly turning to the consumption of plant products rich in bioactive ingredients. Plants, especially fruits, containing bioactive compounds with positive impacts on human health, including polyphenolic compounds and dietary fibers in particular. Therefore, the modern food industry needs new ingredients to enrich existing products. *A. unedo*, due to its chemical

composition and contents of bioactive compounds that contribute to biological potential, can be considered an excellent ingredient for improving and enriching existing products or developing new functional products. Honey from *A. unedo* flowers, for example, is an expensive product with strong, unique and very special sensory properties. It has a characteristic coffee-like flavor, yellow-brown color and sweet-bitter taste characteristic of products containing arbutin [114]. Considering that arbutin was found in 83% of strawberry tree honey samples, it can be considered as a marker for *A. unedo* honey [115]. Due to its antioxidant, biological and antimicrobial properties, *A. unedo* honey can be used for cosmetic and pharmaceutical purposes in addition to its use in food manufacturing [115]. In the study conducted by Mrabti et al. [116], the root of *A. unedo* plant also showed antibacterial and antioxidant activities and therefore has the potential to be used in the production of functional foods and nutraceuticals. However, most of the products made from *A. unedo* were obtained from the fruits and leaves of the plant.

Currently, the development of new technologies and concepts in the context of Industry 4.0, such as 3D printing, opens up various opportunities for the use of fruit and leaf extracts in the production of functional foods and nutraceuticals with the aim of improving health [117]. *A. unedo* certainly has proven its potential, but it is anticipated that this plant will be increasingly used in processing as we strongly move toward sustainable food production.

5.1. Fruits

Because of its high pectin content, strawberry tree fruits are traditionally used to make jams, jellies and marmalades [118,119]. Furthermore, the fruits are traditionally used to produce an alcoholic beverage called "Koumaro" in Greece and "Aguardente de medronho" in Portugal [120]. In the production of spirits, fermentation is the most important step. Since strawberry tree fruit spirits are traditionally produced under uncontrolled conditions, there are significant differences in the alcohol and methanol contents of such beverages [120]. The formation of methanol is a consequence of the activity of specialized enzymes, methyl esterizes during the process of methyl esterification of pectin, which is naturally present in the fruits of *A. unedo* [121]. Methanol is a dangerous product for human health, and according to the European regulation for spirits, the maximum allowed concentration is 1000 g hL^{-1} of pure alcohol [122]. In most cases, the methanol concentration in this alcoholic beverage is below or close to the allowable limit, which depends on the ripeness of the fruit, fermentation conditions and distillation technology [123–126]. In this direction, Anjos et al. [127] created a new spirit by fermentation of strawberry tree fruits and honey with significantly lower contents of methanol and other harmful compounds than the commonly produced spirits of this plant. In order to reduce the variation in alcohol content and to produce a uniform product, Soufleros et al. [120] proposed the systematic production and standardization of the spirit manufacturing process, which opened the possibility for expanding and strengthening the economy of businesses that produce this alcoholic beverage.

As noted previously, fruit extracts have high antioxidant activity due to their high polyphenolic contents; Ganhao et al. [52] investigated the effects of adding *A. unedo* fruit extracts to raw pork burger patties by DPPH and ABTS methods, as well as with thiobarbituric acid reactive substances and color stability during 12 days of storage. They concluded that the extracts exhibited significant antioxidant activity against lipid oxidation and slowed down the color changes of meat caused by oxidation processes, making them suitable ingredients for the production of new meat-based functional products [52]. Similarly, Masmoudi et al. [128] investigated the influences of fruit extracts on antioxidant activity; studying the physicochemical, textural and sensory properties of "Sardaigne" cheese. The addition of the fruit extracts had no negative effects on the color and sensory properties of the product, while improving the firmness and increased the utilization and antioxidant activity of the product. Similar results in terms of antioxidant activity were found by Cossu et al. [129] who added strawberry tree fruit extract to yogurt. In conclusion, *A. unedo* fruit extracts

represent potential for use as a functional ingredient in dairy [128,129] and meat products. Following on, Takwa et al. [130] added strawberry tree fruit extracts to bread and studied the antioxidant and antimicrobial properties. The results showed that the fruit extracts had preservative effects that also enriched the product with bioactive compounds.

Considering the above data, strawberry tree fruits and its extracts can be considered as functional ingredients that can improve existing foods or create new functional products in various fields of food industry.

5.2. Leaves

Due to their rich polyphenolic composition, *A. unedo* leaves are most commonly used for the extraction and enrichment of other products with polyphenolic compounds. To that end, Derbassi et al. [131] incorporated *A. unedo* leaf extracts into Quark cheese and studied their preservation effects for 8 days, together with antioxidant activity. Compared to commercially available additives (potassium sorbate), the extracts showed better antimicrobial properties. Moreover, the antioxidant activity remained strong even after the extracts were incorporated into the product. This confirms the possibility of using the extracts from the leaves of *A. unedo* as natural additives in the production of healthy, functional foods with improved biological properties.

In addition to the antioxidant activity, Dias et al. [132] also investigated the potential of leaf extracts as an anti-browning agent in fresh-cut pears. The results showed that the increased polyphenolic contents correlated with better effectiveness of enzymatic inhibition in the samples. Therefore, these results possibly suggested the application of leaf extracts as an anti-browning agent in fresh products and ultimately prolonging their shorter shelf life.

Erkekoglou et al. studied the contents of phenolic compounds in hot and cold nonalcoholic beverages prepared from these leaves [133]. The authors concluded that decoction is a more suitable method for the preparation of a phenol-rich beverage than infusion. Therefore, these data indicated great possibility for the use of strawberry tree leaves for the preparation of functional infusions and soft drinks.

In addition to the potential use of *A. unedo* leaves in the manufacturing of functional foods, they can also be important for pharmacological and medicinal purposes due to their polyphenolic composition. For instance, the high content of palmitic acid indicates that the leaves are a valuable industrial ingredient for the production of soaps and cosmetics [134]. Similarly, arbutin from leaves is light and pH stable, making it suitable for uses in cosmetics, while through the processes of hydrolysis and oxidation in the aqueous matrix, it can be transformed into benzoquinone, which has antibacterial properties [135,136]. For this reason, and due to the fact that arbutin is a good alternative to hydroquinone, it is suitable for the apical products for the treatment of hyperpigmentation [137]. Figure 5 summarizes the economic properties and further economic prospects of strawberry tree fruits and leaves. In conclusion, the leaves of strawberry tree, as well as the fruits, have great potential for the enrichment of existing or the creation of new functional products, and further research is needed in this area.

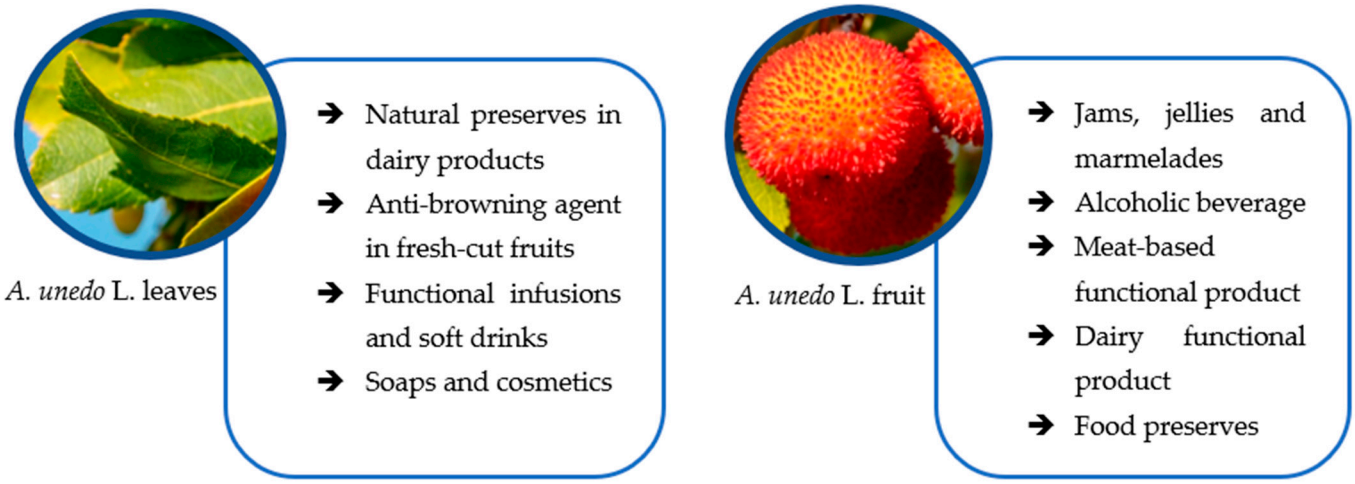

Figure 5. Economic perspective of *A. unedo* L. fruit and leaf utilization [138].

6. Conclusions

With the new trends in the development of functional foods and nutraceuticals, and in line with the emerging global situation influenced by pandemics, ecological problems and energy crises, particular interest of the food (and other) industries for the advanced use of unexploited natural resources is reinforced by the growing of plants. In this context, the strawberry tree (*A. unedo* L.) plant has attracted particular attention and is becoming increasingly appealing to consumers and the industry as a nutrient-rich source of bioactive compounds, with high potential for the production of innovative functional foods and dietary supplements that could greatly contribute to better health. Although both leaves and fruits have significant biological properties, so far, the bioactive compound content and antioxidant activity were significantly higher in the leaves than in fruits.

Although the Mediterranean region is the main growing area for this plant, the strawberry tree has not been sufficiently studied yet. Therefore, future research should focus on fully profiling bioactive constituents and exploring their biological potential in different growing areas to find the species with the greatest potential, and how they can be used in processing. By applying new sustainable manufacturing technologies in the context of Industry 4.0, this plant, whether as fresh or as an extract (both fruits and leaves), could be used in the design of various products. For all of this to be realized in the future, systematic cultivation of this plant would need to be widespread. Therefore, sustainable agronomic practices should be considered to increase cultivation in different geographical regions and to achieve high yields.

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References

1. Jurica, K. Phenolic Compounds in Strawberry Tree (*Arbutus unedo* L.) and Their Biological Effects. Ph.D. Thesis, Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia, 2016.
2. Linnæi, C. *Species Plantarum*; Laurentii Salvii: Stockholm, Sweden, 1753; p. 395.
3. Sulusoglu Durul, M.; Memis, S. Optimization of conditions for in vitro culture of selected *Arbutus unedo* L. genotypes. *Agronomy* **2022**, *12*, 623. [CrossRef]
4. Giovannetti, M.; Lioi, L. The mycorrhizal status of *Arbutus unedo* in relation to compatible and incompatible fungi. *Can. J. Bot.* **1990**, *68*, 1239–1244. [CrossRef]
5. Brčić Karačonji, I.; Jurica, K.; Gašić, U.; Dramićanin, A.; Tešić, Ž.; Milojković Opsenica, D. Comparative study on the phenolic fingerprint and antioxidant activity of strawberry tree (*Arbutus unedo* L.) leaves and fruits. *Plants* **2022**, *11*, 25. [CrossRef] [PubMed]
6. Morgado, S.; Morgado, M.; Plácido, A.I.; Roque, F.; Duarte, A.P. *Arbutus unedo* L.: From traditional medicine to potential uses in modern pharmacotherapy. *J. Ethnopharmacol.* **2018**, *225*, 90–102. [CrossRef] [PubMed]
7. Jurica, K.; Gobin, I.; Kremer, D.; Čepo, D.V.; Grubešić, R.J.; Karačonji, I.B.; Kosalec, I. Arbutin and its metabolite hydroquinone as the main factors in the antimicrobial effect of strawberry tree (*Arbutus unedo* L.) leaves. *J. Herb. Med.* **2017**, *8*, 17–23. [CrossRef]
8. Tenuta, M.C.; Deguin, B.; Loizzo, M.R.; Dugay, A.; Acquaviva, R.; Malfa, G.A.; Bonesi, M.; Bouzidi, C.; Tundis, R. Contribution of flavonoids and iridoids to the hypoglycaemic, antioxidant, and nitric oxide (NO) inhibitory activities of *Arbutus unedo* L. *Antioxidants* **2020**, *9*, 184. [CrossRef] [PubMed]
9. Cappadone, C.; Mandrone, M.; Chiocchio, I.; Sanna, C.; Malucelli, E.; Bassi, V.; Picone, G.; Poli, F. Antitumor potential and phytochemical profile of plants from Sardinia (Italy), a hotspot for biodiversity in the Mediterranean basin. *Plants* **2019**, *9*, 26. [CrossRef]
10. Tenuta, M.C.; Tundis, R.; Xiao, J.; Loizzo, M.R.; Dugay, A.; Deguin, B. *Arbutus* species (*Ericaceae*) as source of valuable bioactive products. *Crit. Rev. Food Sci. Nutr.* **2018**, *59*, 864–881. [CrossRef] [PubMed]
11. Maldini, M.; D'Urso, G.; Pagliuca, G.; Petretto, G.L.; Foddai, M.; Gallo, F.R.; Multari, G.; Caruso, D.; Montoro, P.; Pintore, G. HPTLC-PCA complementary to HRMS-PCA in the case study of *Arbutus unedo* antioxidant phenolic profiling. *Foods* **2019**, *8*, 294. [CrossRef] [PubMed]
12. Naceiri Mrabti, H.; Marmouzi, I.; Sayah, K.; Chemlal, L.; El Ouadi, Y.; Elmsellem, H.; Cherrah, Y.; Faouzi, M.A. *Arbutus unedo* L. aqueous extract is associated with in vitro and in vivo antioxidant activity. *J. Mater. Environ. Sci.* **2017**, *8*, 217–224.
13. Juric, A.; Gasic, U.; Brcic-Karaconji, I.; Jurica, K.; Milojkovic-Opsenica, D. The phenolic profile of strawberry tree (*Arbutus unedo* L.) honey. *J. Serb. Chem. Soc.* **2020**, *85*, 1011–1019. [CrossRef]
14. Granato, D.; Barba, F.J.; Bursać Kovačević, D.; Lorenzo, J.M.; Cruz, A.G.; Putnik, P. Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety. *Annu. Rev. Food Sci. Technol.* **2020**, *11*, 93–118. [CrossRef]
15. Miguel, M.; Faleiro, M.; Guerreiro, A.; Antunes, M. *Arbutus unedo* L.: Chemical and biological properties. *Molecules* **2014**, *19*, 15799–15823. [CrossRef]
16. Caudullo, G.; Welk, E.; San-Miguel-Ayanz, J. Chorological maps for the main European woody species. *Data Brief* **2017**, *12*, 662–666. [CrossRef]
17. Skendrović Babojelić, M.; Bogdanović, S.; Dlačić, I.; Duralija, B.; Prgomet, Ž.; Prgomet, I.; Šic Žlabur, J.; Voća, S. *Strawberry Tree (Arbutus unedo L.) Biological, Chemical and Economic Properties*; University of Zagreb Faculty of Agriculture: Zagreb, Croatia, 2020.
18. Trinajstić, I. *Plant Communities of the Republic of Croatia*; Franjić, J., Ed.; Academy of Forestry Sciences: Zagreb, Croatia, 2008.
19. Flora Croatica Database. Available online: http://hirc.botanic.hr/fcd (accessed on 5 May 2022).
20. Sagbas, H.I.; Ilhan, G.; Zitouni, H.; Anjum, M.A.; Hanine, H.; Necas, T.; Ondrasek, I.; Ercisli, S. Morphological and biochemical characterization of diverse strawberry tree (*Arbutus unedo* L.) genotypes from Northern Turkey. *Agronomy* **2020**, *10*, 1581. [CrossRef]
21. Celikel, G.; Demirsoy, L.; Demirsoy, H. The strawberry tree (*Arbutus unedo* L.) selection in Turkey. *Sci. Hortic.* **2008**, *118*, 115–119. [CrossRef]
22. Songlin, M.; Yuejian, Z.; Senmiao, L.; Huang, X.G.; Wang, S.F.; Miao, S.L.; Zhang, Y.J.; Liang, S.M. Zaose, a promising new Arbutus cultivar. *China Fruits* **1995**, *4*, 3–4.
23. Jihua, H.; Zuyou, L.; Tıanrong, X.; Xıanjun, Z. Study on the characteristics of flower formation and fruit set of Dongkui arbutus variety in western part of Hubei. *S. China Fruits* **1997**, *26*, 33–34.
24. Cai-Huang, C.H. The cultural practices for high and top quality production of Arbutus fruit trees. *China Fruits* **1997**, *3*, 48.
25. Gilman, E.F.; Watson, D.G. *Arbutus unedo*, Strawberry-Tree. In *A Series of the Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences*; University of Florida: Gainesville, FL, USA, 1993; Volume Fact Sheet ST-85.
26. Gomes, F.; Simões, M.; Lopes, M.L.; Canhoto, J.M. Effect of plant growth regulators and genotype on the micropropagation of adult trees of *Arbutus unedo* L. (strawberry tree). *New Biotechnol.* **2010**, *27*, 882–892. [CrossRef]
27. Pato, R.L.; Botelho, G.; Franco, J.; Santos, S.; Ressurreição, S.; Figueiredo, P.; Gama, J.; Gomes, F. Interaction between farming type, nutrient uptake and plant material in strawberry tree fruit production and quality. *Acta Hortic.* **2022**, 275–284. [CrossRef]
28. Mulas, M.; Deidda, P. Domestication of woody plants from Mediterranean maquis to promote new crops for mountain lands. *Acta Hortic.* **1998**, *457*, 295–302. [CrossRef]
29. Karadeniz, T.; Kurt, H.; Kalkışım, Ö. Yomra (Trabzon) çevresinde yetişen kocayemiş (*Arbutus unedo* L.) tiplerinin meyve özellikleri üzerinde çalışmalar. *YYÜZF Derg.* **1996**, *6*, 65–70.

30. Karadeniz, T.; Kalkışım, Ö.; Şişman, T. Trabzon çevresinde yetişen kocayemiş (*Arbutus unedo* L.) tiplerinin meyve özellikleri ve çelikle çoğaltılması. In Proceedings of the Ulusal Kivi ve Üzümsü Meyveler Sempozyumu, Ordu, Turkey, 23–25 October 2003; pp. 476–480.
31. Gozlekci, Ş.; Alkaya, C.E.; Yaş, D. Antalya cevresinde dogal olarak yayılış gosteren çilek ağacı (*Arbutus andrechne* L.)'nin bazı fenolojik ve pomolojik ö zelliklerinin incelenmesi. In Proceedings of the Ü zümsü Kivi ve Ü zümsü Meyveler Sempozyumu, Ordu, Turkey, 23–25 October 2003; pp. 472–475.
32. Sakar, M.K.; Berkman, M.Z.; Calis, I.; Ruedi, P. Constituents of Arbutus andrachne. *Fitoterapia* **1991**, *62*, 176–177.
33. Duralija, B.; Putnik, P.; Brdar, D.; Bebek Markovinović, A.; Zavadlav, S.; Pateiro, M.; Domínguez, R.; Lorenzo, J.M.; Bursać Kovačević, D. The perspective of croatian old apple cultivars in extensive farming for the production of functional foods. *Foods* **2021**, *10*, 708. [CrossRef]
34. Oliveira, I.; Baptista, P.; Malheiro, R.; Casal, S.; Bento, A.; Pereira, J.A. Influence of strawberry tree (*Arbutus unedo* L.) fruit ripening stage on chemical composition and antioxidant activity. *Food Res. Int.* **2011**, *44*, 1401–1407. [CrossRef]
35. Torres, J.A.; Valle, F.; Pinto, C.; García-Fuentes, A.; Salazar, C.; Cano, E. *Arbutus unedo* L. communities in southern Iberian Peninsula mountains. *Plant Ecol.* **2002**, *160*, 207–223. [CrossRef]
36. Molina, M.; Pardo-De-Santayana, M.; Aceituno, L.; Morales, R.; Tardio, J. Fruit production of strawberry tree (*Arbutus unedo* L.) in two Spanish forests. *Forestry* **2011**, *84*, 419–429. [CrossRef]
37. Šic Žlabur, J.; Bogdanović, S.; Voća, S.; Skendrović Babojelić, M. Biological potential of fruit and leaves of strawberry tree (*Arbutus unedo* L.) from Croatia. *Molecules* **2020**, *25*, 5102. [CrossRef]
38. Hart, H. *Inviting All the World's Crops to the Table Supporting Traditional Crops to Supply Future Needs*; CGSpace: Boston, MA, USA, 2007; pp. 1–25.
39. El Haouari, M.; Assem, N.; Changan, S.; Kumar, M.; Daştan, S.D.; Rajkovic, J.; Taheri, Y.; Sharifi-Rad, J.; Kabra, A. An insight into phytochemical, pharmacological, and nutritional properties of *Arbutus unedo* L. from Morocco. *Evid. Based Complement. Altern. Med.* **2021**, *2021*, 1794621. [CrossRef]
40. Jurica, K.; Brčić Karačonji, I.; Tariba, B.; Živković, T.; Brajenović, N.; Pizent, A. A multielement profile of Croatian strawberry tree (*Arbutus unedo* L.) fruit and leaves. In Proceedings of the ISTERH 2015 Conference "Recent Advances in Trace Element Research in Health and Disease", Dubrovnik, Croatia, 18–22 October 2015.
41. El Cadi, H.; El Cadi, A.; Kounnoun, A.; Oulad El Majdoub, Y.; Palma Lovillo, M.; Brigui, J.; Dugo, P.; Mondello, L.; Cacciola, F. Wild strawberry (*Arbutus unedo*): Phytochemical screening and antioxidant properties of fruits collected in northern Morocco. *Arab. J. Chem.* **2020**, *13*, 6299–6311. [CrossRef]
42. Ruiz-Rodríguez, B.-M.; Morales, P.; Fernández-Ruiz, V.; Sánchez-Mata, M.-C.; Cámara, M.; Díez-Marqués, C.; Pardo-de-Santayana, M.; Molina, M.; Tardío, J. Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. *Food Res. Int.* **2011**, *44*, 1244–1253. [CrossRef]
43. Mendes, L.; de Freitas, V.; Baptista, P.; Carvalho, M. Comparative antihemolytic and radical scavenging activities of strawberry tree *(Arbutus unedo* L.) leaf and fruit. *Food Chem. Toxicol.* **2011**, *49*, 2285–2291. [CrossRef]
44. Barros, L.; Carvalho, A.M.; Sá Morais, J.; Ferreira, I.C.F.R. Strawberry-tree, blackthorn and rose fruits: Detailed characterization in nutrients and phytochemicals with antioxidant properties. *Food Chem.* **2010**, *120*, 247–254. [CrossRef]
45. Colak, A.M. Morphological and biochemical diversity in fruits of *Arbutus unedo* L. from east aegean region in Turkey. *Erwerbs-Obstbau* **2019**, *61*, 379–383. [CrossRef]
46. Fortalezas, S.; Tavares, L.; Pimpão, R.; Tyagi, M.; Pontes, V.; Alves, P.; McDougall, G.; Stewart, D.; Ferreira, R.; Santos, C. Antioxidant properties and neuroprotective capacity of strawberry tree fruit (*Arbutus unedo*). *Nutrients* **2010**, *2*, 214–229. [CrossRef]
47. Alarcão-E-Silva, M.L.C.M.M.; Leitão, A.E.B.; Azinheira, H.G.; Leitão, M.C.A. The *Arbutus* berry: Studies on its color and chemical characteristics at two mature stages. *J. Food Compos. Anal.* **2001**, *14*, 27–35. [CrossRef]
48. Pawlowska, A.M.; De Leo, M.; Braca, A. Phenolics of *Arbutus unedo* L. (*Ericaceae*) Fruits: Identification of anthocyanins and gallic acid derivatives. *J. Agric. Food Chem.* **2006**, *54*, 10234–10238. [CrossRef]
49. Pallauf, K.; Rivas-Gonzalo, J.C.; del Castillo, M.D.; Cano, M.P.; de Pascual-Teresa, S. Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits. *J. Food Compos. Anal.* **2008**, *21*, 273–281. [CrossRef]
50. Ayaz, F.A.; Kucukislamoglu, M.; Reunanen, M. Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var.ellipsoidea) fruits. *J. Food Compos. Anal.* **2000**, *13*, 171–177. [CrossRef]
51. Oliveira, I.; Baptista, P.; Bento, A.; Pereira, J.A. *Arbutus unedo* L. and its benefits on human health. *J. Food Nutr. Res.* **2011**, *50*, 73–85.
52. Ganhão, R.; Estévez, M.; Kylli, P.; Heinonen, M.; Morcuende, D. Characterization of selected wild mediterranean fruits and comparative efficacy as inhibitors of oxidative reactions in emulsified raw pork burger patties. *J. Agric. Food Chem.* **2010**, *58*, 8854–8861. [CrossRef] [PubMed]
53. Guimarães, R.; Barros, L.; Dueñas, M.; Carvalho, A.M.; Queiroz, M.J.R.P.; Santos-Buelga, C.; Ferreira, I.C.F.R. Characterisation of phenolic compounds in wild fruits from Northeastern Portugal. *Food Chem.* **2013**, *141*, 3721–3730. [CrossRef] [PubMed]
54. Oliveira, I.; Coelho, V.; Baltasar, R.; Pereira, J.A.; Baptista, P. Scavenging capacity of strawberry tree (*Arbutus unedo* L.) leaves on free radicals. *Food Chem. Toxicol.* **2009**, *47*, 1507–1511. [CrossRef] [PubMed]
55. Bouyahya, A.; Moussaoui, N.; Abrini, J.; Bakri, Y.; Dakka, N. Determination of phenolic contents, antioxidant and antibacterial activities of strawberry tree (*Arbutus unedo* L.) leaf extracts. *Br. Biotechnol. J.* **2016**, *14*, 1–10. [CrossRef]

56. Martins, J.; Batista, T.; Pinto, G.; Canhoto, J. Seasonal variation of phenolic compounds in Strawberry tree (*Arbutus unedo* L.) leaves and inhibitory potential on *Phytophthora cinnamomi*. *Trees* **2021**, *35*, 1571–1586. [CrossRef]
57. Carcache-Blanco, E.J.; Cuendet, M.; Park, E.J.; Su, B.-N.; Rivero-Cruz, J.F.; Farnsworth, N.R.; Pezzuto, J.M.; Douglas Kinghorn, A. Potential cancer chemopreventive agents from *Arbutus unedo*. *Nat. Prod. Res.* **2006**, *20*, 327–334. [CrossRef] [PubMed]
58. Legssyer, A.; Ziyyat, A.; Mekh, H.; Bnouham, M.; Herrenknecht, C.; Roumy, V.; Fourneau, C.; Laurens, A.; Hoerter, J.; Fischmeister, R. Tannins and catechin gallate mediate the vasorelaxant effect of *Arbutus unedo* on the rat isolated aorta. *Phytother. Res.* **2004**, *18*, 889–894. [CrossRef]
59. Males, Z.; Plazibat, M.; Vundac, V.B.; Zuntar, I. Qualitative and quantitative analysis of flavonoids of the strawberry tree–*Arbutus unedo* L. (*Ericaceae*). *Acta Pharm.* **2006**, *56*, 245–250.
60. Sanjust, E.; Mocci, G.; Zucca, P.; Rescigno, A. Mediterranean shrubs as potential antioxidant sources. *Nat. Prod. Res.* **2008**, *22*, 689–708. [CrossRef]
61. Fiorentino, A.; Castaldi, S.; D'Abrosca, B.; Natale, A.; Carfora, A.; Messere, A.; Monaco, P. Polyphenols from the hydroalcoholic extract of *Arbutus unedo* living in a monospecific Mediterranean woodland. *Biochem. Syst. Ecol.* **2007**, *35*, 809–811. [CrossRef]
62. de Falco, B.; Grauso, L.; Fiore, A.; Bonanomi, G.; Lanzotti, V. Metabolomics and chemometrics of seven aromatic plants: Carob, eucalyptus, laurel, mint, myrtle, rosemary and strawberry tree. *Phytochem. Anal.* **2022**, *33*, 696–709. [CrossRef] [PubMed]
63. Asmaa, N.; Abdelaziz, G.; Boulanouar, B.; Carbonell-Barrachina, A.; Cano-Lamadrid, M.; Noguera-Artiaga, L. Chemical composition, antioxidant activity and mineral content of *Arbutus unedo* (leaves and fruits). *J. Microbiol. Biotechnol. Food Sci.* **2019**, *8*, 1335–1339. [CrossRef]
64. Kivcak, B.; Mert, T.; Demirci, B.; Baser, K.H.C. Composition of the essential oil of *Arbutus unedo* L. *Chem. Nat. Compd.* **2001**, *37*, 445–446. [CrossRef]
65. Berka-Zougali, B.; Hassani, A.; Besombes, C.; Allaf, K. Extraction of essential oils from *Algerian myrtle* leaves using instant controlled pressure drop technology. *J. Chromatogr. A* **2010**, *1217*, 6134–6142. [CrossRef] [PubMed]
66. Koukos, D.; Meletiou-Christou, M.-S.; Rhizopoulou, S. Leaf surface wettability and fatty acid composition of *Arbutus unedo* and *Arbutus andrachne* grown under ambient conditions in a natural macchia. *Acta Bot. Gall.* **2015**, *162*, 225–232. [CrossRef]
67. Dib, M.A.; Paolini, J.; Bendahou, M.; Varesi, L.; Allali, H.; Desjobert, J.-M.; Tabti, B.; Costa, J. Chemical composition of fatty acid and unsaponifiable fractions of leaves, stems and roots of *Arbutus unedo* and in vitro antimicrobial activity of unsaponifiable extracts. *Nat. Prod. Commun.* **2010**, *5*, 721. [CrossRef]
68. Kachkoul, R.; Squalli Housseini, T.; Mohim, M.; El Habbani, R.; Miyah, Y.; Lahrichi, A. Chemical compounds as well as antioxidant and litholytic activities of *Arbutus unedo* L. leaves against calcium oxalate stones. *J. Integr. Med.* **2019**, *17*, 430–437. [CrossRef]
69. Zitouni, H.; Hssaini, L.H.; Ouaabou, R.; Viuda-Martos, M.; Hernandez, F.; Ercisli, S.; Hachimi, H.; Zerhoune, M.; Hanine, H. Functionnal and technological properties of five strawberry (*Arbutus unedo* L.) fruit as bioactive ingredients in functional foods. *Int. J. Food Prop.* **2021**, *24*, 380–399. [CrossRef]
70. Izcara, S.; Morante-Zarcero, S.; Casado, N.; Sierra, I. Study of the phenolic compound profile of *Arbutus unedo* L. fruits at different ripening stages by HPLC-TQ-MS/MS. *Appl. Sci.* **2021**, *11*, 11616. [CrossRef]
71. Gündoğdu, M.; Ercisli, S.; Canan, I.; Orman, E.; Sameeullah, M.; Naeem, M.; Ben Ayed, R. Diversity in phenolic compounds, biochemical and pomological characteristics of *Arbutus unedo* fruits. *Folia Hortic.* **2018**, *30*, 139–146. [CrossRef]
72. Maleš, Ž.; Šarić, D.; Bojić, M. Quantitative determination of flavonoids and chlorogenic acid in the leaves of *Arbutus unedo* L. using thin layer chromatography. *J. Anal. Methods Chem.* **2013**, *2013*, 385473. [CrossRef]
73. Maleš, Ž.; Fabijančić, P.; Barman, A.; Gregov, I.; Bojić, M. Antioksidacijski učinak i HPLC analiza listova planike-*Arbutus unedo* L. *Farm. Glas.* **2015**, *71*, 523–528.
74. Jurica, K.; Karačonji, I.B.; Šegan, S.; Opsenica, D.M.; Kremer, D. Quantitative analysis of arbutin and hydroquinone in strawberry tree (*Arbutus unedo* L., Ericaceae) leaves by gas chromatography-mass spectrometry/Kvantitativna analiza arbutina i hidrokinona u listovima obične planike (*Arbutus unedo* L., *Ericaceae*) plinskokromatografskom metodom uz detekciju masenim spektrometrom. *Arch. Ind. Hyg. Toxicol.* **2015**, *66*, 197–202. [CrossRef]
75. Pavlović, R.D.; Lakušić, B.; Došlov-Kokoruš, Z.; Kovačević, N. Arbutin content and antioxidant activity of some Ericaceae species. *Die Pharm. Int. J. Pharm. Sci.* **2009**, *64*, 656–659.
76. Vidrih, R.; Hribar, J.; Prgomet, Ž.; Poklar Ulrih, N. The physico-chemical properties of strawberry tree (*Arbutus unedo* L.) fruits. *Croat. J. Food Sci. Technol.* **2013**, *5*, 29–33.
77. Camejo-Rodrigues, J.S. *Recolha dos 'Saber-Fazer' Tradicionais das Plantas Aromáticas e Medicinais*; Bordeira Concelhos de Aljezur: Bordeira, Portugal, 2006.
78. Carvalho, A.M. Etnobotánica del Parque Natural de Montesinho Plantas, Tradición y Saber Popular en un Territorio del Nordeste de Portugal. Ph.D. Thesis, Universidad Autónoma, Madrid, Spain, 2005.
79. Novais, M.H.; Santos, I.; Mendes, S.; Pinto-Gomes, C. Studies on pharmaceutical ethnobotany in Arrabida Natural Park (Portugal). *J. Ethnopharmacol.* **2004**, *93*, 183–195. [CrossRef] [PubMed]
80. Salem, I.B.; Ouesleti, S.; Mabrouk, Y.; Landolsi, A.; Saidi, M.; Boulilla, A. Exploring the nutraceutical potential and biological activities of *Arbutus unedo* L. (Ericaceae) fruits. *Ind. Crops Prod.* **2018**, *122*, 726–731. [CrossRef]
81. Dib, M.E.A.; Allali, H.; Bendiabdellah, A.; Meliani, N.; Tabti, B. Antimicrobial activity and phytochemical screening of *Arbutus unedo* L. *J. Saudi Chem. Soc.* **2013**, *17*, 381–385. [CrossRef]

82. Kivçak, B.; Mert, T.; Ertabaklar, H.; Balcioğlu, I.C.; Ozensoy Töz, S. In vitro activity of *Arbutus unedo* against *Leishmania tropica* promastigotes. *Turk. J. Parasitol.* **2009**, *33*, 114–115.
83. Zitouni, H.; Hssaini, L.; Messaoudi, Z.; Ourradi, H.; Viuda-Martos, M.; Hernández, F.; Ercisli, S.; Hanine, H. Phytochemical components and bioactivity assessment among twelve strawberry (*Arbutus unedo* L.) genotypes growing in Morocco using chemometrics. *Foods* **2020**, *9*, 1345. [CrossRef] [PubMed]
84. Heinrich, M. Understanding local Mediterranean diets: A multidisciplinary pharmacological and ethnobotanical approach. *Pharmacol. Res.* **2005**, *52*, 353–366.
85. Afkir, S.; Nguelefack, T.B.; Aziz, M.; Zoheir, J.; Cuisinaud, G.; Bnouham, M.; Mekhfi, H.; Legssyer, A.; Lahlou, S.; Ziyyat, A. *Arbutus unedo* prevents cardiovascular and morphological alterations in L-NAME-induced hypertensive rats. *J. Ethnopharmacol.* **2008**, *116*, 288–295. [CrossRef]
86. Guimarães, R.; Barros, L.; Calhelha, R.C.; Carvalho, A.M.; Queiroz, M.J.R.P.; Ferreira, I.C.F.R. Bioactivity of different enriched phenolic extracts of wild fruits from Northeastern Portugal: A comparative study. *Plant Foods Hum. Nutr.* **2013**, *69*, 37–42. [CrossRef]
87. Locatelli, C.; Leal, P.C.; Yunes, R.A.; Nunes, R.J.; Creczynski-Pasa, T.B. Gallic acid ester derivatives induce apoptosis and cell adhesion inhibition in melanoma cells: The relationship between free radical generation, glutathione depletion and cell death. *Chem. Biol. Interact.* **2009**, *181*, 175–184. [CrossRef]
88. Le Marchand, L. Cancer preventive effects of flavonoids—A review. *Biomed. Pharmacother.* **2002**, *56*, 296–301. [CrossRef]
89. El-Hilaly, J.; Hmammouchi, M.; Lyoussi, B. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). *J. Ethnopharmacol.* **2003**, *86*, 149–158. [CrossRef]
90. Hernández-Rodríguez, P.; Pabón, B.; Rodríguez, Á. Chemical and biological properties of *Arbutus unedo*, a potential medicinal plant. *Rev. Cubana Farm.* **2015**, *49*, 144–155.
91. Ait lhaj, Z.; Bchitou, R.; Gaboun, F.; Abdelwahd, R.; Benabdelouahab, T.; Kabbour, M.R.; Pare, P.; Diria, G.; Bakhy, K. Moroccan strawberry tree (*Arbutus unedo* L.) fruits: Nutritional value and mineral composition. *Foods* **2021**, *10*, 2263. [CrossRef]
92. Bouyahya, A.; Bakri, Y.; Et-Touys, A.; Assemian, I.C.C.; Abrini, J.; Dakka, N. In vitro antiproliferative activity of selected medicinal plants from the North-West of Morocco on several cancer cell lines. *Eur. J. Integr. Med.* **2018**, *18*, 23–29. [CrossRef]
93. Bnouham, M.; Merhfour, F.Z.; Legssyer, A.; Mekhfi, H.; Maal-lem, S.; Ziyyat, A. Antihyperglycemic activity of *Arbutus unedo*, *Ammoides pusilla* and *Thymelaea hirsuta*. *Pharm. Int. J. Pharm. Sci.* **2007**, *62*, 630–632.
94. Mekhfi, H.; Haouari, M.E.; Legssyer, A.; Bnouham, M.; Aziz, M.; Atmani, F.; Remmal, A.; Ziyyat, A. Platelet anti-aggregant property of some Moroccan medicinal plants. *J. Ethnopharmacol.* **2004**, *94*, 317–322. [CrossRef]
95. Malheiro, R.; Sá, O.; Pereira, E.; Aguiar, C.; Baptista, P.; Pereira, J.A. *Arbutus unedo* L. leaves as source of phytochemicals with bioactive properties. *Ind. Crops Prod.* **2012**, *37*, 473–478. [CrossRef]
96. Huang, W.-Y.; Cai, Y.-Z.; Zhang, Y. Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutr. Cancer* **2009**, *62*, 1–20. [CrossRef]
97. El Haouari, M.; López, J.J.; Mekhfi, H.; Rosado, J.A.; Salido, G.M. Antiaggregant effects of *Arbutus unedo* extracts in human platelets. *J. Ethnopharmacol.* **2007**, *113*, 325–331. [CrossRef]
98. Tavares, L.; Fortalezas, S.; Carrilho, C.; McDougall, G.J.; Stewart, D.; Ferreira, R.B.; Santos, C.N. Antioxidant and antiproliferative properties of strawberry tree tissues. *J. Berry Res.* **2010**, *1*, 3–12. [CrossRef]
99. Jurica, K.; Brčić Karačonji, I.; Gobin, I. Medicinal herbs and herbal preparations for the treatment of urinary infections. *Med. Flum.* **2018**, *54*, 262–267. [CrossRef]
100. Coimbra, A.T.; Luís, Â.F.S.; Batista, M.T.; Ferreira, S.M.P.; Duarte, A.P.C. Phytochemical characterization, bioactivities evaluation and synergistic effect of *Arbutus unedo* and crataegus monogyna extracts with amphotericin B. *Curr. Microbiol.* **2020**, *77*, 2143–2154. [CrossRef]
101. Oliveira, I.; Nunes, A.; Lima, A.; Borralho, P.; Rodrigues, C.; Ferreira, R.; Ribeiro, A. New lectins from Mediterranean flora. Activity against HT29 colon cancer cells. *Int. J. Mol. Sci.* **2019**, *20*, 3059. [CrossRef]
102. Jurica, K.; Brčić Karačonji, I.; Mikolić, A.; Milojković-Opsenica, D.; Benković, V.; Kopjar, N. In vitro safety assessment of the strawberry tree (*Arbutus unedo* L.) water leaf extract and arbutin in human peripheral blood lymphocytes. *Cytotechnology* **2018**, *70*, 1261–1278. [CrossRef]
103. Jurica, K.; Brčić Karačonji, I.; Kopjar, N.; Shek-Vugrovečki, A.; Cikač, T.; Benković, V. The effects of strawberry tree water leaf extract, arbutin and hydroquinone on haematological parameters and levels of primary DNA damage in white blood cells of rats. *J. Ethnopharmacol.* **2018**, *215*, 83–90. [CrossRef] [PubMed]
104. Jurica, K.; Benković, V.; Sikirić, S.; Kopjar, N.; Brčić Karačonji, I. Liver function and DNA integrity in hepatocytes of rats evaluated after treatments with strawberry tree (*Arbutus unedo* L.) water leaf extract and arbutin. *Drug Chem. Toxicol.* **2018**, *43*, 127–137. [CrossRef] [PubMed]
105. Jurica, K.; Benković, V.; Sikirić, S.; Brčić Karačonji, I.; Kopjar, N. The effects of strawberry tree (*Arbutus unedo* L.) water leaf extract and arbutin upon kidney function and primary DNA damage in renal cells of rats. *Nat. Prod. Res.* **2018**, *34*, 2354–2357. [CrossRef] [PubMed]
106. Orak, H.H.; Yagar, H.; Isbilir, S.S.; Demirci, A.Ş.; Gümüş, T.; Ekinci, N. Evaluation of antioxidant and antimicrobial potential of strawberry tree (*Arbutus Unedo* L.) leaf. *Food Sci. Biotechnol.* **2011**, *20*, 1249–1256. [CrossRef]
107. Kahriman, N.; Albay, C.G.; Dogan, N.; Usta, A.; Karaoglu, S.A.; Yayli, N. Volatile constituents and antimicrobial activities from flower. *Asian J. Chem.* **2010**, *22*, 6422–6437.
108. Mekhfi, H.; ElHaouari, M.; Bnouham, M.; Aziz, M.; Ziyyat, A.; Legssyer, A. Effects of extracts and tannins from *Arbutus unedo* leaves on rat platelet aggregation. *Phytother. Res.* **2006**, *20*, 135–139. [CrossRef]

109. Orak, H.H.; Aktas, T.; Yagar, H.; İsbilir, S.S.; Ekinci, N.; Sahin, F.H. Effects of hot air and freeze drying methods on antioxidant activity, colour and some nutritional characteristics of strawberry tree (*Arbutus unedo* L.) fruit. *Food Sci. Technol. Int.* **2012**, *18*, 391–402. [CrossRef]
110. Pavlović, D.R.; Branković, S.; Kovačević, N.; Kitić, D.; Veljković, S. Comparative study of spasmolytic properties, antioxidant activity and phenolic content of *Arbutus unedo* from Montenegro and Greece. *Phytother. Res.* **2011**, *25*, 749–754. [CrossRef]
111. Mariotto, S.; Ciampa, A.; de Prati, A.; Darra, E.; Vincenzi, S.; Sega, M.; Cavalieri, E.; Shoji, K.; Suzuki, H. Aqueous extract of *Arbutus unedo* inhibits STAT1 activation in human breast cancer cell line MDA-MB-231 and human fibroblasts through SHP2 activation. *Med. Chem.* **2008**, *4*, 219–228. [CrossRef]
112. Mariotto, S.; Esposito, E.; Di Paola, R.; Ciampa, A.; Mazzon, E.; de Prati, A.C.; Darra, E.; Vincenzi, S.; Cucinotta, G.; Caminiti, R. Protective effect of *Arbutus unedo* aqueous extract in carrageenan-induced lung inflammation in mice. *Pharmacol. Res.* **2008**, *57*, 110–124. [CrossRef]
113. Putnik, P.; Lorenzo, J.; Barba, F.; Roohinejad, S.; Režek Jambrak, A.; Granato, D.; Montesano, D.; Bursać Kovačević, D. Novel food processing and extraction technologies of high-added value compounds from plant materials. *Foods* **2018**, *7*, 106. [CrossRef] [PubMed]
114. Tuberoso, C.I.G.; Bifulco, E.; Caboni, P.; Cottiglia, F.; Cabras, P.; Floris, I. Floral markers of strawberry tree (*Arbutus unedo* L.) honey. *J. Agric. Food Chem.* **2009**, *58*, 384–389. [CrossRef]
115. Osés, S.M.; Nieto, S.; Rodrigo, S.; Pérez, S.; Rojo, S.; Sancho, M.T.; Fernández-Muiño, M.Á. Authentication of strawberry tree (*Arbutus unedo* L.) honeys from southern Europe based on compositional parameters and biological activities. *Food Biosci.* **2020**, *38*, 100768. [CrossRef]
116. Mrabti, H.N.; Bouyahya, A.; Ed-Dra, A.; Kachmar, M.R.; Mrabti, N.N.; Benali, T.; Shariati, M.A.; Ouahbi, A.; Doudach, L.; Faouzi, M.E.A. Polyphenolic profile and biological properties of *Arbutus unedo* root extracts. *Eur. J. Integr. Med.* **2021**, *42*, 101266. [CrossRef]
117. Tomašević, I.; Putnik, P.; Valjak, F.; Pavlić, B.; Šojić, B.; Bebek Markovinović, A.; Bursać Kovačević, D. 3D printing as novel tool for fruit-based functional food production. *Curr. Opin. Food Sci.* **2021**, *41*, 138–145. [CrossRef]
118. Seidemann, J. Zur Kenntnis von wenig bekannten exotischen Früchten. 5. (Mitt.): Baumerdbeere (*Arbutus unedo* L.). *Dtsch. Lebensm. Rundsch.* **1995**, *91*, 110–113.
119. TardÍO, J.; Pardo-De-Santayana, M.; Morales, R. Ethnobotanical review of wild edible plants in Spain. *Bot. J. Linn. Soc.* **2006**, *152*, 27–71. [CrossRef]
120. Soufleros, E.H.; Mygdalia, S.A.; Natskoulis, P. Production process and characterization of the traditional Greek fruit distillate "Koumaro" by aromatic and mineral composition. *J. Food Compos. Anal.* **2005**, *18*, 699–716. [CrossRef]
121. Wormit, A.; Usadel, B. The multifaceted role of pectin methylesterase inhibitors (PMEIs). *Int. J. Mol. Sci.* **2018**, *19*, 2878. [CrossRef]
122. European Union. *Regulation EU n° 2019/787 of the European Parliament and of the Council of 17 April 2019 on the Definition, d., Presentation and Labelling of Spirit Drinks, the Use of the Names of Spirit Drinks in the Presentation and Labelling of Other Foodstuffs, the Protection of Geographical Indications for Spirit Drinks, the Use of Ethyl Alcohol and Distillates of Agricultural Origin in Alcoholic Beverages, and Repealing Regulation (EC) No110/2008. O. J. Eur. Union. O. J.*; European Union: Maastricht, The Netherlands, 2019; Volume L130, pp. 1–54.
123. Botelho, G.; Gomes, F.; Ferreira, F.M.; Caldeira, I. Influence of maturation degree of Arbutus (*Arbutus unedo* L.) fruits in spirit composition and quality. *Int. J. Agric. Biol. Eng.* **2015**, *9*, 615–620.
124. Santo, D.E.; Galego, L.; Gonçalves, T.; Quintas, C. Yeast diversity in the Mediterranean strawberry tree (*Arbutus unedo* L.) fruits' fermentations. *Food Res. Int.* **2012**, *47*, 45–50. [CrossRef]
125. Caldeira, I.; Gomes, F.; Botelho, G. *Arbutus unedo* L. spirit: Does the water addition before fermentation matters? In Proceedings of the 1st International Congress on Engineering and Sustainability in the XXI Century—INCREaSE, Faro, Portugal, 11–13 October; Mortal, A., Aníbal, J., Monteiro, J., Sequeira, C., Semião, J., Silva, M.M., Oliveira, M., Eds.; Springer: Berlin/Heidelberg, Germany, 2017; pp. 206–215.
126. Cavaco, T.; Longuinho, C.; Quintas, C.; Saraiva De Carvalho, I. Chemical and microbial changes during the natural fermentation of strawberry tree (*Arbutus unedo* L.) fruits. *J. Food Biochem.* **2007**, *31*, 715–725. [CrossRef]
127. Anjos, O.; Canas, S.; Gonçalves, J.C.; Caldeira, I. Development of a spirit drink produced with strawberry tree (*Arbutus unedo* L.) fruit and honey. *Beverages* **2020**, *6*, 38. [CrossRef]
128. Masmoudi, M.; Ammar, I.; Ghribi, H.; Attia, H. Physicochemical, radical scavenging activity and sensory properties of a soft cheese fortified with *Arbutus unedo* L. extract. *Food Biosci.* **2020**, *35*, 100579. [CrossRef]
129. Cossu, M.; Juliano, C.; Pisu, R.; Alamanni, M.C. Effects of enrichment with polyphenolic extracts from sardinian plants on physico-chemical, antioxidant and microbiological properties of yogurt. *Ital. J. Food Sci.* **2009**, *21*, 447–459.
130. Takwa, S.; Caleja, C.; Barreira, J.C.M.; Soković, M.; Achour, L.; Barros, L.; Ferreira, I.C.F.R. *Arbutus unedo* L. and *Ocimum basilicum* L. as sources of natural preservatives for food industry: A case study using loaf bread. *LWT* **2018**, *88*, 47–55. [CrossRef]
131. Derbassi, N.; Pedrosa, M.C.; Heleno, S.; Fernandes, F.; Dias, M.I.; Calhelha, R.C.; Rodrigues, P.; Carocho, M.; Ferreira, I.C.F.R.; Barros, L. *Arbutus unedo* leaf extracts as potential dairy preservatives: Case study on quark cheese. *Food Funct.* **2022**, *13*, 5442–5454. [CrossRef]
132. Dias, C.; Fonseca, A.M.A.; Amaro, A.L.; Vilas-Boas, A.A.; Oliveira, A.; Santos, S.A.O.; Silvestre, A.J.D.; Rocha, S.M.; Isidoro, N.; Pintado, M. Natural-based antioxidant extracts as potential mitigators of fruit browning. *Antioxidants* **2020**, *9*, 715. [CrossRef]
133. Erkekoglou, I.; Nenadis, N.; Samara, E.; Mantzouridou, F.T. Functional teas from the leaves of *Arbutus unedo*: Phenolic content, antioxidant activity, and detection of efficient radical scavengers. *Plant Foods Hum. Nutr.* **2017**, *72*, 176–183. [CrossRef]

134. Mancini, A.; Imperlini, E.; Nigro, E.; Montagnese, C.; Daniele, A.; Orrù, S.; Buono, P. Biological and Nutritional Properties of Palm Oil and Palmitic Acid: Effects on Health. *Molecules* **2015**, *20*, 17339–17361. [CrossRef]
135. Himejima, M.; Nihei, K.I.; Kubo, I. Hydroquinone, a control agent of agglutination and adherence of *Streptococcus mutans* induced by sucrose. *Bioorganic Med. Chem.* **2004**, *12*, 921–925. [CrossRef] [PubMed]
136. Jin, S.; Sato, N. Benzoquinone, the substance essential for antibacterial activity in aqueous extracts from succulent young shoots of the pear *Pyrus* spp. *Phytochemistry* **2003**, *62*, 101–107. [CrossRef]
137. Migas, P.; Krauze-Baranowska, M. The significance of arbutin and its derivatives in therapy and cosmetics. *Phytochem. Lett.* **2015**, *13*, 35–40. [CrossRef]
138. Pixabay. Available online: https://pixabay.com/photos/fruits-strawberry-tree-sheets-5829002/ (accessed on 7 September 2020).

6. Objectives, hypotheses and expected contribution

This research hypothesized that:

i. strawberry fruits and strawberry juices differ in terms of quality parameters;
ii. the process parameters of the hurdle technology will affect the quality of strawberry juices;
iii. the process parameters of 3D printing technology will influence the quality of strawberry-based functional food.

In order to confirm or refute the proposed hypotheses, the following objectives were defined:

i. to test the quality of strawberry fruit and determine the effects of processing on the quality of strawberry juices;
ii. to optimize the process parameters of the hurdle technology and investigate their influence on the quality of strawberry juices;
iii. to optimize the process parameters of 3D printing technology with regard to the quality of strawberry-based functional food.

Due to its complexity, the research was divided into three distinct parts:

The first part of the study explored the potential of the strawberry cv. 'Albion' for sustainable processing into functional juice using cold pressing technology. This investigation focused on the physicochemical properties (mass and hardness of the fruit, color parameters, total acidity, soluble solids content (SSC) and pH value of the juices), biological properties (spectrophotometric determination of total phenolic compounds, total hydroxycinnamic acids and flavonols as well as monomeric anthocyanins), toxicological properties (analysis of heavy metals and pesticides) and sensory properties (Quantitative descriptive analysis, QDA). These properties were analyzed in fresh strawberries, juice and juice by-products, considering fruit ripeness (technological *vs.* consumer ripeness) and storage conditions for 7 days at 4 °C (*Publication No.3*).

The second part of the study investigated the impact of PEF and HPU treatments, both individually and in combination as a hurdle technology (PEF + HPU), on the quality of strawberry juices. For PEF treatment, the study examined the effects of process parameters such as electric field strength (40 and 50 kV cm^{-1}), frequency (100 and 200 Hz), treatment time

(3 and 6 minutes) and storage duration (0-7 days at 4°C) on the stability of the aforementioned BACs and condensed tannins (*Publication No.4*). In the HPU treatment, the parameters analyzed included amplitude (25, 50, 75 and 100 %), pulse (50 *vs.* 100 %), treatment time (5 *vs.* 10 minutes) and storage (0-7 days at 4°C), focusing on the same BACs as in the PEF study (*Publication No.5*). Based on the results, process parameters were optimized for each technology individually (*Publication No.4* and *Publication No.5*). The hurdle technology, combining PEF and HPU treatments, was then carried out using the selected process parameters. The physicochemical parameters (pH, SSC, color parameters, etc.), browning index, hydroxymethylfurfural content, antioxidant capacity and safety were determined in the juice samples processed using the hurdle technology. The results were statistically evaluated and optimization of the hurdle technology parameters was conducted, considering the sequence of the applied non-thermal technologies (*Publication No.6* and *Publication No.7*).

The third part of the research explored the potential of combining strawberries with strawberry tree fruit (*Arbutus unedo* L.) as a natural thickener to create a fruit matrix with suitable consistency for 3D printing. First, each raw material (strawberries and strawberry tree fruit) was individually adapted to the 3D printing technology by adding hydrocolloids. The effects of 3D process parameters, including the type of starch carrier (wheat *vs.* corn), starch carrier content (0-20 %), printing program (Program 1 *vs.* Program 2), and storage (0-7 days at 4°C), on the quality of 3D printed samples were investigated (*Publication No.8* and *Publication No.9*). Based on the results obtained, the optimal ratio of the two fruit materials was determined and the process of 3D printing a strawberry-based functional product with the addition of strawberry tree fruit as a natural thickening agent was carried out. The optimal 3D printing parameters for the production of a functional 3D product based on strawberries and strawberry tree fruit were identified based on physicochemical and textural properties, BACs content and antioxidant capacities (*Publication No.10*).

The following questions were examined in this dissertation:

1. How does cold pressing technology used to process strawberries into juices affect the biological potential of the juices and by-products? Are strawberries at both ripeness levels (75% and 100%) suitable for juice production? (*Publication No.3*)
2. How do the parameters of PEF technology (electric field strength, frequency and treatment time) influence the quality of strawberry juices in terms of BACs

preservation? What are the optimal PEF treatment parameters that achieve the highest yield of the investigated BACs in strawberry juices? (*Publication No.4*)

3. How do the parameters of HPU technology (amplitude, pulse and treatment time) affect the quality of strawberry juices with regard to the preservation of BACs? What are the optimal HPU treatment parameters that achieve the highest yield of the investigated BACs in strawberry juices? (*Publication No.5*)
4. How do the parameters of hurdle technology in PEF + HPU combination affect the quality of strawberry juices in terms of their physicochemical, biological and antioxidant properties, color parameters, hydroxymethylfurfural formation, browning index and safety? What are the optimal hurdle technology parameters that achieve the highest yield of the studied BACs and antioxidant capacity in strawberry juices? (*Publication No. 6*)
5. How do the parameters of hurdle technology in HPU + PEF combination affect the quality of strawberry juices in terms of their physicochemical, biological and antioxidant properties, color parameters, hydroxymethylfurfural formation, browning index and safety? What are the optimal hurdle technology parameters that achieve the highest yield of the studied BACs and antioxidant capacity in strawberry juices? (*Publication No.7*)
6. How do the parameters of 3D printing (type of starch carrier, starch content and 3D printing program) affect the quality of functional 3D strawberry products in terms of physicochemical parameters, BACs content, antioxidant potential, color parameters and rheological properties? (*Publication No.8*)
7. What is the influence of 3D printing parameters (type of starch carrier, starch content and 3D printing program) on the quality of functional 3D products made from strawberry tree fruit in terms of physicochemical parameters, BAC content, antioxidant potential, color parameters and rheological properties? (*Publication No.9*)
8. How do the 3D printing parameters (starch content and 3D printing program) affect the quality of functional 3D products made from strawberries and strawberry tree fruit in terms of BACs content, antioxidant potential, color parameters, rheological and sensory properties? What are the optimal 3D printing parameters that achieve the highest yield of BACs and antioxidant potential in 3D printed strawberries and strawberry tree fruit products? (*Publication No. 10*)

The following achievements were made through this dissertation:

1. Improved understanding of the effects of PEF treatment process parameters (electric field strength, frequency and treatment time) on the content of BACs in strawberry juices.
2. Identification of the optimal PEF treatment parameters to maximize the yield of BACs in strawberry juices.
3. Enhanced knowledge of the influence of HPU treatment process parameters (amplitude, pulse and treatment time) on the content of BACs in strawberry juices.
4. Identification of the optimal HPU treatment parameters to maximize the yield of BACs in strawberry juices.
5. Improved understanding of the effects of hurdle technology combining PEF and HPU on BACs content and antioxidant capacity in strawberry juices.
6. Determination of the optimal treatment duration for hurdle technology combining PEF and HPU to maximize BACs content and antioxidant capacity in strawberry juices.
7. Enhanced understanding of the impact of 3D printing process parameters (type of starch carrier, starch content and 3D printing program) on the BACs content, antioxidant capacity, color parameters and rheological properties in 3D functional strawberry products.
8. Improved understanding of how 3D printing process parameters (type of starch carrier, starch content and 3D printing program) affect the BACs content, antioxidant capacity, color parameters and rheological properties of the 3D functional strawberry tree fruit product.
9. Better insights into the effects of 3D printing process parameters (starch content and 3D printing program) on the BACs content, antioxidant capacity, color parameters, rheological and sensory properties of functional 3D products made from strawberry with added strawberry tree fruit.
10. Identification of the optimal 3D printing process parameters (type of starch carrier, starch content and 3D printing program) to maximize BACs content and antioxidant capacity in 3D printed products made from strawberries, strawberry tree fruit and combinations of both.

Publication No.3: Chemometric Valorization of Strawberry (*Fragaria x ananassa* Duch.) cv. ‘Albion’ for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value

Publication No.3

Bebek Markovinović, A., Putnik, P., Duralija, B., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., Palac Bešlić, I., Pavlić, B., Manuel Lorenzo, J., Bursać Kovačević, D. (2022) Chemometric Valorization of Strawberry (*Fragaria x ananassa* Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. *Foods*, **11** (5), 640. doi: 10.3390/foods11050640.

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Article

Chemometric Valorization of Strawberry (*Fragaria x ananassa* Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value

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Abstract: Strawberries (*Fragaria x ananassa* Duch. cv. 'Albion') were harvested at two stages of ripeness (75% vs. 100%) and their physicochemical, sensory, toxicological, and bioactive properties were evaluated before and after processing into juice. The fresh fruits and their by-products were also evaluated. During processing into juice, the color change was higher in the fully ripe fruits, confirming the encouraging prospects for using the less ripe strawberries for processing. The analysis of heavy metals (Cu, Zn, Ni, As, Cd, Pb) was carried out, and in juice and by-product samples of 100% maturity, only Pb was higher than the MDK. Of the 566 pesticides analyzed, only cyprodinil was found in the by-products of the strawberries at 75% maturity, while pyrimethanil was detected in all samples. Fresh strawberries of both ripeness levels were rated similarly to the corresponding juices for all sensory attributes studied, indicating that sensory perception was not affected by processing. However, ripeness was found to be an important factor influencing most sensory attributes. The by-products were the materials with the highest levels of all bioactive compounds. Considering all quality parameters evaluated, the chemometric evaluation confirms the suitability of 75% ripe strawberries for processing into functional juice, which could be important for the juice industry.

Keywords: strawberry; ripeness; functional juice; bioactive compounds; sensory; toxicology

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1. Introduction

Strawberries (*Fragaria x ananassa* Duch.) are a very popular fruit among consumers, either as fresh produce for consumption or for processing, e.g., into juices. This raw material is well-aligned with the growing demand for functional foods in the market as consumers increasingly choose products of exceptional quality with added value [1]. Particular attention is being paid to functional products such as strawberry juice, as the strawberry has been shown to be a fruit with numerous health benefits, e.g., anti-inflammatory, anticancer, antioxidant, antidiabetic, antimicrobial, cardioprotective, and neuroprotective effects [2,3].

In addition, the by-product of the strawberry that remains after the fruit is processed into juice has been found to be an excellent source of bioactive compounds with potent antioxidant potential [4,5]. When the strawberry fruit is processed into juice, there is a significant loss of phenolic compounds [6,7]. Most phenolic compounds are contained in the achenes and the receptacles of strawberry fruits, and when strawberry fruits are processed into juice, the phenolic compounds remain bound to the cell wall material. Therefore, most phenolic compounds, including anthocyanins, are better preserved in the by-product than in the juice [8–10]. These bioactive compounds, including phenolic acids, flavonoids (such as anthocyanins and flavonols), and tannins are associated with the above-mentioned beneficial effects, especially due to their antioxidant activity [11]; therefore, the great potential of strawberries lies in the production of functional foods.

On the other hand, strawberries are very fragile fruits, with a short storage time after harvest due to their high respiration rate, and their quality can be affected very quickly, either by handling, storage, or transport, resulting in a short shelf life and high economic losses. Fresh fruits with lower quality are not suitable for processing [12]. Therefore, it is necessary to select cultivars that are more resistant to quality changes during storage and processing. In a recent study, multivariate analysis was successfully applied to select strawberry cultivars suitable for fresh consumption and/or processing. The importance of selecting the appropriate cultivar for the intended purpose was emphasized [13,14]; therefore, this chemometric approach could be an advanced tool for potential industrial purposes [15].

In addition to the genotype, it has been found that growing location, cultivation method and ripening stages have a significant effect on the physicochemical and phytochemical parameters of strawberries [16–18]. Sugar content increases and citric and malic acid content decreases with maturity, which contributes to the sweetness of strawberries, making the sugar-acid ratio an important indicator of fruit quality [19,20]. Moreover, the effects of ripening (e.g., almost ripe, partially red; ripe, red; and fully ripe, dark red) have a strong influence on the type and concentration of individual and total polyphenolic compounds [17,21]. Interestingly, the total antioxidant capacity (TAC) of strawberries on the day of harvest was higher in ripe fruits than in unripe fruits, and with increasing storage time, TAC tended to increase in unripe fruits [22]. Ellagic acid content was found to be the highest in unripe fruits and gradually decreased with increasing ripeness [22]. Although totally unripe strawberries are not processed, they could be used to produce juices due to their higher firmness and biological potential, which could form the basis of functional foods. Besides, fully immature strawberries have a firmer texture and are less sensitive to prolonged storage and transportation [23], so they could be a good raw material for the production of functional foods.

Sensory characteristics are extremely important to consumers, and the color of fresh strawberries, as well as strawberry products, is one of the most important quality indicators that consumers look for first [24]. The overall sensory appearance of strawberries is highly dependent on the visual freshness and shininess of the fruit, most likely because shininess decreases as the strawberries dry out and become wrinkled. Perception of glossiness and visual freshness were found to be negatively correlated with color intensity, with bright orange-red strawberry fruit perceived as fresher than darker, more purple-red strawberry fruit [25]. The overall quality of fresh strawberries is influenced by the intensity of red color and the sensation of sweetness, strawberry flavor and overall flavor, fruit aroma, strawberry aroma and overall aroma, while acidity has no effect on the evaluation of overall quality [19,25,26]. The stage of ripeness significantly affects the sensory quality of strawberries. Unripe strawberries have more acidic, citrus, and green flavor attributes than ripe strawberries due to a high concentration of acids, such as citric acid. A study of six strawberry cultivars at immature and ideal ripening stages revealed that the cultivar 'Albion' has favorable sensory attributes at both ripening stages, making it an excellent cultivar for both fresh consumption and processing [20].

When strawberries are considered from a food safety perspective, the monitoring of pesticide and heavy metal residues should be increased to fully protect consumer health. Heavy metals as naturally occurring elements found throughout the earth's crust are among the largest contaminants in the food supply and are considered the most serious problem facing our environment [27]. They are not biodegradable or thermally degradable and enter the human body through food; thus, they can accumulate in various body organs, leading to undesirable side effects [28]. It was found that among fruits and vegetables for retail sale, strawberries are the fruits with the highest pesticide content [29]. High pesticide residues in fruits are a sign of the ubiquitous and intensive use of pesticides in their production and distribution. However, there is ample evidence that pesticides pose a potential risk to humans and other living organisms and may also have negative effects on the environment [30]. Therefore, the safety and health benefits of strawberries should be verified before their use in the production of functional foods [31]. Consequently, the aim of this study was to investigate the suitability of the strawberry cultivar 'Albion' for the production of functional juice by using chemometrics in quality assessment. For this purpose, the influences of maturity stages, physicochemical properties, toxicology, sensory properties, and bioactive potential were considered.

2. Materials and Methods

2.1. Chemicals and Standards

Formic acid (98%, p.a.), sodium carbonate, anhydrous (99.5–100.5%), and hydrochloric acid (37%, *w*/*w*) were purchased from Lach-Ner (Neratovice, Czech Republic). HPLC grade 99% methanol was purchased from Honeywell (Paris, France). Folin-Ciocalteu reagent was purchased from Fisher Scientific UK (Loughborough, UK). Ethanol (96% pure) was purchased from Gram-Mol (Zagreb, Croatia). Gallic acid standard (97.5–102.5%) and quercetin standard (95%) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Acros Organics (Guangzhou, China), respectively. Chlorogenic acid reference standard (min. 95%), potassium chloride (99.0–100.5%) and sodium acetate anhydride (99%) were purchased from Thermo Fisher (Kandel, Germany).

2.2. Material

The plants were grown in the greenhouse of the private company Jagodar-HB in Donja Lomnica, Zagreb County, (Croatia), in 2021. The growing system was soilless, using plastic bags with coconut coir. Green container plants of the cultivar 'Albion' were planted at a density of 10 plants m^{-2} starting in November of the year before harvest. All plants received standard nutrition; the electrical conductivity of the drainage solution was less than 2 dS m^{-1}, and pH value in the root zone was between 5.5 and 6.5 during growth and harvest.

The fruits were harvested in the early morning on 24 May 2021 at different stages of maturity: (i) technological ripening stage (ripe green fruits, about 25% green), (F1), and (ii) full ripening stage (full red, 100% ripeness), (F2), (Figure 1A,B).

Immediately after harvesting, the fruit samples (5 kg for each stage of ripeness) were delivered to the laboratory where the physicochemical analyses were carried out. The general organization of the entire experiment is shown in Figure 2. The fresh fruits were stored at 4 °C for 3 days to test their suitability for processing after a short storage period. Then, physicochemical, sensory, toxicological, and bioactive analyses were performed. At the end of storage, strawberry samples from both ripening stages were processed into juice (J1 and J2) using cold pressing (Kuvings B6000 Slow Juicer, VerVita d.o.o., Zagreb, Croatia), and strawberry by-products BP1 and BP2 were also prepared and analyzed.

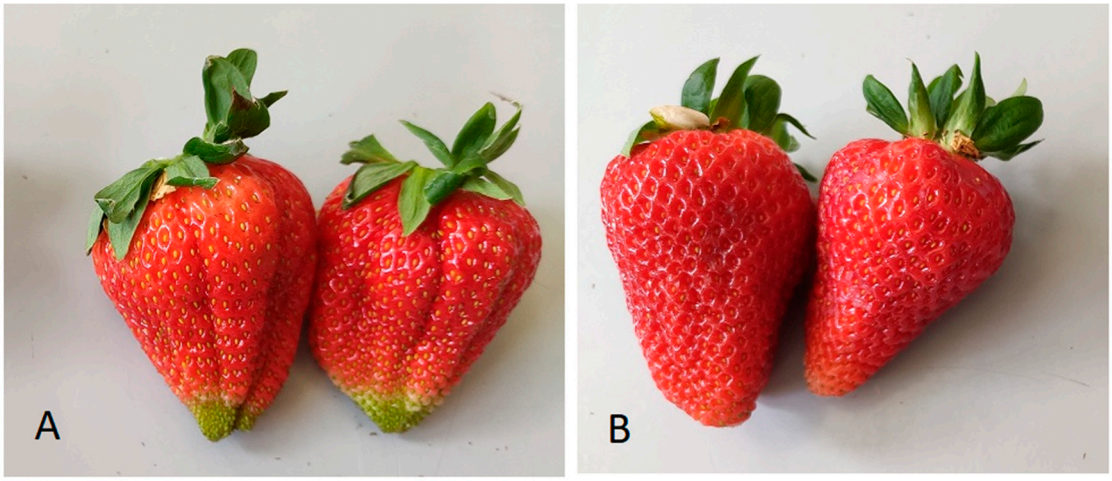

Figure 1. Strawberry fruit (*Fragaria x ananassa* Duch. cv. 'Albion') harvested at the stage of technological ripeness (**A**) and at the stage of full ripeness (**B**).

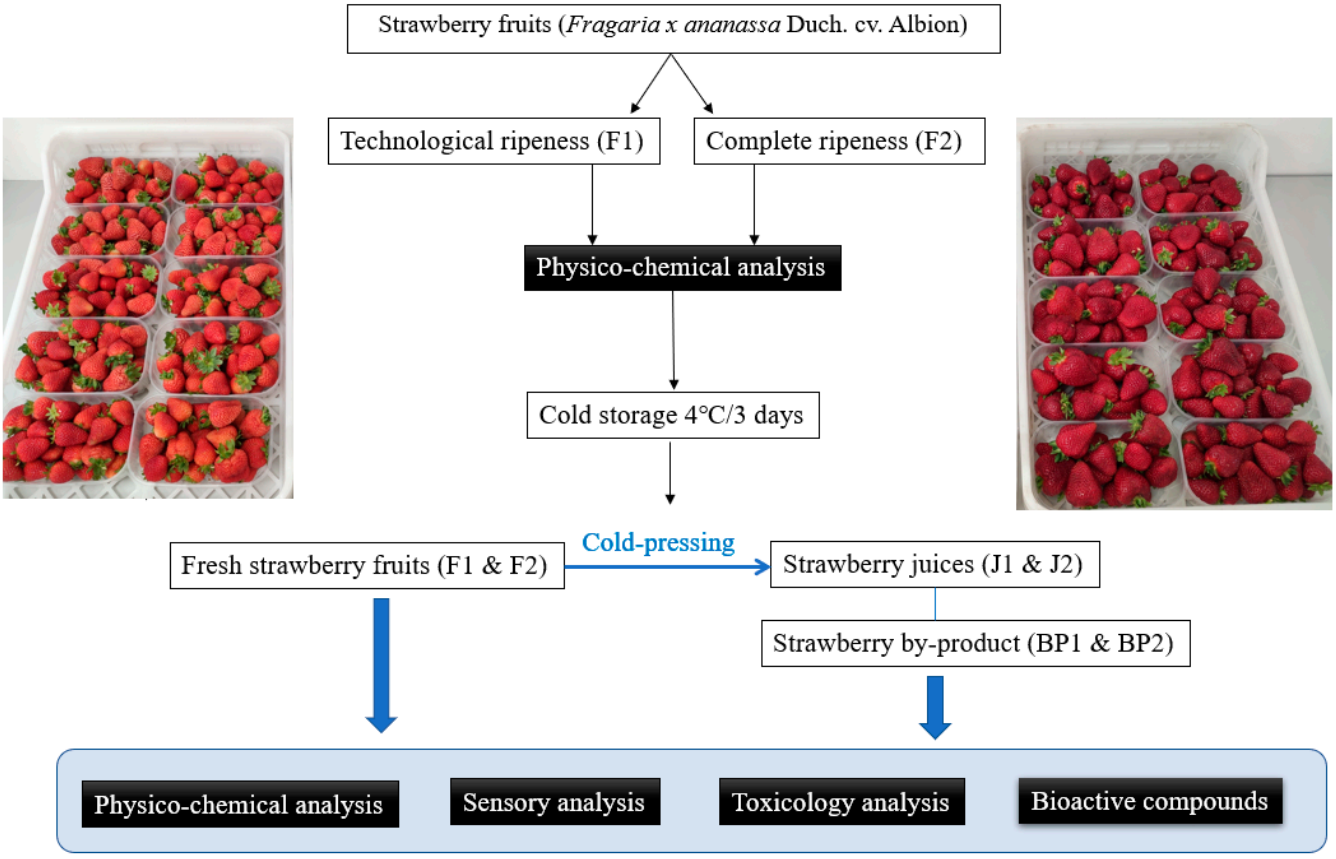

Figure 2. Schematics of the experiment.

2.3. Methods

2.3.1. Juice Production Yield

Strawberry juices (J1 and J2) and their by-products (BP1 and BP2) were prepared by cold pressing the corresponding fresh fruits (F1 and F2). The fresh strawberries (F1 and F2) were weighed before and after removing the calyces, and the removed calyces were weighed separately. The juices obtained (J1 and J2) and their by-products (BP1 and BP2)

were also weighed. The juice yield (%) was calculated based on the total weight of the fresh fruits with (Equation (1)) and without calyces (Equation (2)).

$$\text{Juice yield, \%} = \frac{\text{weight of fruits with calyces (g)}}{\text{weight of juice (g)}} \times 100 \quad (1)$$

$$\text{Juice yield, \%} = \frac{\text{weight of fruits with calyces (g)} - \text{weight of calyces(g)}}{\text{weight of juice (g)}} \times 100 \quad (2)$$

2.3.2. Physicochemical Analysis

Physicochemical analysis for the fresh fruits (F1 and F2) includes the determination of the fruit weight (g), hardness (kg cm^{-2}), colorimetric evaluation (CIEL*a*b*), pH, soluble solids content (Brix%) and total acidity (%). In the juice (J1 and J2) and by-product (BP1 and BP2) samples, colorimetric evaluation (CIEL*a*b*), pH, and soluble solids content (Brix%) were determined.

To determine the fruit weight, color, and firmness at each ripening stage, 10 individual fruits were evaluated. The same fruits were then pureed, homogenized, and filtrated before being used to determine the total soluble solids (TSS), titratable acidity (TA), and pH. The prepared juices (J1 and J2) were also analyzed for color, TSS and pH. The weight of the fruits was determined using an OHAUS AX2202 Adventurer® Precision analytical balance (Ohaus Corporation, Parsippany, NJ, USA).

The color of the fruits and juices was measured using a ColorTec-PCM colorimeter (ColorTec Associates, Clinton, NJ, USA) and expressed as CIEL*a*b* values. Color is defined in three-dimensions using the notation L, a, b. The L* axis represents the lightness of the color (the lower the value, the darker the color). The a* axis represents the balance between red (positive values) and green (negative values) and the b* axis, the balance between yellow (positive values) and blue (negative values). These coordinates allow access to new indices, color change (ΔE*), (Equation (3), chroma (C*), (Equation (4), and hue (H*), (Equation (5)) calculated from:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (3)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

$$H^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (5)$$

Firmness was measured using a PCE-FM 200 penetrometer (PCE Instruments, Germany) with a 6 mm probe. The firmness value for each fruit was the average of two measurements taken on opposite sides of the fruit in the equatorial fruit zone and was expressed in kg cm^{-2}.

The pulp of each fruit and its juices were used to determine TSS (Brix%) using a digital refractometer (ATAGO Pal-3, ATAGO Co., Tokyo, Japan), while the pH of both samples was determined using a Testo 205 manual pH meter (Testo AG, Lenzkirch, Germany) [32]. The pH meter was calibrated with commercial buffer solutions at pH 7.0 and 4.0.

The titratable acidity was measured by titration with 0.1 M NaOH using phenolphthalein as indicator and expressed as percentage of citric acid [32].

2.3.3. Toxicology Analysis

All methods used in this paper for the determination of the toxicological parameters were methods accredited in the flexible scope of accreditation in the Andrija Štampar Teaching Institute of Public Health, Department for Environmental Protection and Health Ecology (accredited since 2003), the National Reference Laboratory for Pesticides in Foods of Plant Origin, for Pesticides in Fruits and Vegetables, Cereals, and the Testing of Pesticides by Individual Methods. For metals, the internal method was used, and for pesticides, the standard method

for evaluating foods of plant origin was used. The determination of pesticide residues was carried out using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and cleanup using the dispersive SPE–QuEChERS EN 15662-2018 method.

Chemicals

A certified reference material (pesticide mixture) for GC-MS/MS and LC-MS/MS analyses (purity above 99%) was purchased from LabStandards (Budapest, Hungary). The concentrations of each pesticide in CRM are 100 μg mL^{-1}. A stock solution was prepared by diluting the CRM 100-fold in acetonitrile to obtain a concentration solution of 1 μg mL^{-1} in 10 mL of acetonitrile. The solution was prepared, and the original mixture of pesticides was stored at a temperature of −20 °C (±2 °C). The stock solution was used to check the recovery of the sample preparation process by spiking samples before extraction. Working solutions of the standard were prepared by the appropriate dilution in a matrix, i.e., acetonitrile extract of strawberry sample. Acetonitrile is 99% pure (pesticide residue grade) and is manufactured by J.T. Baker. QuEChERS salts for extraction and purification of the samples were obtained from Restek Corporation (Bellefonte, PA, USA). QuEChERS salt mixture for sample extraction contains 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, and 0.5 g disodium citrate sesquihydrate. QuEChERS salt mixture for extract purification consists of 25 mg primary secondary amine (PSA), 45 mg graphitized carbon black (GCB), and 900 mg magnesium sulfate ($MgSO_4$).

High-purity concentrated HNO_3 (65% *w*/*w*, Scharlau, Turkey) and certified 30% H_2O_2 (Alkaloids, Skopje, North Macedonia) were used for metal analyses and sample digestion. Standard solutions for calibration were prepared by diluting a stock solution of 100 mg/L (Be, V, Co, Ni, Cu, As, Se, Sr, Mo, Cd, Sb, Ba, Pb, B, Al, Cr, Mn, Fe, Zn, Rb, Sn) from CPAchem.

Sample Preparation

The preparation of samples for the quantitative determination of pesticides is done by extracting the pesticides using the QuEChERS technique (Quick, Easy, Cheap, Effective, Rugged, Safe). Of the homogenized strawberry sample, 10 g was weighed into a polypropylene cuvette with a screw cap. Of the acetonitrile, 10 mL was added to the weighed sample and the cuvette was vortexed vigorously for 1 min. A mixture of QuEChERS salt (4 g MgSO4, 1 g NaCl, 1 g Na-citrate, 0.5 g disodium citrate sesquihydrate) was then added to the cuvette and the cuvette was again vortexed vigorously for 1 min to obtain a crude extract. The extracts obtained must be purified on GC-MS/MS and UPLC-MS/MS before analysis to reduce the concentration of co-extracts.

For pesticide analysis by gas chromatography, the extract was purified by transferring 6 mL of the extract to a dSPE column containing a salt mixture of 25 mg primary secondary amine (PSA), 45 mg graphitized carbon black (GCB), and 900 mg magnesium sulfate (MgSO4).

Afterwards, 6 mL of the extract was transferred to a dSPE purification cuvette; the dSPE cuvette was vortexed vigorously for 1 min and then centrifuged at 3000 rpm for 5 min.

For pesticide analysis by liquid chromatography, 100 μL of the crude extract was diluted with 900 μL of ultra-pure water.

For metal analyses, all samples were homogenized and 0.5 g of a single sample was weighed. Then, 3 mL of HNO_3 and 1 mL of H_2O_2 were added to each sample. UltraWAVE ECR from Milestone was used for microwave digestion. After digestion, the samples were diluted to 20 mL with deionized water.

GC-MS/MS and LC-MS/MS Analysis and ICP/MS Analysis

The pesticide content in the prepared sample was determined using gas and liquid chromatography, coupled with mass spectrometry, using the standard method (HRN EN 15662:2018) and monitoring selected reactions (MRM-multi-reaction monitoring). The gas chromatograph used was the Shimadzu GC-MS-TQ8050 NX coupled with mass spectrometry and the AOC 6000 autosampler. (Shimadzu, Kyoto, Japan). The analytes were

chromatographically separated on a capillary column Rxi-5Sil MS manufactured by Restek (30 m × 0.25 mm inner diameter × 0.25 µm film thickness). Pesticide analysis by liquid chromatography was performed using the UPLC-MS/MS Waters Xevo TQ MS equipped with a thermostatted autosampler, column heater, MS degasser pump (Waters Xevo TQMS), nitrogen generator, and a computer data processing system. The analytes were chromatographically separated on an ACQUITY UPLC BEH C18 150 × 3.0 mm column; 1.7 µm (Waters corporation, Milford, MA, USA).

Identification and quantification were performed using a matrix-matched calibration in which a standard solution was prepared in a strawberry matrix (corresponding to the limit of quantification 0.01 mg kg^{-1}). A strawberry sample that was found to contain pesticides below the limit of detection for recovery tests and for the matrix-matched standard was used. The homogenized blank sample was spiked to a concentration of 0.01 mg kg^{-1} by standard addition prior to the determination procedure. The recovery values must meet the critical limit of 80–120%. For analytes that have been shown to have satisfactory precision (RSD < 20%), a lower recovery may be accepted in accordance with SANTE/12682/2019. The matrix-match standard was analyzed for every 10 samples; the concentrations obtained must not differ by ±20%.

Pesticides were identified based on retention time, a target ion, and two qualifier ions (tolerance ± 0.1 min for retention time). The ratio of the selected ion transitions in the sample must correspond to the ratio of the same ion transitions in the MM standards, with a tolerance of ± 30%. The maximum reporting limit (MRLs) of pesticides in strawberries were checked on the day the results were issued in Regulation 396/2005, https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr (accessed on 25 October 2021).

Inductively coupled plasma with mass spectrometry (Agilent 7900 ICP-MS), and high-purity argon and helium (≥99.99%) were used for metal analyses. The ICP-MS measurements were performed using the Micro Mist Nebulizer, with the Rf power, plasma, nebulizer, and auxiliary gas set to 1180 W, 15.0 L/min, 1.07 L/min, and 0.90 L min^{-1}, respectively. Before the samples were measured, the instrument was calibrated. The reagent blank solution contained 1% HNO_3. Mixed standard solutions were prepared in reagent blank solutions. Linearity was tested by injecting seven concentrations of the working standard. Each concentration was injected three times and the regression line and correlation coefficient were determined. A correlation coefficient of ≥0.99 was obtained for each element. The matrix effect was compensated for by adding internal standards (mixture 100 µg L^{-1} Bi, Ge, In, Li6, Sc, Tb, Y from Agilent) to the solutions.

The precision parameter was determined by preparing a sample (n = 6) multiple times before measuring each sample and giving it an RSD of 4.5%. The limits of quantification ranged from 0.01–0.25 mg kg^{-1}.

2.3.4. Sensory Evaluation

Sensory analysis of the fresh strawberries (F1 and F2) and juices (J1 and J2) was performed using a Quantitative Descriptive Analysis (QDA), with a total of 13 sensory descriptors evaluated [19,33]. The descriptive terms are listed in Table 1. A team of 16 professional panelists from the Faculty of Agriculture and the Faculty of Food Technology and Biotechnology at the University of Zagreb, aged 20–52, was selected based on their sensory acuity, sensitivity, and ability to distinguish small differences in the intensity of a sensory attribute. Panelists scored the samples for each characteristic in the vocabulary, using an appropriate line intensity scale, with scores assigned on a scale of 0–7 to indicate the relative intensity of each attribute, with 0 indicating the complete absence ('none') of the sensory attribute and 7 indicating a very distinct attribute ('intense'). Fresh fruit samples consisting of four fresh fruits with sepals removed were served on white porcelain plates, while juice samples (30 mL) were served in two identical cups. The panelists cleansed their mouths with salt-free bread and water between each sample.

Table 1. Sensory attributes used for sensory evaluation.

Sensory Attribute	Descriptive Term
Color	Color intensity
Flavor	Flavor intensity Floral flavor Fruity flavor Green flavor Off-flavor
Taste	Taste intensity Acid taste Sweet taste Harmonious Off-taste
Texture	Firmness/homogeneity
Overall sensory quality	Overall sensory quality

2.3.5. Determination of Bioactive Compounds (BACs)

All absorbance measurements were conducted with an UV/Vis spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch & Holm, Meckenheim, Germany). For each sample, duplicate measurements were performed.

Extraction Procedure

The extraction of bioactive compounds from fresh strawberry fruits (F1 and F2), strawberry juices (J1 and J2), and strawberry by-products (BP1 and BP2) was performed according to modified protocols from the literature [34]. Briefly, 5 g of the sample were mixed with 20 mL of 1% formic acid in 80% methanol (v/v). The mixture was vortexed for 1 min and extracted for 15 min at 50 °C in an ultrasonic bath (DT 514 H SONOREX DIGITEC 13.5 L, 860 W, 40 kHz, Bandelin electronic, Germany). The mixture was then centrifuged at 10,000 rpm/10 min (Thermo Scientific™, Megafuge™ 16R, Kalkberg, Germany) and the supernatant was filtered through Whatman filter paper No. 40 (Whatman International Ltd., Kent, UK), and increased to 25 mL in volumetric flask using an extraction solvent. All extracts were prepared in duplicate. Prior to analysis, the extracts were stored at −18 °C in an inert gas atmosphere.

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using a modified spectrophotometric method described in the literature [35]. Briefly, 400 µL of the properly diluted extract was mixed with 400 µL of FC reagent (previously diluted 5 times with distilled water) and 4 mL of 7.5% sodium carbonate solution (w/v). The reaction mixture was allowed to stand at room temperature for 20 min and the absorbance was measured at 725 nm using a spectrophotometer. A calibration curve was prepared using a standard solution of gallic acid (10–250 mg L^{-1}) and the results were expressed as mg gallic acid equivalent (GAE) per 100 g or 100 mL of the sample.

Determination of Total Hydroxycinnamic Acids (HCA) and Total Flavonols (TF)

HCA and FL were determined using a modified spectrophotometric assay [36]. Briefly, 250 µL of extract and 250 µL of solution were stirred in a vortex for 10 s and then allowed to react in the dark at room temperature for 30 min. The absorbance of the solution was then measured at 320 nm for HCA and 360 nm for FL in a spectrophotometer. A blank was prepared in the same way, but an extraction solvent was used instead of the extract.

For quantification of HCA, a standard solution of chlorogenic acid (10–600 mg L^{-1}) was used to prepare the calibration curve and the results were expressed as mg chlorogenic

acid equivalent (CAE) per 100 g or 100 mL of the sample. For the quantification of FL, a standard solution of quercetin (10–600 mg L^{-1}) was used to construct the calibration curve and the results were expressed as mg quercetin equivalent (QE) per 100 g or 100 mL of the sample.

Determination of Monomeric Anthocyanins (MA)

The determination of MA was performed using a spectrophotometric pH differential method [37]. Briefly, 1 mL of the extract was mixed with 4 mL of 0.025 M potassium chloride buffer (pH 1.0) and also separately with 4 mL of 0.4 M sodium acetate buffer (pH 4.5). The reaction mixture was allowed to stand at room temperature for 20 min and absorbance was measured using a spectrophotometer at 520 nm and 700 nm, using deionized water as a blank. The concentration of monomeric anthocyanins in the sample was calculated as equivalent of pelargonidin-3-glucoside (Pg-3-G) (mg L^{-1}) according to Formula (6):

$$\frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (6)$$

where: $A = (A_{520nm} - A_{700nm})_{pH=1.0} - (A_{520nm} - A_{700nm})_{pH=4.5}$; MW = molecular weight (for pelargonidine-3-glucoside $C_{21}H_{21}ClO_{10}$ = 468.8 g mol^{-1}); DF = dilution factor; 10^3 = factor for conversion g to mg; ε = molar absorption extinction coefficient (for pelargonidine-3-glucoside 22,400 L mol^{-1} cm^{-1}); l = cuvette thickness (1 cm).

2.3.6. Statistical Analysis

Descriptive statistics were used for the characterization of the sample. Discrete variables and factor scores were tested using MANOVA. Ward's method of exploratory hierarchical cluster analysis was used for measuring standardized similarities in the samples. The level of significance for all tests was $\alpha \leq 0.05$, and results were analyzed using SPSS software (v.22).

3. Results and Discussion

3.1. Physiochemical Assessment of Strawberry Fruits

The results for changes in the physiochemical parameters in strawberry cv. 'Albion' at different levels of maturity and storage are shown in Table 2. As can be seen, the average mass of sampled strawberries was 51.79 ± 1.07 g. As expected, the fruit weight with the calyx was almost 40% higher in strawberries at full maturity, while this mass decreased by 5% from the baseline at 4 days of storage. The different degree of ripeness did not affect this parameter during storage. The average calyx mass was 0.80 ± 0.03 g. Here, the weight of the calyces of the fully ripe strawberries was 37% higher than that for fruits with a lower degree of ripeness, while their mass decreased by 25% during storage. The differences in the ripeness of the fruits did not affect the loss of calyx mass during storage. Next, the mass of strawberry fruits without calyces was 23% higher in the fully ripe samples than in their less ripe counterparts, while it remained constant during 4 days of storage. Their average mass in the data set was 50.99 ± 1.05 g.

The maximum acceptable weigh loss for strawberries stored at 20 °C and 85–95% relative humidity was 2.5–3% within 2.5–3 days, resulting in a softening of the flesh, darkening of the color, and drying of the calyx and skin [17]. In a study by Kelly et al. [38], weight loss of strawberries during 9 days of storage at 1.5 °C was also found to be in the range of 2.97–5.97%. The reduction in fruit weight during storage is caused by the loss of moisture from the fruit, which may have a negative effect on their appearance and processing costs. The morphological characteristics of the fruit, its size, and initial moisture content, as well as the integrity of the skin, affect the rate of moisture loss from the fruit [38].

The average firmness of the samples was 0.40 ± 0.14 kg cm^{-2}. Samples that were less ripe had 35% higher firmness than fully ripe samples, while total hardness increased by 20% during storage. Moreover, there was a significant decrease in hardness during

storage of strawberries, making them softer and more susceptible to spoilage, which is in agreement with other literature data [22,39,40].

The average TSS in the samples was 8.98 ± 0.14° Brix, which is slightly higher than the values obtained by Ornelas-Paz [39] for the same cultivar, where higher TSS content was found in strawberries at a higher maturity stage compared to fruits with lower maturity. About a 5% higher level of soluble solids content was found in fully ripe fruits, while it decreased by nearly 7% during storage. Other researchers also confirmed that TSS increases with fruit ripeness [22,39,40]. Similarly, TSS in strawberry fruit decreases during storage of 9 days at 1.5 °C. However, the relationship between the ripeness levels of strawberries and the days of storage was not statistically confirmed [38].

Fully ripe strawberries had a slightly higher pH, which tended to decrease during storage. This parameter averaged 3.25 ± 0.01 in the samples. Other researchers also confirmed that pH increased with fruit ripeness [22,39]. Olsson et al. noted a decrease in pH in ripe strawberries after 3 days of storage at +4 °C. A possible explanation for this trend could be that the low temperature contributes to the stabilization of pH during storage [41]. On the other hand, the total acidity was about 21% lower in more mature samples, which essentially means that the total acidity decreased by 1% for almost every percent of ripeness. In contrast to SSC, titratable acidity tended to decrease during maturation [22,39]. Storage had a significant effect on total acidity, which tended to decrease by 12% during 4 days of storage, which is in agreement with the results of Kelly et al. [38]. As for the other parameters, their pH and TA were not altered by the simultaneous influence of ripening and storage.

Table 2. The changes of the physiochemical parameters in strawberry cv. 'Albion' with different ripeness levels and storage.

Strawberry Fruit Parameters	M1	M2	M3	H_{avg}	TSS	pH	TA
Maturity	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.09$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †
F1	43.34 ± 1.52 ^{b}	0.68 ± 0.04 ^{b}	42.67 ± 1.49 ^{b}	0.48 ± 0.02 ^{a}	8.75 ± 0.19 ^{a}	3.25 ± 0.01 ^{b}	1.10 ± 0.01 ^{a}
F2	60.23 ± 1.52 ^{a}	0.93 ± 0.04 ^{a}	59.30 ± 1.49 ^{a}	0.31 ± 0.02 ^{b}	9.22 ± 0.19 ^{a}	3.38 ± 0.01 ^{a}	0.87 ± 0.01 ^{b}
Storage	$p = 0.24$ ‡	$p \leq 0.01$ †	$p = 0.27$ ‡	$p = 0.02$ †	$p = 0.02$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
0 days	53.08 ± 1.52 ^{a}	0.92 ± 0.04 ^{a}	52.17 ± 1.49 ^{a}	0.43 ± 0.02 ^{a}	9.32 ± 0.19 ^{a}	3.38 ± 0.01 ^{a}	1.05 ± 0.01 ^{a}
4 days	50.50 ± 1.52 ^{a}	0.69 ± 0.04 ^{b}	49.80 ± 1.49 ^{a}	0.36 ± 0.02 ^{b}	8.65 ± 0.19 ^{b}	3.26 ± 0.01 ^{b}	0.92 ± 0.01 ^{b}
Maturity by Storage	$p = 0.79$ ‡	$p = 0.67$ ‡	$p = 0.79$ ‡	$p = 0.17$ ‡	$p = 0.34$ ‡	$p = 0.10$ ‡	$p = 0.70$ ‡
F1; 0 days	44.35 ± 2.14 ^{a}	0.78 ± 0.06 ^{a}	43.57 ± 2.1 ^{a}	0.43 ± 0.03 ^{a}	8.95 ± 0.27 ^{a}	3.30 ± 0.01 ^{a}	1.16 ± 0.01 ^{a}
F1; 4 days	42.34 ± 2.14 ^{a}	0.58 ± 0.06 ^{a}	41.77 ± 2.1 ^{a}	0.54 ± 0.03 ^{a}	8.54 ± 0.27 ^{a}	3.21 ± 0.01 ^{a}	1.04 ± 0.01 ^{a}
Maturity by Storage	$p = 0.79$ ‡	$p = 0.67$ ‡	$p = 0.79$ ‡	$p = 0.17$ ‡	$p = 0.34$ ‡	$p = 0.10$ ‡	$p = 0.70$ ‡
F2; 0 days	61.82 ± 2.14 ^{a}	1.06 ± 0.06 ^{a}	60.76 ± 2.1 ^{a}	0.30 ± 0.03 ^{a}	9.69 ± 0.27 ^{a}	3.45 ± 0.01 ^{a}	0.93 ± 0.01 ^{a}
F2; 4 days	58.65 ± 2.14 ^{a}	0.81 ± 0.06 ^{a}	57.84 ± 2.1 ^{a}	0.32 ± 0.03 ^{a}	8.75 ± 0.27 ^{a}	3.31 ± 0.01 ^{a}	0.81 ± 0.01 ^{a}
Average in samples	51.79 ± 1.07	0.80 ± 0.03	50.99 ± 1.05	0.40 ± 0.14	8.98 ± 0.14	3.25 ± 0.01	0.98 ± 0.01

F1—fresh fruits with 75% maturity; F2—fresh fruits with 100% maturity. Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. M1—Fruit weight with calyx (g); M2—Calyx weight (g); M3—Fruit weight without calyx (g); H_{avg}—Average value for hardness measured from frontal and rear part of the fruit (kg/cm^{-2}); TSS—Total Soluble Solids (% Brix). TA—total acidity (%); For M1–M4; H_{avg} and SSC $n = 40$ for pH, and TA $n = 12$.

In conclusion, the obtained results show that the storage of fresh strawberries intended for processing is significantly affected by the degree of ripeness and storage time; therefore, these parameters must be carefully considered in order to produce a functional strawberry juice of the highest quality.

3.2. Colorimetric Assessment of Strawberry Fruits

The color of fresh strawberries, as well as strawberry products, is one of the most important quality indicators that consumers percieve first [25]. Therefore, in this work, the color parameters of fresh strawberries were monitored on the day of harvest and during a 4-day storage at 4 °C (Table 3). On average, all samples were in the darker, more reddish–yellow range of the CIELAB light spectrum. At the same time, the samples with a lower degree of ripeness were 14% brighter than their fully ripe counterparts. They also had a 13% higher a* value and an 18% higher b* value, while chroma was 17% higher in these samples. There was no difference in hue between the fully ripe and 75% ripe fruits.

Table 3. Changes in the CIELAB parameters in strawberry cv. 'Albion' at different stages of ripeness and storage.

Strawberry Fruit Parameters	*n*	L*	a*	b*	C*	H*
Maturity		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.55$ ‡
F1	40	33.83 ± 0.49 ^{a}	19.15 ± 0.39 ^{a}	22.98 ± 0.83 ^{a}	30.15 ± 0.67 ^{a}	49.43 ± 1.35 ^{a}
F2	40	29.68 ± 0.49 ^{b}	16.55 ± 0.39 ^{b}	19.33 ± 0.83 ^{b}	25.79 ± 0.67 ^{b}	48.29 ± 1.35 ^{a}
Storage		$p \leq 0.01$ †	$p = 0.91$ ‡	$p = 0.72$ ‡	$p = 0.81$ ‡	$p = 0.42$ ‡
0 days	40	33.18 ± 0.49 ^{a}	17.82 ± 0.39 ^{a}	21.36 ± 0.83 ^{a}	28.09 ± 0.67 ^{a}	49.64 ± 1.35 ^{a}
4 days	40	30.33 ± 0.49 ^{b}	17.88 ± 0.39 ^{a}	20.94 ± 0.83 ^{a}	27.86 ± 0.67 ^{a}	48.08 ± 1.35 ^{a}
Maturity by Storage		$p = 0.17$ ‡	$p = 0.89$ ‡	$p = 0.04$†	$p = 0.05$†	$p = 0.64$ ‡
F1; 0 days	20	34.51 ± 0.69 ^{a}	19.08 ± 0.55 ^{a}	21.18 ± 1.17 ^{a}	28.75 ± 0.95 ^{b}	47.23 ± 1.91 ^{a}
F1; 4 days	20	33.15 ± 0.69 ^{a}	19.22 ± 0.55 ^{a}	24.78 ± 1.17 ^{b}	31.55 ± 0.95 ^{a}	51.63 ± 1.91 ^{a}
Maturity by Storage		$p \leq 0.01$ †	$p = 0.89$ ‡	$p \leq 0.01$ †	$p = 0.02$ †	$p = 0.02$ †
F2; 0 days	20	31.84 ± 0.70 ^{a}	16.56 ± 0.55 ^{a}	21.55 ± 1.17 ^{a}	27.43 ± 0.95 ^{a}	52.05 ± 1.91 ^{a}
F2; 4 days	20	27.51 ± 0.70 ^{b}	16.54 ± 0.55 ^{a}	17.10 ± 1.17 ^{b}	24.16 ± 0.95 ^{b}	44.53 ± 1.91 ^{b}
Average	80	31.75 ± 0.35	17.85 ± 0.27	21.15 ± 0.58	27.97 ± 0.48	48.86 ± 0.96

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. L*—CIELAB lightness; a*—CIELAB green–red parameter; b*—CIELAB blue–yellow parameter; C*—CIELAB chroma; H*—CIELAB hue.

During storage, the fruits became darker, while other CIELAB parameters remained the same. Changes in strawberry fruit color are most commonly observed during fruit ripening, while the results of studies comparing strawberry fruit color before and after storage for different ripening stages have not been found. However, a possible explanation for this observed trend could be the decomposition of hexoses during storage, which is more pronounced in fruits with higher ripeness (higher SSC) due to the Maillard reaction, which may consequently manifest itself in the darkening of the color of the strawberry [42].

Interestingly, the color changes seem to be more pronounced in the already fully ripe fruits because in 75% of the ripe strawberries, L*, a*, and H* did not change during storage, while a change was only observed in parameter b*, shifting the less ripe fruits towards the yellowish part of the spectrum. On the other hand, the fully ripe fruits showed changes in all CIELAB variables except a*. Here, the fully ripe fruits became 13% darker and moved away from the yellowish part of the spectrum (21%), while hue and chroma decreased by 12% and 14%, respectively.

Finally, it is interesting to note that the color change (ΔE) is greater in fully ripe strawberries (6.07) than in strawberries with a lower degree of ripeness (3.85), implying that less ripe strawberries, if they meet other quality parameters, would potentially be more interesting for processing [22,40].

3.3. Physiochemical and Color Assessment of Strawberry Juices

Stored strawberries of both ripening stages were processed into juice and the yield and the physicochemical analysis of all samples were determined. Because the goal was to produce functional strawberry juices, cold pressing was chosen as the technology for juice production, as this technology produces thick and pulpy juices with no temperature rise during the production process. Considering the yield of the process in terms of juice production, the results showed that strawberries of both ripening stages had a similar yield (68.47% at 75% ripeness vs. 69.87% at 100% ripeness) (Table 4). These results confirm that strawberries from both maturity levels are suitable for processing.

Table 4. Yield in the production of strawberry juice.

Parameter	Ripeness 75%	Ripeness 100%
Strawberry mass with calyces (g)	1004.73	1011.72
Strawberry mass without calyces (g)	989.73	998.37
Calyx weight (g)	14.74	13.12
Juice weight (g)	677.68	697.61
Pomace weight (g)	304.04	316.03
Proportion of calyces in relation to whole strawberries (%)	1.47	1.30
Proportion of juice in relation to strawberries with calyces (%)	67.45	68.95
Proportion of stalks in relation to strawberries without calyces (%)	68.47	69.87
Proportion of pomace in relation to strawberries with calyces (%)	30.26	31.24
Proportion of pomace in relation to strawberries without calyces (%)	30.72	31.66

Previous studies have confirmed that the yield of strawberries processed into juice is highly dependent on the cultivar, with the authors obtaining juice yields ranging from 48.22% to 89.98% for 15 different strawberry cultivars [43].

Without the influence of ripeness, the average SSC and pH of the juice samples were 8.38 ± 0.06 and 3.31 ± 0.01, respectively. Juices from less ripe fruits (75%) had TSS and pH levels of 7.85 ± 0.08 and 3.22 ± 0.02, respectively, while for fully ripe fruits, both parameters were significantly higher and were 8.90 ± 0.08 and 3.39 ± 0.02, respectively. However, comparing these values for juice with the results for fresh fruits of the same degree of ripeness, it is observed that during processing into juice, both the TSS and pH of the juice decreased.

3.4. Toxicology Analysis

3.4.1. Heavy Metals

Analysis of heavy metals (Cu, Zn, Ni, As, Cd, Pb) in fresh strawberry fruit, juice, and by-product samples showed that the concentrations for Ni and Cd were below the detection limits. The ICP-MS method (Table 5) detected the following metals Cu (0.077–0.415 mg kg^{-1}), Zn (0.988–3.12 mg kg^{-1}), As (<0.02–0.04 mg kg^{-1}) and Pb (<0.03–0.076 mg kg^{-1}), which is in agreement with the results of a group of authors from China who found lead, cadmium, and nickel in most strawberry samples, with detection rates of 75.76, 92.93, and 92.93%, respectively [44]. In a paper from Poland, a group of authors found that the average content of heavy metals in strawberry fruits grown in the Lublin region was 0.023 mg Pb, 0.020 mg Cd, 0.091 mg Ni, 1.228 mg Zn, 0.358 mg Cu,

0.0015 mg As, and 0.00011 mg Hg per kg of fresh weight indicating that the threshold for products of this type was not exceeded [45].

Table 5. The concentration of heavy metals in the fresh strawberry, strawberry juice, and by-product samples.

Sample	Cu *m*/*z* 63	Zn *m*/*z* 67	Ni *m*/*z* 60	As *m*/*z* 75	Cd *m*/*z* 111	Pb *m*/*z* 208
F1	0.159 ± 0.006	1.20 ± 0.015	<0.04 ± 0.000	0.022 ± 0.009	<0.01 ± 0.000	<0.03 ± 0.000
F2	0.144 ± 0.008	1.24 ± 0.026	<0.04 ± 0.000	0.026 ± 0.008	<0.01 ± 0.000	<0.03 ± 0.000
J1	0.077 ± 0.003	1.03 ± 0.049	<0.04 ± 0.000	<0.02 ± 0.000	<0.01 ± 0.000	<0.03 ± 0.000
J2	0.132 ± 0.000	0.988 ± 0.033	<0.04 ± 0.000	0.038 ± 0.007	<0.01 ± 0.000	0.035 ± 0.002
BP1	0.412 ± 0.011	3.12 ± 0.091	<0.04 ± 0.000	0.040 ± 0.007	<0.01 ± 0.000	<0.03 ± 0.000
BP2	0.371 ± 0.003	2.40 ± 0.018	<0.04 ± 0.000	<0.02 ± 0.000	<0.01 ± 0.000	0.076 ± 0.005

F—fresh strawberry fruit; J—strawberry juice; BP—strawberry by-product; 1—ripeness 75%; 2—ripeness 100%. Results are expressed as the mean concentration ± standard deviation in mg kg^{-1} (n = 3).

Among 250 samples of fruit and vegetable products from the Libyan market, the highest Pb concentrations were found in mangoes, followed by strawberries (0.53 ± 0.2 mg kg^{-1}). Moreover, the authors detected several heavy metals in the strawberry samples: Cd 0.01 ± 0.02 mg kg^{-1}, Ni 1.818 ± 0.103 mg kg^{-1}, Zn 1.32 ± 3.12 mg kg^{-1}, Cu 3.14 ± 0.58 mg kg^{-1}, and Co 0.272 ± 0.58 mg kg^{-1} [46].

In 2021, new, stricter MRLs for Pb and Cd in food came into force in the EU, amending Commission Regulation (EC) No 1881/2006 of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs [47], with Commission Regulation (EU) 2021/1317 of 9 August 2021 amending Regulation (EC) No 1881/2006 [48] regarding maximum levels for lead in certain foodstuffs, and Commission Regulation (EU) 2021/1323 of 10 August 2021 amending Regulation (EC) No 1881/2006 regarding maximum levels for cadmium in certain foodstuffs. [49].

In samples of fresh strawberries fruits, Pb was not found, but in sample J2 strawberry juice from fruits of 100% ripeness, the level for Pb exceeded the permissible MDK for juice. The concentration in sample BP2 from fruits of 100% ripeness was even higher than in J2, but below the MDK for strawberries fruits. On 18 March 2010, the European Food Safety Authority ('the Authority') adopted an opinion on lead in food [50]. The Authority found that lead may cause developmental neurotoxicity in young children and cardiovascular problems and nephrotoxicity in adults. The risk assessment for lead was based on these potentially critical adverse effects.

3.4.2. Pesticides

The strawberry is a perishable fruit that is easily attacked by fungi after harvest, so it is often treated with fungicides, cyprodinil, pyrimethanil, and fludioxonil being the most commonly used [51–53]. Pyrimethanil and cyprodinil belong to the class of anilinopyrimidine fungicides that prevent protein formation and cell division in fungal pathogens (such as gray mold, powdery mildew, scab, downy mildew, and Phomopsis leaf spot on a variety of crops, including apples, oranges, strawberries, root crops, and tubers) by inhibiting methionine biosynthesis [54,55]. Botrytis fruit rot, caused by *Botrytis cinerea*, is one of the most threatening strawberry diseases worldwide. To control the disease, fungicides containing pyrimethanil or cyprodinil as active ingredients are commonly used in commercial strawberry production [55]. Pyrimethanil and cyprodinil have low acute toxicity to humans, but there are some toxicological concerns related to their antiandrogenic properties [54–60]. According to the PubChem open chemistry database, pyrimethanil is classified as a Group C 'possible human carcinogen,' while there is no evidence of carcinogenic potential for cyprodinil at any dose [45]. Both substances are toxic to aquatic organisms [53], and they are both toxic to aquatic life [53,61]. The results from the literature indicate that the risk of using pyrimethanil in strawberries at the recommended dosage is negligible for humans [60].

The strawberry samples were analyzed for 261 pesticides at GC-MS/MS and 305 pesticides at LC-MS/MS. The pesticides detected in the strawberry samples were cyprodinil and pyrimethanil (LC-MS/MS). No pesticides were detected using GC-MS/MS (Table 6). The results are listed with the measurement uncertainty for each result.

Table 6. Pesticides detected in the strawberry samples (mg kg^{-1}).

Pesticides/RT (min)/Recovery%	F1	F2	J1	J2	BP1	BP2
Cyprodinil/ 13.77/68	ND	ND	ND	ND	0.013 ± 0.0065	ND
Pyrimethanil/ 10.06/109	0.037 ± 0.0185	0.035 ± 0.0175	0.033 ± 0.165	0.034 ± 0.017	0.060 ± 0.030	0.053 ± 0.0265

F—fresh strawberry fruit; J—strawberry juice; BP—strawberry by-product; 1—ripeness 75%; 2—ripeness 100%. Results are expressed as the mean concentration ± measurement uncertainty in mg kg^{-1} (n = 6). ND—not determined.

The MRL for cyprodinil and pyrimethanil in strawberries given in the EU database is 5 mg kg^{-1}. Strawberries are included in the 'high acidity and water content' category [62]. Cyprodinil was detected only in the by-product BP1by LC-MS/MS. The average recovery for cyprodinil in strawberries is 68% and the RSD for sample preparation repeatability is 4.2%. Since the preparation repeatability < is 20%, this recovery can be accepted, but the results for cyprodinil in strawberries need to be corrected based on the recovery. The average recovery for pyrimethanil in the 'high acidity and water content' commodity group, which includes strawberries, is 109%. This means that the results do not need to be corrected for recovery. Overall, it can be concluded that the processed raw materials and juices were toxicologically safe.

3.5. Sensorial Comparison of Strawberry Fruits and Juices

The sensory characteristics of fresh fruit can be degraded when processed into juice, affecting consumers' sensory perception of the final products [63,64]. In this study, the fresh samples and corresponding juices were sensory evaluated using 13 sensory descriptors and the results are shown in Table 7.

The average color intensity score of the samples was 5.66/7, representing 81% of the total score. The testers were not able to identify the difference in color intensity of the juices compared to the fresh fruits, as they gave them the same score with an average of 5.70. However, they were able to distinguish different ripeness levels as they gave 38% fewer points for less ripe samples and opposed to the riper samples (4.75 vs. 6.56). On the other hand, panelists were unable to differentiate the redness of the fruit processed into juice by ripeness. Next, the taste of all samples (fruits and juices) was assessed with 72% of the total points. Similar to color, panelists gave 13% more points to samples with lower ripeness. However, as before, they were unable to consistently distinguish the flavor intensity of the fruits and juices with different levels of ripeness. On average, fruit flavor received 77% of the total score, while panelists were unable to consistently distinguish the degree of floral flavor among fruits and juices. However, they were able to identify a 22% higher intensity of fruit flavor in fully ripe samples than in samples with a ripeness score of only 75%. The average intensity of greenish flavor in the samples was 41% of the total score. For the fruits and juices, this intensity was the same, averaging 2.88 points. The testers evaluated the intensity of greenish flavor by 56% in samples with lower ripeness, but were not able to evaluate the intensity of this flavor in relation to ripeness and conversion in strawberry juices. The intensity of off-flavor and beige flavor in the samples was 19% and 20% of the maximum value, respectively, although this was not related to the type of samples, i.e., whether they were fruits or juices, and independent of the degree of ripeness and their mutual influences. The greater presence of more intense sour or green taste attributes may be attributed to a higher concentration of acid in unripe fruits, along with a lower presence

of sugars [39]. Therefore, in determining the perceived sweet and sour taste of strawberries, the ratio of sugar to acid plays an important role [39].

The average value for flavor intensity and sour taste of the samples was 75% and 57% of the maximum intensity, respectively. Ripeness was the only thing panelists could relate to the difference in flavor intensity and sourness, so they gave 18% fewer points to samples with lower ripeness. Interestingly, samples with full ripeness were rated as more acidic than their 75% ripe counterparts. However, neither flavor intensity nor acidity were related to the mutual influence of ripeness and whether or not the samples were fruits or juices. Ripeness was another parameter by which panelists could distinguish the intensity of sweet and harmonic tasting samples, but as with other samples, the ability to associate different levels of ripeness with fruits and juices was not evident. Here, lower ripeness samples had 27% lower intensity for sweetness and 24% for harmony than the fully ripe samples. Overall, the intensity for these two parameters was 64% and 70% of the total intensity, respectively. Thus, an increase in sugar content and a simultaneous decrease in organic acids should lead to an increased perception of sweetness in ripe strawberries [39,65].

In addition, texture (as firmness for fresh samples and as homogeneity for juice samples) was rated as 79% of the total score for all samples, and this rating was similar for all samples of fruits and juices, regardless of ripeness. As expected, ripeness was again the only variable that allowed testers to distinguish between samples, as they gave fully ripened samples a 20% higher score for texture, while this score did not vary for other fruits and juices, either alone or in combination with ripeness.

Finally, the overall sensory quality of all samples was rated as 75% of the total score. As with the rest of the dataset, ripeness was the only parameter associated with overall sensory quality. Here, more mature samples received almost 20% more points than the less mature samples. This parameter did not differ significantly between juices and fruits with different degrees of ripeness. In other words, juices made of 75% and 100% ripe fruits were practically indistinguishable by the consumers.

3.6. Biologically Active Compounds in Fresh Strawberries, Their Juices, and By-Products

The highest content of all bioactive compounds (BAC) was found in strawberry by-products and the lowest in fruit juices (Table 8). This is a very interesting result, as it indicates the great importance of the use of strawberry by-products in food production. By-products contained either the same or higher amounts of all the tested BACs when compared to raw fruits or juices. This was particularly evident in total flavonols (FL), which were 11 times higher in by-product samples than in juices. Total polyphenols (TPC) and especially anthocyanins (ANT) contents did not differ from that in raw fruits.

Higher ripeness was associated with lower levels of TPC, higher levels of ANT, lower levels of hydroxycinnamic acids (HCA), and no difference in FL. This relationship was maintained only for HCA in the fruit samples tested, while the other BACs remained the same in the other raw fruit samples. ANT maintained this relationship when raw strawberries were converted to juices, but with a greater discrepancy in the concentration between the lower and fully ripened samples than previously noted in the raw fruit samples. This may be supported by the fact that the phenolic components are mainly synthesized in the skin of the fruit; therefore, their content decreases with increasing fruit weight or ripeness [66]. Thus, the content of phenolic compounds is higher in some unripe fruits, such as grapes [67], kiwifruit [68], apples [69], and pomegranates [70], than in ripe fruits. Juice production requires the disruption of fruit cells to release the juice, which is usually achieved by mechanical action on the fruit [57]. Since the hardness of the fruit decreases as it ripens [22,39,40,71], it is possible to achieve better cell disruption and ultimately, better extraction, of water-soluble components such as anthocyanins. This would be a possible explanation for the results obtained.

Table 7. Results for the sensorial comparison of strawberry fresh fruits and corresponding juices.

Strawberry Fruit Parameters	*n*	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Material		$p = 0.55$ ‡	$p = 0.85$ ‡	$p = 0.32$ ‡	$p = 0.85$ ‡	$p = 0.82$ ‡	$p = 0.40$ ‡	$p = 0.06$ ‡	$p = 0.75$ ‡	$p = 0.45$ ‡	$p = 0.11$ ‡	$p = 0.36$ ‡	$p = 0.49$ ‡	$p = 0.06$ ‡
Fruit	16	5.75 ± 0.22 [a]	5.06 ± 0.24 [a]	4.63 ± 0.35 [a]	5.44 ± 0.25 [a]	2.94 ± 0.39 [a]	1.25 ± 0.16 [a]	5.63 ± 0.2 [a]	4.06 ± 0.27 [a]	4.63 ± 0.23 [a]	5.19 ± 0.24 [a]	1.25 ± 0.16 [a]	5.63 ± 0.25 [a]	5.63 ± 0.25 [a]
Juice	16	5.56 ± 0.22 [a]	5.00 ± 0.24 [a]	5.13 ± 0.35 [a]	5.38 ± 0.25 [a]	2.81 ± 0.39 [a]	1.44 ± 0.16 [a]	5.06 ± 0.2 [a]	3.94 ± 0.27 [a]	4.38 ± 0.23 [a]	4.63 ± 0.24 [a]	1.50 ± 0.16 [a]	5.38 ± 0.25 [a]	4.94 ± 0.25 [a]
Maturity		$p \leq 0.01$ †	$p = 0.05$ †	$p = 0.09$ ‡	$p \leq 0.01$ †	$p = 0.03$ †	$p = 0.78$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 1.00$ ‡	$p = 0.73$ ‡	$p = 0.01$ †
75%	16	4.75 ± 0.22 [b]	4.68 ± 0.24 [b]	4.44 ± 0.35 [a]	4.88 ± 0.25 [b]	3.50 ± 0.39 [a]	1.38 ± 0.16 [a]	4.81 ± 0.2 [b]	4.88 ± 0.27 [a]	3.69 ± 0.23 [b]	4.25 ± 0.24 [b]	1.38 ± 0.16 [a]	5.44 ± 0.25 [a]	4.81 ± 0.25 [b]
100%	16	6.56 ± 0.22 [a]	5.38 ± 0.24 [a]	5.31 ± 0.35 [a]	5.94 ± 0.25 [a]	2.25 ± 0.39 [b]	1.31 ± 0.16 [a]	5.88 ± 0.2 [a]	3.13 ± 0.27 [b]	5.31 ± 0.23 [a]	5.56 ± 0.24 [a]	1.38 ± 0.16 [a]	5.56 ± 0.25	5.75 ± 0.25 [a]
Material by Maturity		$p = 0.17$ ‡	$p = 0.85$ ‡	$p = 0.80$ ‡	$p = 0.85$ ‡	$p = 0.82$ ‡	$p = 0.78$ ‡	$p = 0.83$ ‡	$p = 0.75$ ‡	$p = 0.71$ ‡	$p = 0.36$ ‡	$p = 1.00$ ‡	$p = 0.09$ ‡	$p = 0.86$ ‡
Fruit; 75%	8	4.63 ± 0.31 [a]	4.75 ± 0.33 [a]	4.13 ± 0.5 [a]	4.88 ± 0.36 [a]	3.63 ± 0.55 [a]	1.25 ± 0.22 [a]	5.13 ± 0.28 [a]	5.00 ± 0.39 [a]	3.88 ± 0.33 [a]	4.38 ± 0.34 [a]	1.25 ± 0.22 [a]	5.88 ± 0.36 [a]	5.13 ± 0.35 [a]
Fruit; 100%	8	6.88 ± 0.31 [a]	5.38 ± 0.33 [a]	5.13 ± 0.5 [a]	6.00 ± 0.36 [a]	2.25 ± 0.55 [a]	1.25 ± 0.22 [a]	6.13 ± 0.28 [a]	3.13 ± 0.39 [a]	5.38 ± 0.33 [a]	6.00 ± 0.34 [a]	1.25 ± 0.22 [a]	5.38 ± 0.36 [a]	6.13 ± 0.35 [a]
		$p = 0.17$ ‡	$p = 0.85$ ‡	$p = 0.80$ ‡	$p = 0.85$ ‡	$p = 0.82$ ‡	$p = 0.78$ ‡	$p = 0.83$ ‡	$p = 0.75$ ‡	$p = 0.71$ ‡	$p = 0.36$ ‡	$p = 1.00$ ‡	$p = 0.09$ ‡	$p = 0.86$ ‡
Juice; 75%	8	4.88 ± 0.31 [a]	4.63 ± 0.33 [a]	4.75 ± 0.5 [a]	4.88 ± 0.36 [a]	3.38 ± 0.55 [a]	1.50 ± 0.22 [a]	4.50 ± 0.28 [a]	4.75 ± 0.39 [a]	3.50 ± 0.33 [a]	4.13 ± 0.34 [a]	1.50 ± 0.22 [a]	5.00 ± 0.36 [a]	4.50 ± 0.35 [a]
Juice; 100%	8	6.25 ± 0.31 [a]	5.38 ± 0.33 [a]	5.50 ± 0.5 [a]	5.88 ± 0.36 [a]	2.25 ± 0.55 [a]	1.38 ± 0.22 [a]	5.63 ± 0.28 [a]	3.13 ± 0.39 [a]	5.25 ± 0.33 [a]	5.13 ± 0.34 [a]	1.50 ± 0.22 [a]	5.75 ± 0.36 [a]	5.38 ± 0.35 [a]
Average in samples	32	5.66 ± 0.15	5.03 ± 0.17	4.88 ± 0.25	5.41 ± 0.25	2.88 ± 0.27	1.34 ± 0.11	5.34 ± 0.14	4.00 ± 0.19	4.50 ± 0.17	4.91 ± 0.17	1.38 ± 0.11	5.50 ± 0.18	5.28 ± 0.18

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. S—sensory evaluation; 1—intensity of red color; 2—flavor intensity; 3—flowery flavor; 4—fruity flavor; 5—green flavor; 6—off-flavor; 7—Taste; 8—acidic taste; 9— sweetness; 10—harmonious taste; 11—off-taste; 12—texture of sample firmness for fruits/homogeneity for juices; 13—overall sensory quality.

Interestingly, the increase/decrease in HCA content and ripeness reversed after conversion to juices. Here, the HCA content increased in the juices prepared from fruits with full ripeness, while this relationship was completely lost in the by-products. This means that all samples of the by-products had the same HCA content, regardless of ripeness. As with anthocyanins, a possible explanation could be the lower tissue hardness of fully ripe fruits compared to 75% ripe fruits and the consequent better extraction of HCA components [71].

The processing of the fruit into juice was the only factor that affected the content of FL in the samples, while the ripeness of the fruit had no effect on FL. This was also true when the dataset was broken down by the type of material assessed (e.g., fruits, juices, or by-products).

A hierarchical cluster analysis using the standardized Ward's method revealed that when considering average values for CIELAB L*; a*; b*; C*; H*; pH; contents of total phenolic compounds (mg GAE 100 mL^{-1}); anthocyanins (mg Pg-3–100 mL^{-1}); hydroxycinnamic acids (mg CAE 100 mL^{-1}); flavonols (mg QE 100 mL^{-1}); and overall sensory quality at different ripeness levels of the fruits and their juices, the results were clustered in an interesting manner. As expected, the samples of the juices and the corresponding fruits at 100% ripeness were clustered closely together. However, the next-closest neighbors included samples of juices at 75% maturity, suggesting that even at lower maturity, strawberry juices were similar to those of 100% maturity (see Figure 3).

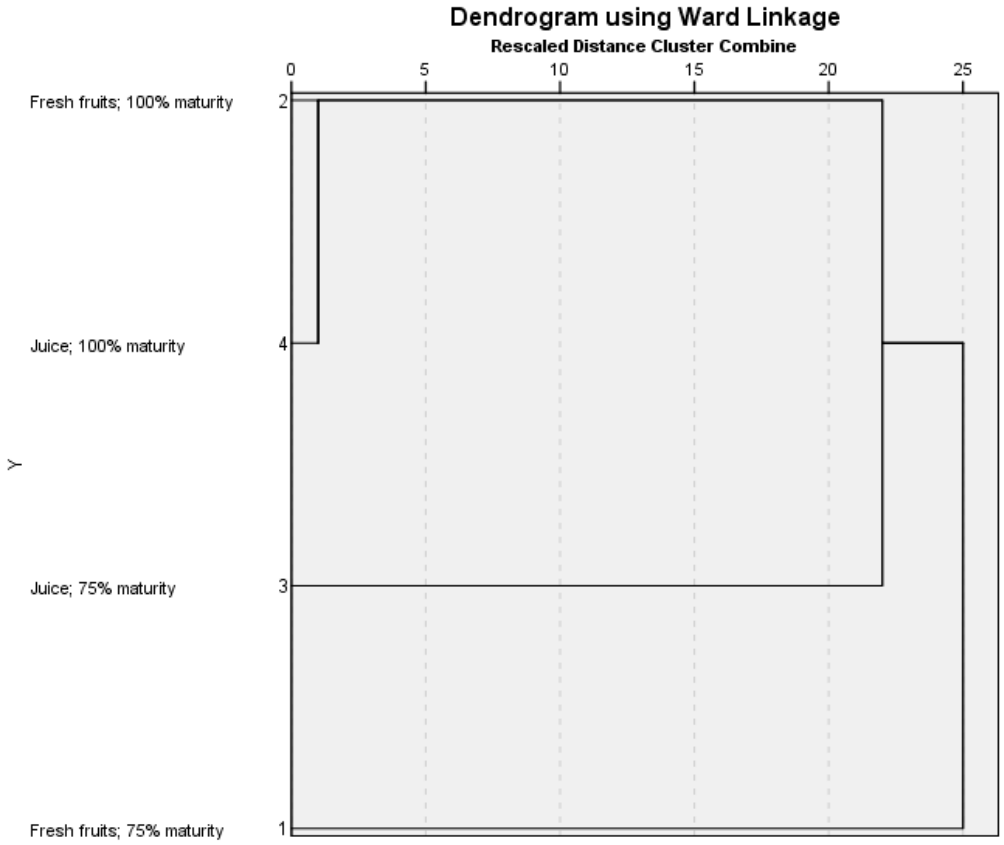

Figure 3. Clustering of samples of strawberry fruits and juices with various maturity with regards to average values for CIELAB L*; a*; b*; C*; H*; pH; contents of total phenolic compounds (mg GAE 100 mL^{-1}); anthocyanins (mg Pg-3–100 mL^{-1}); hydroxycinnamic acids (mg CAE 100 mL^{-1}); flavonols (mg QE 100 mL^{-1}); and overall sensory quality.

Further away from this cluster were fruits with a ripeness level of 75%. Although still close to the corresponding juice samples with 75% ripeness, they are outside the cluster that includes fruits and juices with 100% ripeness. In other words, it appears that the strawberries with lower ripeness, when processed into juices, are of similar quality

to fruit with higher ripeness. This could be important for industrial growers and juice processors, saving them the time it takes strawberries to reach full maturity from the 75% mark on, allowing them to harvest earlier without any particular loss of quality. Moreover, in comparison to 75% ripe fruits, the lower mechanical strength and susceptibility to fungal attack of fully ripe strawberries can be a limiting factor in production, especially in the transportation and marketing stages, resulting in significant losses [72,73]. The perspective of the use of unripe fruits is also being increasingly considered for various industrial purposes, for example, thinning of unripe fruits has shown great potential in food processing, bringing economic benefits and reducing environmental impact [66].

Table 8. Biologically active compounds in fresh strawberries, their juices, and by-products.

Parameters	*n*	TPC	ANT	HCA	FL
Material		$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.05$ †	$p \leq 0.01$ †
Fruit	4	58.19 ± 1.5 ^{a}	26.75 ± 0.47 ^{a}	14.51 ± 0.29 ^{b}	2.92 ± 0.25 ^{b}
Juice	4	35.27 ± 1.5 ^{b}	22.08 ± 0.47 ^{b}	9.06 ± 0.29 ^{c}	0.55 ± 0.25 ^{c}
By-Product	4	55.56 ± 1.5 ^{a}	26.90 ± 0.47 ^{a}	24.61 ± 0.29 ^{a}	6.29 ± 0.25 ^{a}
Maturity		$p = 0.02$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.68$ ‡
75%	6	52.51 ± 1.22 ^{a}	23.45 ± 0.38 ^{b}	16.43 ± 0.23 ^{a}	3.23 ± 0.21 ^{a}
100%	6	46.83 ± 1.22 ^{b}	27.04 ± 0.38 ^{a}	15.69 ± 0.23 ^{b}	3.28 ± 0.21 ^{a}
Material by Maturity		$p = 0.07$ ‡	$p = 0.16$ ‡	$p = 0.01$ †	$p = 0.07$ ‡
Fruit; 75%	2	64.36 ± 2.39 ^{a}	28.36 ± 1.04 ^{a}	16.96 ± 0.38 ^{a}	2.73 ± 0.36 ^{a}
Fruit; 100%	2	52.02 ± 2.39 ^{a}	25.13 ± 1.04 ^{a}	12.06 ± 0.38 ^{b}	3.12 ± 0.36 ^{a}
		$p = 0.09$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.07$ ‡
Juice; 75%	2	32.56 ± 1.23 ^{a}	15.80 ± 0.31 ^{b}	7.36 ± 0.20 ^{b}	1.10 ± 0.36 ^{a}
Juice; 100%	2	37.97 ± 1.23 ^{a}	28.36 ± 0.31 ^{a}	10.76 ± 0.20 ^{a}	1.00 ± 0.36 ^{a}
		$p = 0.10$ ‡	$p = 0.11$ ‡	$p = 0.47$ ‡	$p = 0.07$‡
By-product; 75%	2	60.62 ± 2.49 ^{a}	26.19 ± 0.37 ^{b}	24.96 ± 0.55 ^{a}	6.87 ± 0.36 ^{a}
By-product; 100%	2	50.51 ± 2.49 ^{a}	27.62 ± 0.37 ^{a}	24.26 ± 0.55 ^{a}	5.71 ± 0.36 ^{a}
Average in samples	12	49.67 ± 0.86	25.24 ± 0.27	16.06 ± 0.17	3.25 ± 0.15

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. TPC—total phenolic compounds (mg GAE 100 mL^{-1}); ANT—anthocyanins (mg Pg-3-G 100 mL^{-1}); HCA—hydroxycinnamic acids (mg CAE 100 mL^{-1}); FL—flavonols (mg QE 100 mL^{-1}).

4. Conclusions

It has been found that the degree of fruit ripeness has a greater influence on the color characteristics of fresh fruit than does storage, implying that less ripe 'Albion' strawberries, if they meet other quality parameters, could be suitable for processing.

Out of the analyzed concentrations of detected heavy metals (Cu, Zn, Ni, As, Cd, Pb) in strawberries, the permissible values assigned (MDK) to these types of products were not exceeded, but in the juice and by-product, there was a slightly higher amount of Pb with 100% ripeness, while the other metal concentrations were consistent with the results in the literature. Cd and Ni were not detected in any sample. Of the 566 pesticides analyzed, only two pesticides were detected with LC-MS/MS, cyprodinil and pyrimethanil.

As for the sensory analysis, the fresh fruits did not differ from the corresponding juices in any of the sensory attributes studied, confirming that sensory perception was not affected during the processing of the fresh fruits into juice. Nevertheless, ripeness proved to be a significant factor influencing most sensory attributes, with the 100% ripe fruits and their juices exhibiting an almost 20% higher overall sensory quality than the less ripe fruits and their juices.

The highest content of all bioactive compounds (BAC) was found in strawberry by-products and the lowest in fruit juices, indicating the great importance of using by-products in the production of functional foods.

A chemometric evaluation was successfully applied to strawberry cv. 'Albion' for processing into functional juice in terms of physicochemical parameters, sensory analysis,

and bioactive compounds. The results confirmed that, in addition to fully ripe fruits, strawberries with a lower degree of ripeness (75%) are also suitable for processing with respect to all quality parameters evaluated. These results are important for industrial juice producers because strawberries are a very delicate fruit with a tender texture that is easily damaged during transportation and storage. Therefore, by processing less ripe strawberries that better survive transport and storage due to their better textural properties, high-quality functional strawberry juices could also be produced.

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References

1. Basu, A.; Nguyen, A.; Betts, N.M.; Lyons, T.J. Strawberry As a Functional Food: An Evidence-Based Review. *Crit. Rev. Food Sci. Nutr.* **2013**, *54*, 790–806. [CrossRef] [PubMed]
2. Afrin, S.; Gasparrini, M.; Forbes-Hernandez, T.Y.; Reboredo-Rodriguez, P.; Mezzetti, B.; Varela-López, A.; Giampieri, F.; Battino, M. Promising health benefits of the strawberry: A focus on clinical studies. *J. Agric. Food Chem.* **2016**, *64*, 4435–4449. [CrossRef] [PubMed]
3. Giampieri, F.; Forbes-Hernandez, T.Y.; Gasparrini, M.; Alvarez-Suarez, J.M.; Afrin, S.; Bompadre, S.; Quiles, J.L.; Mezzetti, B.; Battino, M. Strawberry as a health promoter: An evidence based review. *Food Funct.* **2015**, *6*, 1386–1398. [CrossRef] [PubMed]
4. Villamil-Galindo, E.; Van de Velde, F.; Piagentini, A.M. Strawberry agro-industrial by-products as a source of bioactive compounds: Effect of cultivar on the phenolic profile and the antioxidant capacity. *Bioresour. Bioprocess.* **2021**, *8*, 61. [CrossRef]
5. Lorenzo, J.M.; Pateiro, M.; Domínguez, R.; Barba, F.J.; Putnik, P.; Kovačević, D.B.; Shpigelman, A.; Granato, D.; Franco, D. Berries extracts as natural antioxidants in meat products: A review. *Food Res. Int.* **2018**, *106*, 1095–1104. [CrossRef]
6. Oszmiański, J.; Wojdyło, A. Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. *Eur. Food Res. Technol.* **2008**, *228*, 623–631. [CrossRef]
7. Skrovankova, S.; Sumczynski, D.; Mlcek, J.; Jurikova, T.; Sochor, J. Bioactive compounds and antioxidant activity in different types of berries. *Int. J. Mol. Sci.* **2015**, *16*, 24673–24706. [CrossRef]
8. Aaby, K.; Skrede, G.; Wrolstad, R.E. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). *J. Agric. Food Chem.* **2005**, *53*, 4032–4040. [CrossRef]
9. Aaby, K.; Wrolstad, R.E.; Ekeberg, D.; Skrede, G. Polyphenol composition and antioxidant activity in strawberry purees; Impact of achene level and storage. *J. Agric. Food Chem.* **2007**, *55*, 5156–5166. [CrossRef]
10. Hartmann, A.; Patz, C.-D.; Andlauer, W.; Dietrich, H.; Ludwig, M. Influence of processing on quality parameters of strawberries. *J. Agric. Food Chem.* **2008**, *56*, 9484–9489. [CrossRef]
11. Fierascu, R.C.; Temocico, G.; Fierascu, I.; Ortan, A.; Babeanu, N.E. Fragaria genus: Chemical composition and biological activities. *Molecules* **2020**, *25*, 498. [CrossRef]
12. Azam, M.; Ejaz, S.; Naveed Ur Rehman, R.; Khan, M.; Qadri, R. Postharvest Quality Management of Strawberries. In *Strawberry-Pre- and Post-Harvest Management Techniques for Higher Fruit Quality*. Available online: https://www.intechopen.com/books/6996 (accessed on 15 November 2021).
13. Barth, E.; Resende, J.T.V.d.; Moreira, A.F.P.; Mariguele, K.H.; Zeist, A.R.; Silva, M.B.; Stulzer, G.C.G.; Mafra, J.G.M.; Simões Azeredo Gonçalves, L.; Roberto, S.R.; et al. Selection of experimental hybrids of strawberry using multivariate analysis. *Agronomy* **2020**, *10*, 598. [CrossRef]

14. Šamec, D.; Maretić, M.; Lugarić, I.; Mešić, A.; Salopek-Sondi, B.; Duralija, B. Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis. *Food Chem.* **2016**, *194*, 828–834. [CrossRef]
15. Granato, D.; Putnik, P.; Kovačević, D.B.; Santos, J.S.; Calado, V.; Rocha, R.S.; Cruz, A.G.D.; Jarvis, B.; Rodionova, O.Y.; Pomerantsev, A. Trends in Chemometrics: Food Authentication, Microbiology, and Effects of Processing. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 663–677. [CrossRef]
16. Gündüz, K.; Özbay, H. The effects of genotype and altitude of the growing location on physical, chemical, and phytochemical properties of strawberry. *Turk. J. Agric. For.* **2018**, *42*, 145–153. [CrossRef]
17. Aaby, K.; Mazur, S.; Nes, A.; Skrede, G. Phenolic compounds in strawberry (*Fragaria x ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chem.* **2012**, *132*, 86–97. [CrossRef]
18. Bursać Kovačević, D.; Putnik, P.; Dragović-Uzelac, V.; Vahčić, N.; Babojelić, M.S.; Levaj, B. Influences of organically and conventionally grown strawberry cultivars on anthocyanins content and color in purees and low-sugar jams. *Food Chem.* **2015**, *181*, 94–100. [CrossRef]
19. Bursać Kovaćević, D.; Vahčić, N.; Levaj, B.; Dragović-Uzelac, V. The effect of cultivar and cultivation on sensory profiles of fresh strawberries and their purées. *Flavour Fragr. J.* **2008**, *23*, 323–332. [CrossRef]
20. Oliver, P.; Cicerale, S.; Pang, E.; Keast, R. Developing a strawberry lexicon to describe cultivars at two maturation stages. *J. Sens. Stud.* **2018**, *33*, e12312. [CrossRef]
21. Kosar, M.; Kafkas, E.; Paydas, S.; Baser, K.H.C. Phenolic composition of strawberry genotypes at different maturation stages. *J. Agric. Food Chem.* **2004**, *52*, 1586–1589. [CrossRef]
22. Hwang, H.; Kim, Y.-J.; Shin, Y. Influence of ripening stage and cultivar on physicochemical properties, sugar and organic acid profiles, and antioxidant compositions of strawberries. *Food Sci. Biotechnol.* **2019**, *28*, 1659–1667. [CrossRef]
23. Paniagua, C.; Santiago-Doménech, N.; Kirby, A.R.; Gunning, A.P.; Morris, V.J.; Quesada, M.A.; Matas, A.J.; Mercado, J.A. Structural changes in cell wall pectins during strawberry fruit development. *Plant Physiol. Biochem.* **2017**, *118*, 55–63. [CrossRef]
24. Bhat, R.; Geppert, J.; Funken, E.; Stamminger, R. Consumers perceptions and preference for strawberries—A case study from Germany. *Int. J. Fruit Sci.* **2015**, *15*, 405–424. [CrossRef]
25. Lewers, K.S.; Newell, M.J.; Park, E.; Luo, Y. Consumer preference and physiochemical analyses of fresh strawberries from ten cultivars. *Int. J. Fruit Sci.* **2020**, *20*, 733–756. [CrossRef]
26. Fan, Z.; Hasing, T.; Johnson, T.S.; Garner, D.M.; Barbey, C.R.; Colquhoun, T.A.; Sims, C.A.; Resende, M.F.R.; Whitaker, V.M. Strawberry sweetness and consumer preference are enhanced by specific volatile compounds. *Hortic. Res.* **2021**, *8*, 66. [CrossRef]
27. Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K.; Sutton, D.J. Heavy Metal Toxicity and the Environment. In *Molecular, Clinical and Environmental Toxicology*; Experientia Supplementum; Birkhäuser Basel: Basel, Switzerland, 2012; pp. 133–164.
28. Bekele Bahiru, D.; Yegrem, L. Levels of Heavy Metal in Vegetable, Fruits and Cereals Crops in Ethiopia: A Review. *Int. J. Environ. Monit. Anal.* **2021**, *9*, 96–103. [CrossRef]
29. Parker, C. Strawberry fields forever: Can consumers see pesticides and sustainability as an issue? *Sustain. Sci.* **2014**, *10*, 285–303. [CrossRef]
30. Aktar, W.; Sengupta, D.; Chowdhury, A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip. Toxicol.* **2009**, *2*, 1–12. [CrossRef]
31. Abbasi, H.; Shah, M.H.; Mohiuddin, M.; Elshikh, M.S.; Hussain, Z.; Alkahtani, J.; Ullah, W.; Alwahibi, M.S.; Abbasi, A.M. Quantification of heavy metals and health risk assessment in processed fruits' products. *Arab. J. Chem.* **2020**, *13*, 8965–8978. [CrossRef]
32. Tiwari, B.K.; Muthukumarappan, K.; O'Donnell, C.P.; Cullen, P.J. Colour degradation and quality parameters of sonicated orange juice using response surface methodology. *LWT-Food Sci. Technol.* **2008**, *41*, 1876–1883. [CrossRef]
33. Bursać, D.; Vahčić, N.; Levaj, B.; Dragović-Uzelac, V.; Biško, A. The influence of cultivar on sensory profiles of fresh and processed strawberry fruits grown in Croatia. *Flavour Fragr. J.* **2007**, *22*, 512–520. [CrossRef]
34. Bursać Kovačević, D.; Putnik, P.; Dragović-Uzelac, V.; Pedisić, S.; Režek Jambrak, A.; Herceg, Z. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chem.* **2016**, *190*, 317–323. [CrossRef] [PubMed]
35. Škegro, M.; Putnik, P.; Bursać Kovačević, D.; Kovač, A.P.; Salkić, L.; Čanak, I.; Frece, J.; Zavadlav, S.; Ježek, D. Chemometric Comparison of High-Pressure Processing and Thermal Pasteurization: The Nutritive, Sensory, and Microbial Quality of Smoothies. *Foods* **2021**, *10*, 1167. [CrossRef] [PubMed]
36. Howard, L.R.; Clark, J.R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238–1247. [CrossRef]
37. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278. [CrossRef]
38. Kelly, K.; Whitaker, V.M.; Nunes, M.C.d.N. Physicochemical characterization and postharvest performance of the new Sensation® 'Florida127' strawberry compared to commercial standards. *Sci. Hortic.* **2016**, *211*, 283–294. [CrossRef]
39. De Jesús Ornelas-Paz, J.; Yahia, E.M.; Ramírez-Bustamante, N.; Pérez-Martínez, J.D.; del Pilar Escalante-Minakata, M.; Ibarra-Junquera, V.; Acosta-Muñiz, C.; Guerrero-Prieto, V.; Ochoa-Reyes, E. Physical attributes and chemical composition of organic strawberry fruit (*Fragaria x ananassa* Duch, cv. Albion) at six stages of ripening. *Food Chem.* **2013**, *138*, 372–381. [CrossRef]

40. Nunes, M.C.N.; Brecht, J.K.; Morais, A.M.M.B.; Sargent, S.A. Physicochemical changes during strawberry development in the field compared with those that occur in harvested fruit during storage. *J. Sci. Food Agric.* **2006**, *86*, 180–190. [CrossRef]
41. Olsson, M.E.; Ekvall, J.; Gustavsson, K.-E.; Nilsson, J.; Pillai, D.; Sjöholm, I.; Svensson, U.; Åkesson, B.; Nyman, M.G.L. Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (*Fragaria x ananassa*): Effects of cultivar, ripening, and storage. *J. Agric. Food Chem.* **2004**, *52*, 2490–2498. [CrossRef]
42. Concha–Meyer, A.A.; D'Ignoti, V.; Saez, B.; Diaz, R.I.; Torres, C.A. Effect of storage on the physico–chemical and antioxidant properties of strawberry and kiwi leathers. *J. Food Sci.* **2016**, *81*, C569–C577. [CrossRef]
43. Belakud, B.; Bahadur, V.; Prasad, V.M. Performance of strawberry (*Fragaria x ananassa* Duch.) varieties for yield and biochemical parameters. *Pharma Innov. J.* **2015**, *4*, 5–8.
44. Shao, W.-C.; Zang, Y.-Y.; Ma, H.-Y.; Ling, Y.E.; Kai, Z.-P. Concentrations and related health risk assessment of pesticides, phthalates, and heavy metals in strawberries from Shanghai, China. *J. Food Prot.* **2021**, *84*, 2116–2122. [CrossRef] [PubMed]
45. Bystricka, J.; Musilova, J.; Trebichalsky, P.; Tomas, J.; Stanovic, R.; Bajcan, D.; Kavalcova, P. The relationships between content of heavy metals in soil and in strawberries. *Int. J. Phytoremediation* **2015**, *18*, 553–558. [CrossRef] [PubMed]
46. Elbagermi, M.A.; Edwards, H.G.M.; Alajtal, A.I. Monitoring of Heavy Metal Content in Fruits And Vegetables Collected from Production and Market Sites in the Misurata Area of Libya. *ISRN Anal. Chem.* **2012**, *2012*, 827645. [CrossRef]
47. Official Journal of the European Union, L 364, 20 December 2006. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2006:364:TOC (accessed on 15 November 2021).
48. Commission Regulation (EU) 2021/1317. Available online: https://eur-lex.europa.eu/eli/reg/2021/1317/oj (accessed on 15 November 2021).
49. Commission Regulation (EU) 2021/1323. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021R1323 (accessed on 15 November 2021).
50. EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on Lead in Food. *EFSA J.* **2010**, *8*, 1570. [CrossRef]
51. Fernández-Ortuño, D.; Chen, F.; Schnabel, G. Resistance to Pyraclostrobin and Boscalid in *Botrytis cinerea* Isolates from Strawberry fields in the Carolinas. *Plant Dis.* **2012**, *96*, 1198–1203. [CrossRef] [PubMed]
52. Krieger, R. *Hayes' Handbook of Pesticide Toxicology*; Academic Press: Cambridge, MA, USA, 2010.
53. Available online: https://www.fao.org/fileadmin/user_upload/IPM_Pesticide/JMPR/Evaluations/2007/Pyrimethanil.pdf (accessed on 15 November 2021).
54. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Pyrimethanil (accessed on 15 November 2021).
55. Baggio, J.S.; Peres, N.A.; Amorim, L. Sensitivity of Botrytis cinerea Isolates from Conventional and Organic Strawberry Fields in Brazil to Azoxystrobin, Iprodione, Pyrimethanil, and Thiophanate-Methyl. *Plant Dis.* **2018**, *102*, 1803–1810. [CrossRef] [PubMed]
56. Faniband, M.; Ekman, E.; Littorin, M.; Maxe, M.; Larsson, E.; Lindh, C.H. Biomarkers of Exposure to Pyrimethanil After Controlled Human Experiments. *J. Anal. Toxicol.* **2019**, *43*, 277–283. [CrossRef]
57. Weber, F.; Larsen, L.R. Influence of fruit juice processing on anthocyanin stability. *Food Res. Int.* **2017**, *100*, 354–365. [CrossRef]
58. Orton, F.; Rosivatz, E.; Scholze, M.; Kortenkamp, A. Widely Used Pesticides with Previously Unknown Endocrine Activity Revealed asin VitroAntiandrogens. *Environ. Health Perspect.* **2011**, *119*, 794–800. [CrossRef]
59. Gupta, P.K. Toxicity of Fungicides. In *Veterinary Toxicology*; Gupta, R.C., Ed.; Academic Press: Cambridge, MA, USA, 2018; pp. 569–580.
60. Waechter, F.; Weber, E.; Hertner, T. Cyprodinil: A Fungicide of the Anilinopyrimidine Class. In *Handbook of Pesticide Toxicology*; Academic Press: London, UK, 2001; pp. 1701–1710.
61. Seeland, A.; Oehlmann, J.; Müller, R. Aquatic ecotoxicity of the fungicide pyrimethanil: Effect profile under optimal and thermal stress conditions. *Environ. Pollut.* **2012**, *168*, 161–169. [CrossRef]
62. Malhat, F.M.; Loutfy, N.M.; Thabet, W. Dissipation Profile and Human Risk Assessment of Pyrimethanil Residues in Cucumbers and Strawberries. *J. Health Pollut.* **2014**, *4*, 36–41. [CrossRef]
63. Aprea, E.; Biasioli, F.; Carlin, S.; Endrizzi, I.; Gasperi, F. Investigation of volatile compounds in two raspberry cultivars by two headspace techniques: Solid-phase microextraction/gas chromatography−mass spectrometry (SPME/GC−MS) and proton-transfer reaction−mass spectrometry (PTR−MS). *J. Agric. Food Chem.* **2009**, *57*, 4011–4018. [CrossRef]
64. Lu, H.; Ban, Z.; Wang, K.; Li, D.; Li, D.; Poverenov, E.; Li, L.; Luo, Z. Aroma volatiles, sensory and chemical attributes of strawberry (*Fragaria x ananassa* Duch.) achenes and receptacle. *Int. J. Food Sci. Technol.* **2017**, *52*, 2614–2622. [CrossRef]
65. Aubert, C.; Bruaut, M.; Chalot, G.; Cottet, V. Impact of maturity stage at harvest on the main physicochemical characteristics, the levels of vitamin C, polyphenols and volatiles and the sensory quality of Gariguette strawberry. *Eur. Food Res. Technol.* **2020**, *247*, 37–49. [CrossRef]
66. Wei, M.; Wang, H.; Ma, T.; Ge, Q.; Fang, Y.; Sun, X. Comprehensive utilization of thinned unripe fruits from horticultural crops. *Foods* **2021**, *10*, 2043. [CrossRef]
67. Jančářová, I.; Jančář, L.; Náplavová, A.; Kubáň, V. Changes of organic acids and phenolic compounds contents in grapevine berries during their ripening. *Open Chem.* **2013**, *11*, 1575–1582. [CrossRef]
68. Jiao, Y.; Chen, D.; Fan, M.; Young Quek, S. UPLC-QqQ-MS/MS-based phenolic quantification and antioxidant activity assessment for thinned young kiwifruits. *Food Chem.* **2019**, *281*, 97–105. [CrossRef]
69. Zheng, H.-Z.; Kim, Y.-I.; Chung, S.-K. A profile of physicochemical and antioxidant changes during fruit growth for the utilisation of unripe apples. *Food Chem.* **2012**, *131*, 106–110. [CrossRef]

70. Labbé, M.; Ulloa, P.A.; López, F.; Sáenz, C.; Peña, Á.; Salazar, F.N. Characterization of chemical compositions and bioactive compounds in juices from pomegranates (Wonderful, Chaca and Codpa) at different maturity stages. *Chil. J. Agric. Res.* **2016**, *76*, 479–486. [CrossRef]
71. Heng Koh, T.; Melton, L.D. Ripening-related changes in cell wall polysaccharides of strawberry cortical and pith tissues. *Postharvest Biol. Technol.* **2002**, *26*, 23–33. [CrossRef]
72. Siebeneichler, T.J.; Crizel, R.L.; Camozatto, G.H.; Paim, B.T.; da Silva Messias, R.; Rombaldi, C.V.; Galli, V. The postharvest ripening of strawberry fruits induced by abscisic acid and sucrose differs from their in vivo ripening. *Food Chem.* **2020**, *317*, 126407. [CrossRef]
73. Haile, Z.M.; Nagpala-De Guzman, E.G.; Moretto, M.; Sonego, P.; Engelen, K.; Zoli, L.; Moser, C.; Baraldi, E. Transcriptome Profiles of Strawberry (Fragaria vesca) Fruit Interacting With Botrytis cinerea at Different Ripening Stages. *Front. Plant Sci.* **2019**, *10*, 1131. [CrossRef]

Publication No.4: The Application and Optimization of HIPEF Technology in the Processing of Juice from Strawberries Harvested at Two Stages of Ripeness

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The Application and Optimization of HIPEF Technology in the Processing of Juice from Strawberries Harvested at Two Stages of Ripeness

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Abstract: The aim of this study was to investigate the influence of high intensity pulsed electric field (HIPEF) technology on the stability of total phenols, anthocyanins, hydroxycinnamic acids, flavonols, and condensed tannins in strawberry juices (*Fragaria x ananassa* Duch. cv. 'Albion') with different ripening stages (75% and 100%) and stored at +4 °C for 7 days. The HIPEF parameters studied were: (i) electric field strength (40 and 50 kV cm^{-1}), (ii) frequency (100 and 200 Hz), and (iii) treatment duration (3 and 6 min). Of the HIPEF parameters studied, electric field strength and frequency had a statistically significant effect on the content of all phenolic compounds. Treatment duration showed no statistically significant effects on phenolic compounds except for flavonols and condensed tannins. Storage had a positive effect on the stability of most of the phenolic compounds, with the exception of flavonols. Optimization of HIPEF processing showed that strawberry samples at both ripeness levels were suitable for HIPEF treatment to obtain functional fruit juices with a high content of polyphenols.

Keywords: high intensity pulsed electric field (HIPEF); bioactive compounds; strawberry juice; maturity; storage

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1. Introduction

Given the global pandemic of chronic diseases, many of which are directly related to obesity, immense efforts are being made to highlight the importance of higher consumption of fruit and vegetables as a preventive measure [1,2]. In this case, foods with distinct functional properties have attracted particular attention because of their promising health benefits [3,4]. Strawberry (*Fragaria x ananassa* Duch.) is widely recognized as a fruit with functional properties, mainly due to its high concentration of bioactive compounds such as polyphenols and/or vitamins [1,5,6]. Environmental conditions, cultivation technique and harvest time can have a great influence on the fruit quality of cv. 'Albion' [7]. There is ample evidence of the antioxidant, antihypertensive, antihyperlipidemic, and antiproliferative effects of strawberries [1]. Among the numerous bioactive compounds, ascorbic acid, ellagitannins, and anthocyanins are the most potent in yielding certain health benefits [1]. Considering the short harvest season, but also due to consumer preferences, strawberries are processed into various products, of which jams, purees, and juices are the most common [1]. For example, Zhao et al. investigated the antioxidant and antibacterial potential of functional strawberry juice inoculated with a starter culture of lactic acid bacteria (LAB) and yeasts [8]. A similar methodology was used by Cataldo et al. in

their work in which strawberry juice was inoculated with *Levilactobacillus brevis* to study the immunomodulatory effect of gamma-aminobutyric acid on rodent cell cultures [9]. In addition to processing into juice, studies have also shown the potential of processing strawberries into dry powders [10]. A considerable number of studies, including human intervention studies and cell lines using fresh or frozen fruits, extracts, purees, etc., some of which are covered in the 2014 review by Basu et al., illustrated the great potential and, what is now evident, the great interest in using strawberries as valuable health promoters [1].

However, it is important to minimize the loss of bioactive compounds of strawberries during processing to preserve the "functional character" of the final product. Additionally, the selection of the appropriate cultivar and degree of ripeness is of great importance when developing a product with a high content of bioactive compounds from strawberries. For example, Mazur et al. evaluated the suitability of three different strawberry cultivars ('Blink', 'Polka', and 'Senga Sengana') at three stages of ripeness ('nearly ripe', 'ripe', and 'fully ripe') for the production of jam [11]. Although there were differences in total phenolic content (TP) among different cultivars, still they were no statistically different nor did they differ significantly with maturation. Consequently, no significant differences were found in the TP content of the jams produced, although jams made from 'fully ripped' strawberries had the highest TP content. Nevertheless, this study suggests that fully ripe fruits are more suitable for processing to better preserve product color during storage. However, Bebek Markovinović et al. showed that the strawberry cultivar 'Albion' harvested at 75% ripeness had a significantly higher content of TP and was also suitable for processing, although the juice produced from 100% ripe fruit had a higher TP content, which indeed was not statistically significant from that which was produced from 75% ripe fruit [12]. Interestingly, the authors addressed the potential of strawberry by-products, which also showed a high content of bioactive compounds. However, the strength of this study lies in the fact that the selection of partially ripened fruits can be extremely useful for the industry, as strawberries are highly susceptible to damage during transportation and storage, which consequently has a negative impact on the sensory quality of the final product. Therefore, less ripe fruits may be more suitable for industrial purposes, as they are more "resistant" to logistical factors.

Several factors must be considered when processing strawberries into functional products. First of all, the strawberry itself is an excellent substrate for bacterial growth due to its high-water content and the amount of sugar present [13]. These factors must be considered especially for designing of strawberry juice, where the aforementioned issues are even more intensified. Attention should be paid not only to the biochemical composition and its preservation, but also to microbiological safety. To date, thermal pasteurization is one of the safest processing methods, but at the same time it results in a deterioration of the nutritional and bioactive contents of the final product, in this case juices [13,14]. Thermal processing is also responsible for color changes in juices, such as browning, that can make the product unacceptable to consumers seeking a fresh and attractive red color of the juice [15]. In view of this, novel non-thermal methods of food preservation such as high-intensity pulsed electric field processing (HIPEF) are being developed and tested to meet the high demand for functional products with nutritional and sensory properties similar to those of fresh fruits [16–18]. The results of a previous study [12] have shown that both ripening grades (75% and 100%) of strawberries (*Fragaria x ananassa* Duch., cultivar 'Albion') are suitable for the production of functional juices. Therefore, the aim of this work was to study the influence of different parameters of HIPEF technology on the content of bioactive compounds in strawberry juices produced at different degrees of ripeness and stored for 7 days at +4 °C. Based on the obtained results, the optimal parameters for HIPEF treatment that ensure the best preservation of bioactive compounds in the juices were also calculated.

2. Materials and Methods

2.1. Chemicals and Standards

Sodium carbonate, anhydrous (99.5–100.5%), hydrochloric acid (37%, *w*/*w*), sulfuric acid (96%, p.a.), and formic acid (98%, p.a.) were obtained from Lach-ner (Neratovice, Czech Republic). HPLC 99% pure methanol purchased from Honeywell (Paris, France) was used as the extraction solvent. For spectrophotometric analysis of total phenols used Folin–Ciocalteu reagent procured from Fisher Scientific UK (Loughborough, UK). Ethanol (96% pure) was procured from Gram-mol (Zagreb, Croatia). Quercetin (95%) and gallic acid standard (97.5–102.5%) were purchased from Acros Organics (Guangzhou, China) and Sigma-Aldrich (St. Louis, MO, USA). Potassium chloride (99.0–100.5%), vanillin (99%), sodium acetate anhydride (99%), and chlorogenic acid (min. 95%) were obtained from Thermo Fisher (Kandel, Germany).

2.2. Strawberry Juice Preparation

Strawberry fruits (*Fragaria x ananassa* Duch., cv. 'Albion') were harvested in 2021 at the company Jagodar HB in Donja Lomnica, Zagreb County (Croatia). The strawberries were harvested at two different stages of ripeness: (i) at the technological ripening stage, where the fruits are 75% red (F1), and (ii) at the full ripening stage, where the fruits are 100% red (F2). Immediately after harvest, strawberries were delivered to the laboratory, where they were removed from the stems, washed with tap water and dried with cellulose. Then, juices (J1 and J2) were prepared from the strawberries of the appropriate degree of ripeness (F1 and F2) by cold pressing (Kuvings B6000 Slow Juicer, VerVita d.o.o., Zagreb, Croatia). The juices produced in this way were subjected to HIPEF treatment whose process parameters are described in Section 2.3. All juices were filled in sterile glass bottles and hermetically sealed.

2.3. High-Intensity Pulsed Electric Field (HIPEF) Processing of Strawberry Juice Samples

The HIPEF device used in this research was HVG60/1 HIPEF (Impel d.o.o., Zagreb, Croatia). HIPEF treatments were performed by placing the strawberry juice samples in the treatment chamber at room temperature. The treatment chamber configuration was two stainless steel electrodes (Figure 1) with a diameter of 68 mm, a gap distance of 1 cm, and a volume of 100 mL. Treatments were conducted using square wave pulses, duration of 0.5 μs at the frequencies of 100 and 200 Hz. The applied electric field strength ranges were 40 and 50 kV cm^{-1}. Total treatment time was 3 and 6 min, and the total applied energy ranged from 1.425×10^2–6.750×10^2 kJ L^{-1}.

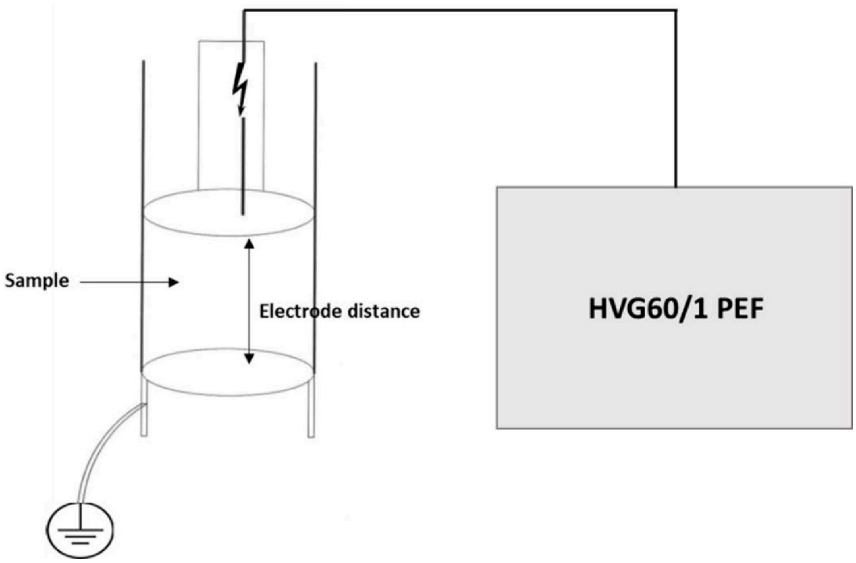

Figure 1. Schematic depiction of HIPEF reactor.

The control samples were untreated juices, while the HIPEF treatment conditions were as follows: electric field 40 kV cm^{-1} and 50 kV cm^{-1}, frequency 100 Hz and 200 Hz with pulse duration 3 min and 6 min, according to design of the experiment (Table 1). A batch of juices was analyzed immediately after HIPEF processing, and another batch of juices were stored at 4 °C for 7 days. The stored juice samples were evaluated during the storage period to determine the evolution of the quality indices related to the physiochemical aspects and the stability of the bioactive compounds.

Table 1. Design of the experiment.

Sample	Juice	Storage (Days)	Treatment	Electric Field Strength (kV cm^{-1})	Frequency (Hz)	Pulse Duration (min)
1	J1	0	Control	/	/	/
2	J1	0	HIPEF	40	100	3
3	J1	0	HIPEF		200	3
4	J1	0	HIPEF		100	6
5	J1	0	HIPEF		200	6
6	J1	0	HIPEF	50	100	3
7	J1	0	HIPEF		200	3
8	J1	0	HIPEF		100	6
9	J1	0	HIPEF		200	6
10	J2	0	Control	/	/	/
11	J2	0	HIPEF	40	100	3
12	J2	0	HIPEF		200	3
13	J2	0	HIPEF		100	6
14	J2	0	HIPEF		200	6
15	J2	0	HIPEF	50	100	3
16	J2	0	HIPEF		200	3
17	J2	0	HIPEF		100	6
18	J2	0	HIPEF		200	6
19	J1	7	Control	/	/	/
20	J1	7	HIPEF	40	100	3
21	J1	7	HIPEF		200	3
22	J1	7	HIPEF		100	6
23	J1	7	HIPEF		200	6
24	J1	7	HIPEF	50	100	3
25	J1	7	HIPEF		200	3
26	J1	7	HIPEF		100	6
27	J1	7	HIPEF		200	6
28	J2	7	Control	/	/	/
29	J2	7	HIPEF	40	100	3
30	J2	7	HIPEF		200	3
31	J2	7	HIPEF		100	6
32	J2	7	HIPEF		200	6
33	J2	7	HIPEF	50	100	3
34	J2	7	HIPEF		200	3
35	J2	7	HIPEF		100	6
36	J2	7	HIPEF		200	6

J1—strawberry juice prepared from 75% ripe strawberries; J2—strawberry juice prepared from 100% ripe strawberries. Control—untreated samples.

2.4. Characterization of Untreated and HIPEF-Treated Strawberry Juices

2.4.1. Determination of pH and Soluble Solids Content (SSC)

pH was determined with a Mettler Toledo FiveEasy pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland) previously calibrated with commercial buffer solutions at pH 4.0 and 7.0. SSC (Brix%) was determined with a digital refractometer ATAGO Pal-3 (ATAGO Co., Tokyo, Japan). Duplicate measurements were performed for each sample.

2.4.2. Extraction of Bioactive Compounds

According to a modified protocol from the literature [14], the extractions of biologically active compounds from prepared strawberry juices (J1 and J2) were performed. A total of 5 mL of the sample and 20 mL of 1% formic acid in 80% methanol (v/v) were mixed and vortexed for 1 min. Then, the reaction mixture was extracted at 50 °C for 15 min in an ultrasonic bath (DT 514 H Sonorex Digitec 13.5 L, Bandelin electronic, Berlin, Germany) and centrifuged at 10,000 rpm/10 min (Thermo Scientific™, Megafuge™ 16R, Kalkberg, Germany). The supernatant was then filtered and made up to 25 mL with extraction solvent in a volumetric flask. All extracts were prepared in duplicates. The extracts were stored at −18 °C under inert gas atmosphere until analysis.

2.4.3. Determination of Total Phenolic Content (TPC)

Total phenolic content was measured using the Folin–Ciocalteau modified spectrophotometric assay described in the literature [19]. Volume of 400 µL of the extract was mixed with 400 µL of the FC reagent (previously diluted 5 -fold with distilled water) and 4 mL of a 7.5% sodium carbonate solution (w/v). The reaction mixture was allowed to stand at room temperature for 20 min and the absorbance of the colored reaction was measured at 725 nm using a spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch & Holm, Meckenheim, Germany). Duplicate measurements were performed for each sample. The TPC in the extracts was calculated using a standard calibration curve generated with different concentrations of gallic acid (10–250 mg L^{-1}). Results were expressed as mg gallic acid equivalent (GAE) per 100 g or 100 mL of sample.

2.4.4. Determination of Total Monomeric Anthocyanins (ANT)

Anthocyanins were determined by the spectrophotometric differential pH method [20]. A total of 1 mL of the extract was mixed with 4 mL of 0.4 M sodium acetate buffer (pH 4.5) and separately with 4 mL of 0.025 M potassium chloride buffer (pH = 1.0). The reaction mixture was allowed to stand at room temperature for 20 min and absorbance was measured at 520 and 700 nm using a spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch & Holm, Meckenheim, Germany). Deionized water was used as a blank. Duplicate measurements were performed for each sample. The concentration of monomeric anthocyanins in the sample was expressed as pelargonidin-3-glucoside equivalent (Pg-3-G) (mg L^{-1}) according to calculation as described in the literature [21].

2.4.5. Determination of Total Hydroxycinnamic Acids (HCA)

HCA was determined by a modified spectrophotometric method [22]. A volume of 250 µL of the extract was mixed with 250 µL of solution 1 (1 g L^{-1} solution of HCl dissolved in 96% ethanol) and 4.55 mL of solution 2 (2 g L^{-1} HCl dissolved in distilled water). The reaction was vortexed for 10 s and then allowed to react in the dark at room temperature for 30 min. The absorbance of the reaction was measured at 320 nm in a spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch & Holm, Meckenheim, Germany). For the blank sample, the extraction solvent was used instead of the extract, and the rest of the procedure remained the same as for the sample. Duplicated measurements were performed for each sample. The HCA content was calculated using a calibration curve generated with different concentrations of chlorogenic acid (10–600 mg L^{-1}). The results were expressed as mg chlorogenic acid equivalent (CAE) per 100 g or 100 mL of the sample.

2.4.6. Determination of Total Flavonols (FL)

FL were determined by modified spectrophotometric method [22]. A volume of 250 µL of the extract was mixed with 250 µL of solution 1 (1 g L^{-1} solution of HCl dissolved in 96% ethanol) and 4.55 mL of solution 2 (2 g L^{-1} HCl dissolved in distilled water). The reaction was vortexed for 10 s and then allowed to react in the dark at room temperature for 30 min. The absorbance of the reaction was measured at 360 nm in a spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch & Holm, Meckenheim, Germany). For the blank sample, the extraction solvent was used instead of the extract, and the rest of the procedure remained the same as for the sample. Duplicated measurements were made for each sample. The FL in the extracts were calculated from a calibration curve obtained with different concentrations of quercetin solution (10–600 mg L^{-1}). Results were expressed as mg quercetin equivalent (QE) per 100 g or 100 mL of the sample.

2.4.7. Determination of Condensed Tannins (CT)

CT was determined by a modified spectrophotometric method [23]. A volume of 2.5 mL of reagent 1 (1% vanillin solution in methanol) was mixed with reagent 2 (2.5 mL of 25% H_2SO_4 solution in methanol) and 1 mL of extract. The reaction mixture was stirred with a vortex for 1 min and then allowed to react at room temperature for 10 min. Then, the absorbances were measured at 500 nm in a spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch & Holm, Meckenheim, Germany). A blank sample was prepared in the same way, except that the extraction solvent was used instead of the sample. Duplicated measurements were made for each sample. A catechin standard solution (10–120 mg L^{-1}) was used to prepare the calibration curve, and the results were expressed as mg catechin equivalent (CA) per 100 g or 100 mL of the sample.

2.5. Statistical Analysis

Experiments were designed as full factorial randomized experimental design. Dependent variables for multivariate analysis were: pH; SSC—soluble solids content (%); TPC—total phenolic compounds (mg 100 mL^{-1}); ANT—anthocyanins (mg 100 mL^{-1}); HCA—hydroxycinnamic acids (mg 100 mL^{-1}); FL—flavonols (mg 100 mL^{-1}), and CT—condensed tannins (mg 100 mL^{-1}). Independent variables were: sample maturity (75%, 100%); storage (0 and 7 days). HIPEF settings evaluated were: electric field strength (40 and 50 kV cm^{-1}), frequency (100 Hz, 200 Hz), and treatment time (3 and 6 min). The significance levels for all tests were $\alpha \leq 0.05$. Analyses were performed with IBM SPSS Statistics (v.24). The RSM optimization analysis was done with STATGRAPHICS Centurion XVIII. Occurrence of overparameterization was tested with variance inflation factors that were all lower than acceptable value (V.I.F. ≤ 4) so that all models are precise and with very good predictive power.

3. Results and Discussion

3.1. Changes of the Physicochemical Parameters in Untreated and HIPEF-Treated Strawberry Juices during Storage

Of the physicochemical parameters in untreated strawberry juices during storage, pH and SSC were studied (Table 2). The average value for SSC in untreated juices was 7.09%, while in HIPEF-treated samples it was 6.87%. Untreated strawberry juices had a pH of 3.41 and HIPEF-treated samples had a pH of 3.47. As can be seen, the J2 strawberry juices had higher SSC and pH values than the J1 juices, which can be attributed to the higher ripeness of the strawberries used for J2 production. Since SSC value is strongly correlated with fruit ripeness [12], it was expected that a higher SSC value would be observed in riper fruits. In addition, as the fruit ripens, total acidity decreases, which is reflected in an increase in pH [24,25].

Table 2. Changes in physicochemical parameters in untreated strawberry juices during storage.

Variables	n	SSC (%)	pH
Maturity		$p \leq 0.01$ †	$p \leq 0.01$ †
75% (Juice J1)	4	6.15 ± 0.03 ^{b}	3.38 ± 0.01 ^{b}
100% (Juice J2)	4	8.03 ± 0.03 ^{a}	3.53 ± 0.01 ^{a}
Storage	4	$p \leq 0.01$ †	$p = 0.02$ †
0 days	4	7.20 ± 0.03 ^{a}	3.48 ± 0.01 ^{a}
7 days	8	6.98 ± 0.03 ^{b}	3.43 ± 0.01 ^{a}
Dataset average		7.09 ± 0.02	3.41 ± 0.01

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. SSC—soluble solids content (%).

A storage period of 7 days affected the reduction of SSC in untreated strawberry juices. Similarly, previous work reported that SSC may gradually decrease during cold storage [26], which may be due to the denaturation of enzymes [27]. The pH of the untreated juice samples remained stable during storage. However, Aaby et al. found that the pH of strawberry purée decreased during storage, which can be attributed to the formation of small amounts of organic acids as a result of initial microbial contamination [28]. The decrease in pH during storage can also be attributed to the stabilization of pH due to low temperature during storage [23].

Similar to the untreated samples, HIPEF samples with higher maturity (J2) were determined to have higher SSC and pH (Table 3). The effect of storage on physicochemical parameters for HIPEF-treated strawberry juices was identical to that of untreated juices, with SSC decreasing and pH remaining unchanged during storage. Therefore, the treated samples had lower SSC (6.78 ± 0.01%) than at the beginning of storage (6.97 ± 0.01%). These results are in agreement with those of Mtaou et al. who also found a statistically significant decrease in the SSC value of date juice treated with HIPEF (35 kV cm^{-1}, 100 Hz, 1000 ms) during 5 weeks of storage at 4–5 °C [29].

Table 3. Changes in physiochemical parameters in HIPEF-treated strawberry juices during storage.

Variables	n	SSC (%)	pH
Maturity		$p \leq 0.01$ †	$p \leq 0.01$ †
75% (Juice J1)	32	5.82 ± 0.01 ^{b}	3.44 ± 0.01 ^{b}
100% (Juice J2)	32	7.92 ± 0.01 ^{a}	3.51 ± 0.01 ^{a}
Storage		$p \leq 0.01$ †	$p = 0.22$ ‡
0 days	32	6.97 ± 0.01 ^{a}	3.48 ± 0.01 ^{a}
7 days	32	6.78 ± 0.01 ^{b}	3.46 ± 0.01 ^{a}
Electric field strength		$p \leq 0.01$ †	$p = 0.41$ ‡
40 kV cm^{-1}	32	6.82 ± 0.01 ^{b}	3.46 ± 0.01 ^{a}
50 kV cm^{-1}	32	6.92 ± 0.01 ^{a}	3.48 ± 0.01 ^{a}
Frequency		$p = 0.39$ ‡	$p = 0.23$ ‡
100 Hz	32	6.86 ± 0.01 ^{a}	3.46 ± 0.01 ^{a}
200 Hz	32	6.89 ± 0.01 ^{a}	3.48 ± 0.01 ^{a}
Treatment time		$p = 0.31$ ‡	$p = 0.21$ ‡
3 min	32	6.85 ± 0.01 ^{a}	3.48 ± 0.01 ^{a}
6 min	32	6.89 ± 0.01 ^{a}	3.46 ± 0.01 ^{a}
Dataset average	64	6.87 ± 0.01	3.47 ± 0.01

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. SSC—soluble solids content (%).

On the other hand, these results are in contrast with the results of Geveke et al. who found an opposite trend in SSC values of strawberry puree during 24 weeks of accelerated storage at 40 °C [30]. These discrepancies in the results could be explained by different storage conditions, or more precisely, in this case, different storage temperatures. Namely, there is a possibility that due to the higher storage temperature, there was a greater evaporation of water from the product and consequently an increase in the SSC value compared to storing of the product at lower temperatures.

Increasing the electric field strength from 40 kV cm^{-1} to 50 kV cm^{-1} resulted in a significant increase in SSC (6.82 ± 0.01% vs. 6.92 ± 0.01%), which can be explained by the HIPEF phenomenon of electrophoresis that enhanced the release of cell biomaterial by degrading the cellular structures in the colloid system of the juice [31,32]. However, frequency and treatment duration had no effect on SSC in the juice samples.

Storage, electric field strength, and duration of treatment did not affect the pH of the samples. Similarly, Odriozola Serrano et al. showed that HIPEF treatment (35 kV cm^{-1}, 100 Hz and 1700 µs) did not produce significant differences in pH change before (3.39 ± 0.05) and after HIPEF treatment (3.38 ± 0.03) [33]. The obtained results are in agreement with the results of Geveke et al. who studied the influence of HIPEF technology on the physicochemical parameters of strawberry puree during accelerated storage for a period of 24 weeks. In their study, the pH values of HIPEF-treated strawberry puree did not change during storage [30].

3.2. The Changes in the Stability of Bioactive Compounds of Untreated Strawberry Juices during Storage

Strawberry juice is a valuable source of biologically active compounds, as confirmed by the results of this study, in which the total phenolic content (TPC) in untreated samples was 79.78 ± 1.25 mg GAE 100 mL^{-1} (Table 4). Considering the subgroups of phenolic compounds, condensed tannins (CT) were the most abundant (75.82%), followed by anthocyanins (ANT) (33.63%), hydroxycinnamic acids (HCA) (14.09%), and flavonols (FL) (2.54%). Although juice J1 from the less ripe strawberries had higher TPC content than juice J2 from fully ripe strawberries (85.19 vs. 74.39 mg GAE 100 mL^{-1}), all other bioactive compounds were detected at higher concentrations in strawberry juices from fully ripe strawberries (J2). Since there is no evidence in the literature that strawberries from different stages of ripeness were processed into juice, it can be assumed that these results are consistent with previous findings for fresh fruit at different stages of ripeness [24].

Table 4. Changes in the contents of bioactive compounds in untreated strawberry juices during storage.

Variables	n	TPC	ANT	HCA	FL	CT
Maturity		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
75% (Juice J1)	4	85.19 ± 1.77 ^{a}	16.87 ± 0.20 ^{b}	9.76 ± 0.44 ^{b}	1.78 ± 0.10 ^{b}	56.28 ± 0.58 ^{b}
100% (Juice J2)	4	74.39 ± 1.77 ^{b}	36.80 ± 0.20 ^{a}	12.71 ± 0.44 ^{a}	2.29 ± 0.10 ^{a}	64.70 ± 0.58 ^{a}
Storage		$p \leq 0.01$ †	$p \leq 0.01$†	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
0 days	4	63.55 ± 1.77 ^{b}	23.82 ± 0.20 ^{b}	8.76 ± 0.44 ^{b}	2.94 ± 0.10 ^{a}	41.20 ± 0.58 ^{b}
7 days	4	96.02 ± 1.77 ^{a}	29.85 ± 0.20 ^{a}	13.71 ± 0.44 ^{a}	1.13 ± 0.10 ^{b}	79.78 ± 0.58 ^{a}
Dataset average	8	79.78 ± 1.25	26.83 ± 0.14	11.24 ± 0.31	2.03 ± 0.07	60.49 ± 0.40

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. ANT—anthocyanins (mg Pg-3-G 100 mL^{-1}); TPC—total phenolic compounds (mg GAE 100 mL^{-1}); HCA—hydroxycinnamic acids (mg CAE 100 mL^{-1}); FL—flavonols (mg QE 100 mL^{-1}); CT—condensed tannins (mg CA 100 mL^{-1}).

The ANT concentration in J2 was 1.25-fold higher than in J1, which is consistent with previous results in strawberries at different stages of ripening [34]. The significantly higher

HCA concentration in J2 than in J1 was explained by Pradas et al. [34] in terms of phenolic acid accumulation, since these compounds serve as precursors for the different branches of the phenylpropanoid pathways and phenolic compound metabolism [35]. In addition, the different contents of FL in strawberries from different ripening stages seemed to correlate with variations in cultivars, as there is evidence in the literature that the content of FL in strawberries increases or decreases with ripening depending on the cultivar type [34].

During storage, the content of TPC, ANT, HCA, and CT generally increased significantly by about 20–36%, regardless of the maturity stage at harvest. After 7 days at 4 °C, the TPC content in strawberry juices (96.02 mg 100 mL^{-1}) was 1.5 times higher ($p \leq 0.05$) than at the beginning of storage (63.55 mg 100 mL^{-1}). In strawberry fruit (*Fragaria x ananassa* Duch. cv. 'Gariguette'), an increase in TPC was also observed during 2 days of storage at 10 °C [36].

3.3. The Influence of HIPEF Processing on the Stability of Bioactive Compounds in Strawberry Juices during Storage

The effect of HIPEF treatment, ripeness, and storage parameters on the bioactive compounds of the HIPEF-treated samples is shown in Table 5. The average content of TPC in the treated samples was 89.49 ± 0.50 mg GAE 100 mL^{-1}. As maturity increased from 75% to 100%, the TPC content decreased by 15% (96.91 mg 100 mL^{-1} vs. 82.08 mg 100 mL^{-1}). These results are most likely related to the fact that phenolic compounds are synthesized in the superficial part of the fruit tissue, and, as the fruit ripens, their mass increases, so that the proportion of phenolic compounds is higher in unripe fruit than in ripe fruit [37]. On the other hand, TPC levels increased by 15% during prolonged storage from the 1st to the 7th day of storage. Odriozola Serrano et al. studied the effects of HIPEF technology (35 kV cm^{-1}, 100 Hz and 1700 µs) on the quality of strawberry juice during 56 days of storage at +4 °C. The data show an increase in TPC up to 21 days of storage, after which the TPC begins to decrease [33]. With increasing electric field strength, the TPC increased significantly, which can be explained by electroporation and facilitated leakage of biomaterial due to cell wall damages [38–40]. These results are also in agreement with the results of a study by Siddeeg et al. who investigated the effect of different electric field strengths (1, 2 and 3 kV cm^{-1}, 10 Hz, 100 µs) on the bioactive components of date palm fruit [41]. Here, a decrease when the frequency was increased from 100 Hz (90.69 mg/100 mL^{-1}) to 200 Hz (88.31 mg 100 mL^{-1}) of almost 3% was observed, while the treatment time had no effect on the content of TPC.

Since the color of the juice is the first visual impression that consumers perceive and determines their perception of the product, the content of anthocyanins in HIPEF-treated juice and the effects of HIPEF parameters on anthocyanin content are very important. The average content of anthocyanins in the treated samples was 28.49 ± 0.13 mg 100 mL^{-1} (Table 5). As expected, samples with higher ripeness had higher contents of anthocyanins, thus samples with 100% ripeness (37.98 mg 100 mL^{-1}) had almost 100% more anthocyanins than samples with 75% ripeness (19.01 mg 100 mL^{-1}). With longer storage, the content of anthocyanins was higher (29.63 mg 100 mL^{-1}) than at the beginning of storage (27.35 mg 100 mL^{-1}).

When considering the influence of electric field strength (40 vs. 50 kV cm^{-1}), the results showed that higher electric field strength resulted in higher anthocyanin content, likely due to increased extraction of anthocyanins from the matrix by HIPEF treatment [42]. A previous report showed that the contents of anthocyanins in strawberry juices after HIPEF treatment with an electric field strength between 20 and 35 kV cm^{-1} for up to 2000 µs, varied only slightly and was 96 to 100% of the original content, depending on the processing intensity [33]. Similarly, the results of this study showed that the content of anthocyanins in untreated strawberry juices was 26.83 mg 100 mL^{-1}, while this concentration in HIPEF-treated juices was 28.60 mg 100 mL^{-1}. The content of anthocyanins decreased with increasing frequency, as a slight decrease of 2% was observed when the frequency was doubled from 100 Hz (28.79 mg 100 mL^{-1}) to 200 Hz (28.19 mg 100 mL^{-1}). Odriozola Ser-

rano et al. showed that a range between 100 and 250 Hz in HIPEF treatment of strawberry juices positively affected the stability of the initial anthocyanin content. However, the effect of frequency on anthocyanin retention strongly depended on the pulse width used [43]. The treatment time did not change the anthocyanin content.

Table 5. Changes in the contents of bioactive compounds in HIPEF-treated strawberry juices during storage.

Variables	n	TPC	ANT	HCA	FL	CT
Maturity		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
75% (Juice J1)	32	96.91 ± 1.52 ^{a}	19.01 ± 0.18 ^{b}	10.62 ± 0.12 ^{b}	1.81 ± 0.04 ^{b}	64.99 ± 0.22 ^{b}
100% (Juice J2)	32	82.08 ± 1.52 ^{b}	37.97 ± 0.18 ^{a}	14.10 ± 0.12 ^{a}	2.48 ± 0.04 ^{a}	69.99 ± 0.22 ^{a}
Storage		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
0 days	32	83.11 ± 1.52 ^{b}	27.35 ± 0.18 ^{b}	10.14 ± 0.12 ^{b}	3.29 ± 0.04 ^{a}	54.79 ± 0.22 ^{b}
7 days	32	95.88 ± 1.52 ^{a}	29.63 ± 0.18 ^{a}	14.58 ± 0.12 ^{a}	1.00 ± 0.04 ^{b}	80.18 ± 0.22 ^{a}
Electric field strength		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
40 kV cm^{-1}	32	85.89 ± 1.52 ^{b}	27.73 ± 0.18 ^{b}	11.59 ± 0.12 ^{b}	1.91 ± 0.04 ^{b}	63.98 ± 0.22 ^{b}
50 kV cm^{-1}	32	93.11 ± 1.52 ^{a}	29.26 ± 0.18 ^{a}	13.12 ± 0.12 ^{a}	2.38 ± 0.04 ^{a}	70.99 ± 0.22 ^{a}
Frequency		$p = 0.27$ ‡	$p = 0.03$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
100 Hz	32	90.69 ± 1.52 ^{a}	28.79 ± 0.18 ^{a}	12.60 ± 0.12 ^{a}	2.29 ± 0.04 ^{a}	68.57 ± 0.22 ^{a}
200 Hz	32	88.31 ± 1.52 ^{a}	28.19 ± 0.18 ^{a}	12.12 ± 0.12 ^{b}	2.00 ± 0.04 ^{b}	66.40 ± 0.22 ^{b}
Time		$p = 0.99$ ‡	$p = 0.40$ ‡	$p = 0.30$ ‡	$p \leq 0.01$ †	$p = 0.05$ †
3 min	32	89.49 ± 1.52 ^{a}	28.38 ± 0.18 ^{a}	12.45 ± 0.12 ^{a}	2.29 ± 0.04 ^{a}	67.78 ± 0.22 ^{a}
6 min	32	89.5 ± 1.52 ^{a}	28.60 ± 0.18 ^{a}	12.27 ± 0.12 ^{a}	2.00 ± 0.04 ^{b}	67.19 ± 0.22 ^{b}
Dataset average	64	89.49 ± 0.50	28.60 ± 0.13	12.36 ± 0.09	2.15 ± 0.03	67.49 ± 0.14

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis. ANT—anthocyanins (mg Pg-3-G 100 mL^{-1}); TPC—total phenolic compounds (mg GAE 100 mL^{-1}); HCA—hydroxycinnamic acids (mg CAE 100 mL^{-1}); FL—flavonols (mg QE 100 mL^{-1}); CT—condensed tannins (mg CA 100 mL^{-1}).

Next, the contents of hydroxycinnamic acids were examined, which averaged 12.36 ± 0.09 mg 100 mL^{-1} in the treated samples (Table 5). As with the other polyphenols, the hydroxycinnamic acid content increased with maturity (10.62 mg 100 mL^{-1} for 75% maturity vs. 14.10 mg 100 mL^{-1} for 100% maturity). Similar to before, longer storage time also increased the hydroxycinnamic acid contents in the samples, by almost 43%. Galani et al. found that cold storage of fruits and vegetables had positive effect on the stability of phenolic acids, which was due to the accumulation of sugars, released after HIPEF treatment that could serve as substrates for their synthesis [44]. As observed with other polyphenol groups, the content of hydroxycinnamic acids increased with increasing electric field strength. Although no data comparing the effects of different electric field strengths on hydroxycinnamic acid content were found in the literature, changes in hydroxycinnamic acid content could be due to the degradation of more complex polyphenolic structures in HIPEF treatments [45]. Another emerging pattern similar to all studied polyphenols seem to be inverse relation among increased frequency and content of polyphenols in the samples. This is evident from the evaluation of hydroxycinnamic acids, whose content decreased slightly when the frequency was doubled from 100 Hz (12.60 ± 0.12 mg 100 mL^{-1}) to 200 Hz (12.12 ± 0.12 mg 100 mL^{-1}), or for about 4%. As with the other polyphenols, treatment duration had no effect on hydroxycinnamic acid content.

In addition, the average value of flavonols was 2.15 ± 0.03 mg 100 mL^{-1} in the data set of treatment samples (Table 5). As with other polyphenols, higher maturity yields more flavonols, as samples with 100% maturity (2.48 ± 0.04 mg 100 mL^{-1}) had 37% more flavonols than samples with 75% maturity (1.81 ± 0.04 mg 100 mL^{-1}; Table 4). In contrast to all other polyphenols, the content of flavonols decreased three-fold with prolonged storage, as their initial content before storage was 3.29 ± 0.04 mg 100 mL^{-1}, while after 7 days it was only 0.99 ± 0.04 mg 100 mL^{-1}. This was in agreement with the results of Odriozola Serrano et al. who found a 44% retention of quercetin during storage of HIPEF-treated strawberry juice (56 days at 4 °C) [33]. As with all other polyphenols, the same trend with increasing electric field strength was observed for flavonols as well. Here, a field strength of 50 kV cm^{-1} (2.38 ± 0.04 mg 100 mL^{-1}) resulted in a 1.3-fold higher contents of flavonols than the strength of 40 kV cm^{-1} (1.91 ± 0.04 mg 100 mL^{-1}). As before, a pattern for field frequency was observed for flavonols as for other polyphenols. Consequently, the field at 100 Hz frequency had 2.29 ± 0.04 mg 100 mL^{-1} flavonols (or 13% more) than 2.00 ± 0.04 mg 100 mL^{-1} for 200 Hz. In contrast to all other polyphenolic groups, the duration of HIPEF treatment decreased the flavonols content from 2.29 ± 0.04 mg 100 mL^{-1} (3 min) to 1.99 ± 0.04 mg 100 mL^{-1} (6 min) or by 13%. Odriozola Serrano et al. found a low flavonol content in HIPEF-treated strawberry juice (35 kV cm^{-1}, 100 Hz and 1700 µs) compared to phenolic acids and anthocyanins, which is in agreement with our results [33].

The last group of polyphenols evaluated were condensed tannins, which accounted for the majority of the total the polyphenol content (or 67.49 ± 0.14 mg 100 mL^{-1}) in the treated samples, and also represented the largest group of compounds in these experiments (Table 5). Similar to anthocyanins and hydroxycinnamic acids, the content of condensed tannins increased with increasing ripeness of strawberries. Interestingly, the content of all polyphenols increased during storage, except flavonols, whose concentration decreased (Table 5). Flavonols decreased by almost 70% after 7 days of storage, while condensed tannin content increased by 46%. This is somewhat expected since flavonols are known to condense to tannins [46], which was probably captured in the experiments and data. As with all other polyphenols, the content of condensed tannins increased with increasing electric field strength, which makes sense in the context of electroporosity and its well-documented relationship with field strength [40]. Although there are no data in the literature on the effects of different electric field strengths on condensed tannin contents, a study conducted by Manzoor et al. showed a significant increase in condensed tannin content in almond extracts compared to untreated samples [47].

Increasing the frequency, degraded 4% of the condensed tannin content, similar to what was previously observed for all other polyphenols. This strongly suggests that the electric field frequency has an optimal value that must be determined to obtain the maximum nutritional value, e.g., as measured by the polyphenol content in this case [48]. Similarly, for the HIPEF treatment time, it can be observed that when the time was doubled, 3% of polyphenols were degraded, from which we can also conclude that these parameters of the HIPEF treatment are sufficiently effective at a shorter treatment time (3 min). Moreover, in almost all cases, the treatment time did not show a significant effect on the content of bioactives (with the exception of flavonols and condensed tannins). This could perhaps be explained by the fact that the HIPEF process achieves its maximum extraction effect with a shorter treatment time. Increasing the treatment time from 3 min to 6 min had a negative effect on the flavonols and condensed tannins content, indicating possible degradation of these compounds at longer treatment time.

3.4. Comparison of the Stability of Bioactive Compounds in Treated vs. Untreated Strawberry Juices during Storage

When considering the stability of the bioactive compounds, it can be seen that the untreated samples had the same stability patterns (Tables 4 and 5). However, the HIPEF treatment resulted in slightly higher values of all the bioactive compounds determined, so this technology is not only an alternative to pasteurization, but can also be successfully

used to better maintain the stability of bioactive compounds in strawberry juices. Overall, HIPEF treatment increased bioactive compound levels by an average of 8.41% in all treated strawberry juices, regardless of ripeness and processing parameters.

However, an interesting observation was made in the data set regarding changes in polyphenols with increasing maturity and storage. In the untreated J2 juice samples, condensed tannins increased by 15% (at 75% to 100% maturity). This can be considered a normal condensation rate in the samples due to the increasing ripening of the strawberries. However, in the samples treated with HIPEF with the same ripening variation, this value was halved (8% condensation). The situation was similar for the storage and condensation of tannins. Here, the normal condensation rate for prolonged storage (from 0 to 7 days) was 94%. When the samples were treated with HIPEF, the condensation rate was halved to 46% for the same storage period.

Similarly, anthocyanins increased 25% in controls throughout storage, while they decreased three-fold in HIPEF-treated samples (only 8% increase for the same storage period). Increased degradation of cyanidin-3-glucoside under the influence of HIPEF in aqueous methanol medium was found by Zhang et al. while the increase of field strength and treatment duration had no significant effect on the food system [49]. This may indicate the influence of abundance on the decrease of anthocyanins, which was shown in our results by a decrease in the content of several groups of biologically active compounds. Accordingly, the content of total phenolics increased by 51% with storage (0–7 days) in the controls, while this rate was 15% in the HIPEF samples for the same storage parameters (in other words, HIPEF decreased the increase of all polyphenols threefold).

It is likely that anthocyanins and condensed tannins defined the above relations in the samples (i.e., they were mainly responsible for the observed changes in total phenolic content). For instance, observed data strongly suggested that HIPEF in some way halved the spontaneous increase and condensation of polyphenols (potentially important for personal customization of foods or synthesis of nutraceuticals if combined with data for particular bioavailability). This occurrence was potentially associated with previously mentioned increase in HIPEF frequency, which showed a tendency to decrease polyphenolic concentrations. A possible explanation could be sought in the differently charged species generated by HIPEF, which are capable of shifting electrostatic stability toward the degradation of tannins and anthocyanins when generated at sufficient concentrations. This makes even more sense considering that these two groups of polyphenols were the most represented in the samples. Another important conclusion from the data is the need to determine the optimal value for the aforementioned HIPEF treatment parameters.

3.5. Optimization of HIPEF Processing Parameters for Strawberry Juice Treatment

HIPEF technology belongs to the group of non-thermal technologies, which means that it operates at room temperature, or below or slightly above room temperature, thus enabling the preservation of heat-sensitive bioactive compounds [50]. Table 6. shows the average values of the HIPEF settings, where temperature can be highlighted as the most important factor influencing the quality of the final product. The average temperature of the treated samples was T2 = 22.93 $\pm$ 0.40 °C (room temperature) and no significant temperature differences were observed before and after HIPEF treatment (ΔT = 1.68 $\pm$ 1.00 °C), which is consistent with other literature data [14,16]. Since no statistically significant temperature variations were observed during HIPEF treatment, this confirms that temperature did not affect the stability of bioactive compounds in HIPEF-treated samples.

Table 6. Average values for experimental HIPEF settings.

Variables	T1 (°C)	T2 (°C)	ΔT (°C)	Voltage (kV)	Current (mA)	Power (W)
Electric field strength	$p = 0.30$ ‡	$p = 0.21$ ‡	$p = 0.80$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
40 kV cm^{-1}	20.11 ± 1.04 ^{a}	22.06 ± 0.57 ^{a}	1.95 ± 1.42 ^{a}	39.89 ± 0.02 ^{b}	3.00 ± 0.02 ^{b}	119.75 ± 0.72 ^{b}
50 kV cm^{-1}	21.75 ± 1.04 ^{a}	23.16 ± 0.57 ^{a}	1.41 ± 1.42 ^{a}	49.93 ± 0.02 ^{a}	3.84 ± 0.02 ^{a}	190.00 ± 0.72 ^{a}
Frequency	$p = 0.32$ ‡	$p = 0.44$ ‡	$p = 0.30$ ‡	$p = 0.58$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †
100 Hz	20.15 ± 1.04 ^{a}	22.94 ± 0.57 ^{a}	2.79 ± 1.42 ^{a}	44.90 ± 0.02 ^{a}	2.83 ± 0.02 ^{b}	128.38 ± 0.72 ^{b}
200 Hz	21.71 ± 1.04 ^{a}	22.29 ± 0.57 ^{a}	0.58 ± 1.42 ^{a}	44.91 ± 0.02 ^{a}	4.01 ± 0.02 ^{a}	181.38 ± 0.72 ^{a}
Treatment time	$p = 0.50$ ‡	$p = 0.49$ ‡	$p = 0.82$ ‡	$p = 0.12$ ‡	$p = 0.58$ ‡	$p = 0.25$ ‡
3 min	20.41 ± 1.04 ^{a}	22.33 ± 0.57 ^{a}	1.91 ± 1.42 ^{a}	44.89 ± 0.02 ^{a}	3.41 ± 0.02 ^{a}	154.25 ± 0.72 ^{a}
6 min	21.45 ± 1.04 ^{a}	22.90 ± 0.57 ^{a}	1.45 ± 1.42 ^{a}	44.93 ± 0.02 ^{a}	3.43 ± 0.02 ^{a}	155.50 ± 0.72 ^{a}
Dataset average	20.93 ± 0.74	22.93 ± 0.40	1.68 ± 1.00	44.91 ± 0.11	3.42 ± 0.01	154.88 ± 0.51

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; †—significant factor in multifactor analysis; ‡—not significant factor in multifactor analysis.

The average voltage in the samples was 44.91 ± 0.11 kV with an average expenditure of 3.42 ± 0.01 mA and power of 154.88 ± 0.51 W. Since voltage, current, and power are related to the electric field strength and frequency (excluding voltage) parameters, it is not surprising that there are statistically significant variations in these values. Voltage (kV) or electric field strength (kV cm^{-1}) has a direct effect on the electroporation of membrane cells in a plant, which translates into elevated release of bioactive compounds. A stronger electric field with a longer treatment time leads to irreversible electroporation of tissue cells and consequently tissue damages, which is the basis of how HIPEF technology works [51].

In order to find out which parameters of HIPEF treatment are most favorable for preservation of bioactive compounds in strawberry juice, process optimization was performed. Figure 2 shows response surface plots with the optimal HIPEF parameters for each group of bioactive compounds. The highest content of total polyphenols can be obtained at settings slightly different for each polyphenol group. For example, 113.75 mg 100 mL^{-1} of total polyphenols can be best obtained after almost 7 days of storage at lower ripeness (near 75% ripeness) with HIPEF settings of 49.90 kV cm^{-1} at a frequency of 199.74 Hz and a treatment time of 3.02 min (Table 7).

Table 7. Optimal HIPEF parameters for maximum mg 100 mL^{-1} of polyphenols in strawberry juices.

Analytical Variable	TPC	ANT	HCA	FL	CT
Content (mg 100 mL^{-1})	113.75	41.04	18.00	4.85	86.07
Maturity (%)	75.40	100.00	100.00	100.00	100.00
Storage (days)	6.96	7.00	7.00	0.00	7.00
Field (kV cm^{-1})	49.90	50.00	50.00	50.00	50.00
Frequency (Hz)	199.74	100.00	100.00	100.00	100.00
Time (min)	3.02	3.00	6.00	3.00	3.00

TPC—total phenolic compounds (mg GAE 100 mL^{-1}); ANT—anthocyanins (mg Pg-3-G 100 mL^{-1}); HCA—hydroxycinnamic acids (mg CAE 100 mL^{-1}); FL—flavonols (mg QE 100 mL^{-1}); CT—condensed tannins (mg CA 100 mL^{-1}).

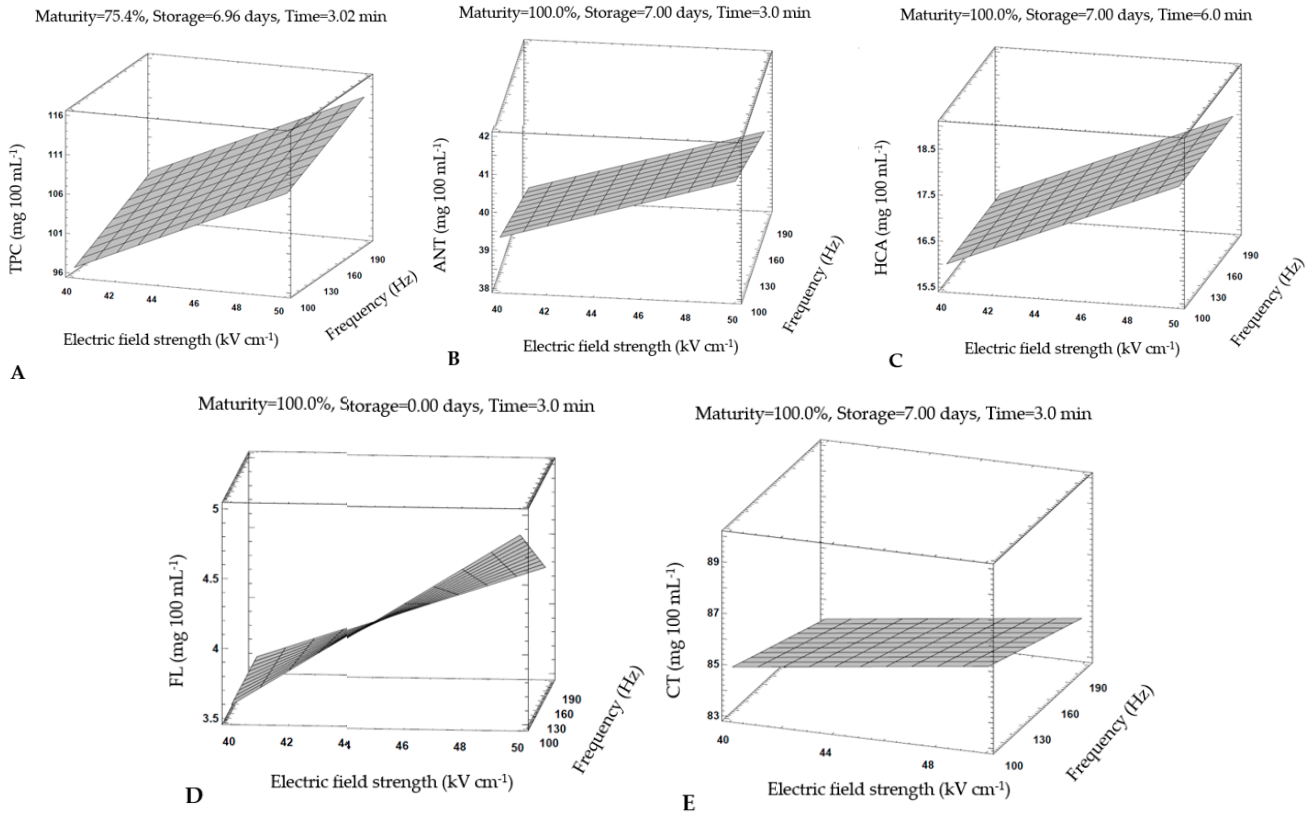

Figure 2. Response surface plots with optimal HIPEF parameters for all bioactive compounds in strawberry juices: (**A**) total phenolic compounds; (**B**) anthocyanins; (**C**) hydroxycinnamic acids; (**D**) flavonols; (**E**) condensed tannins.

This was similar to other individual polyphenol groups, except that all other polyphenols had the highest concentrations at lower frequency (100 Hz) and higher maturity (100%). These results suggested that strawberry juices of both ripeness levels are suitable for HIPEF treatment, and depending on which bioactive constituents are to be better preserved in the final product, the strawberry raw material for juice production could be selected based on its ripeness level.

All increases in polyphenol content favored longer storage (except flavonols) and shorter treatment times (except hydroxycinnamic acids). Regarding the storage and its positive effects on bioactive compound contents (except flavonols), a possible explanation could be in the subsequent extraction or leakage of bioactive compounds from damaged tissue cells. Odriozola Serrano et al. performed a kinetic study on anthocyanins, vitamin C and antioxidant activity of strawberry juice treated with HIPEF technology (20, 25, 30, and 35 kV cm^{-1} during 100, 300, 600, 1000, 1500 and 2000 µs at 232 Hz). The results of their studies agree with ours, confirming that anthocyanins are better preserved with a shorter treatment time and a stronger electric field. Moreover, they proposed a mathematical model based on Weibull equation with electric field strength and treatment time parameters to predict changes in anthocyanin content during HIPEF treatment [52]. Regardless of the type of bioactive component, in almost all cases (Figure 2), the maximum values of bioactive compounds were at highest value of electric field strength (50 kV cm^{-1}), which is consistent with other literature data [48]. Better permeabilization is associated with an increase in tissue electrical conductivity due to HIPEF treatment [53]; therefore, an increase in cell disruption and ultimately better extraction of bioactive compounds has been observed with an increase in electric field strength [38,54].

When considering the influence of frequency on bioactive compounds, the lower frequency (100 Hz) had a positive effect on the content of individual bioactive compounds than the higher frequency (200 Hz). These results are confirmed by the research of Martin-

Garcia et al. who performed an optimization of the HIPEF parameters of brewers' spent grain (BSG): electric field strength (0.5, 1.5, and 2.5 kV cm^{-1}), frequency (50, 100, and 150 Hz) and total treatment time (5, 10, and 15 s), where the maximum values of total polyphenolic compounds were at the highest value of electric field strength (2.5 kV cm^{-1}) and the lowest frequency tested (50 Hz) [48]. A possible explanation for this trend could be that lower frequencies result in a higher degree of permeabilization of the plant tissue compared to higher frequencies, which consequently translates into better extraction of the respective compounds [55,56].

4. Conclusions

Strawberry juices from different ripening stages differed significantly in terms of physicochemical parameters and bioactive compound contents. Regarding the influence of HIPEF parameters on physicochemical properties of strawberry juice, no statistically significant changes in pH were observed, while higher electric field strength and longer treatment duration influenced the increase in SSC value.

In general, HIPEF-treated strawberry juices showed an 8.41% higher content of bioactive compounds compared to untreated juices, regardless of ripeness and processing parameters. Considering the influence of HIPEF parameters on the content of bioactive compounds, the increase in electric field strength led to an increase in the content of most bioactive compounds (except flavonols). Moreover, frequency, in contrast to electric field strength, showed an inverse effect on the content of all observed bioactive compounds. Treatment duration had no effect on the content of most observed bioactive compounds (except flavonols and condensed tannins). Storage at +4 °C for 7 days resulted in an increase in all phenols studied (except flavonols) and a decrease in SSC.

Based on the obtained results, the optimal parameters for carrying out the HIPEF treatment that affected the total phenolic content were as follows: electric field strength of 49.90 kV cm^{-1}, frequency 199.74 Hz, treatment time 3.02 min with storage for 7 days. However, the optimization parameters indicated that the strawberry juices obtained at both ripening stages are suitable for HIPEF treatment, which can preserve the quality of strawberry juices.

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References

1. Basu, A.; Nguyen, A.; Betts, N.M.; Lyons, T.J. Strawberry as a functional food: An evidence-based review. *Crit. Rev. Food Sci. Nutr.* **2013**, *54*, 790–806. [CrossRef] [PubMed]
2. Tomašević, I.; Putnik, P.; Valjak, F.; Pavlić, B.; Šojić, B.; Markovinović, A.B.; Kovačević, D.B. 3d printing as novel tool for fruit-based functional food production. *Curr. Opin. Food Sci.* **2021**, *41*, 138–145. [CrossRef]
3. Morales-Quintana, L.; Ramos, P. Chilean strawberry (*Fragaria chiloensis*): An integrative and comprehensive review. *Food Res. Int.* **2019**, *119*, 769–776. [CrossRef]

4. Milić, A.; Daničić, T.; Horecki, A.T.; Šumić, Z.; Teslić, N.; Kovačević, D.B.; Putnik, P.; Pavlić, B. Sustainable extractions for maximizing content of antioxidant phytochemicals from black and red currants. *Foods* **2022**, *11*, 325. [CrossRef] [PubMed]
5. Miller, K.; Feucht, W.; Schmid, M. Bioactive compounds of strawberry and blueberry and their potential health effects based on human intervention studies: A brief overview. *Nutrients* **2019**, *11*, 1510. [CrossRef] [PubMed]
6. Šamec, D.; Maretić, M.; Lugarić, I.; Mešić, A.; Salopek-Sondi, B.; Duralija, B. Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis. *Food Chem.* **2016**, *194*, 828–834. [CrossRef] [PubMed]
7. Jurić, S.; Vlahoviček-Kahlina, K.; Duralija, B.; Maslov Bandić, L.; Nekić, P.; Vinceković, M. Stimulation of plant secondary metabolites synthesis in soilless cultivated strawberries (*Fragaria* × *ananassa* duchesne) using zinc-alginate microparticles. *Turk. J. Agric. For.* **2021**, *45*, 324–334. [CrossRef]
8. Zhao, Z.; Wu, X.; Chen, H.; Liu, Y.; Xiao, Y.; Chen, H.; Tang, Z.; Li, Q.; Yao, H. Evaluation of a strawberry fermented beverage with potential health benefits. *PeerJ* **2021**, *9*, 11974. [CrossRef]
9. Cataldo, P.G.; Villena, J.; Elean, M.; de Giori, G.S.; Saavedra, L.; Hebert, E.M. Immunomodulatory properties of a γ-aminobutyric acid-enriched strawberry juice produced by *Levilactobacillus brevis* crl 2013. *Front. Microbiol.* **2020**, *11*, 610016. [CrossRef]
10. Leyva-Porras, C.; Saavedra-Leos, M.Z.; López-Martinez, L.A.; Espinosa-Solis, V.; Terán-Figueroa, Y.; Toxqui-Terán, A.; Compeán-Martínez, I. Strawberry juice powders: Effect of spray-drying conditions on the microencapsulation of bioactive components and physicochemical properties. *Molecules* **2021**, *26*, 5466. [CrossRef]
11. Mazur, S.P.; Nes, A.; Wold, A.-B.; Remberg, S.F.; Martinsen, B.K.; Aaby, K. Effects of ripeness and cultivar on chemical composition of strawberry (*Fragaria* × *ananassa* duch.) fruits and their suitability for jam production as a stable product at different storage temperatures. *Food Chem.* **2014**, *146*, 412–422. [CrossRef] [PubMed]
12. Markovinović, A.B.; Putnik, P.; Duralija, B.; Krivohlavek, A.; Ivešić, M.; Andačić, I.M.; Bešlić, I.P.; Pavlić, B.; Lorenzo, J.M.; Kovačević, D.B. Chemometric valorization of strawberry (*Fragaria x ananassa* duch.) cv. 'Albion' for the production of functional juice: The impact of physicochemical, toxicological, sensory, and bioactive value. *Foods* **2022**, *11*, 640. [CrossRef] [PubMed]
13. Yildiz, S.; Pokhrel, P.R.; Unluturk, S.; Barbosa-Cánovas, G.V. Changes in quality characteristics of strawberry juice after equivalent high pressure, ultrasound, and pulsed electric fields processes. *Food Eng. Rev.* **2020**, *13*, 601–612. [CrossRef]
14. Odriozola-Serrano, I.; Aguiló-Aguayo, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Pulsed electric fields processing effects on quality and health-related constituents of plant-based foods. *Trends Food Sci. Technol.* **2013**, *29*, 98–107. [CrossRef]
15. Aguiló-Aguayo, I.; Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. *LWT Food Sci. Technol.* **2009**, *42*, 813–818. [CrossRef]
16. Gabrić, D.; Barba, F.; Roohinejad, S.; Gharibzahedi, S.M.T.; Radojčin, M.; Putnik, P.; Kovačević, D.B. Pulsed electric fields as an alternative to thermal processing for preservation of nutritive and physicochemical properties of beverages: A review. *J. Food Process Eng.* **2018**, *41*, e12638. [CrossRef]
17. Elez-Martínez, P.; Odriozola-Serrano, I.; Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Effects of pulsed electric fields processing strategies on health-related compounds of plant-based foods. *Food Eng. Rev.* **2017**, *9*, 213–225. [CrossRef]
18. Asl, P.J.; Rajulapati, V.; Gavahian, M.; Kapusta, I.; Putnik, P.; Khaneghah, A.M.; Marszałek, K. Non-thermal plasma technique for preservation of fresh foods: A review. *Food Control* **2022**, *134*, 108560. [CrossRef]
19. Škegro, M.; Putnik, P.; Kovačević, D.B.; Kovač, A.P.; Salkić, L.; Čanak, I.; Frece, J.; Zavadlav, S.; Ježek, D. Chemometric comparison of high-pressure processing and thermal pasteurization: The nutritive, sensory, and microbial quality of smoothies. *Foods* **2021**, *10*, 1167. [CrossRef]
20. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the ph differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278. [CrossRef]
21. Oszmiański, J.; Wojdyło, A. Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. *Eur. Food Res. Technol.* **2008**, *228*, 623–631. [CrossRef]
22. Howard, L.R.; Clark, J.R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238–1247. [CrossRef]
23. Sun, B.; Ricardo-da-Silva, J.M.; Spranger, I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. [CrossRef]
24. Hwang, H.; Kim, Y.-J.; Shin, Y. Influence of ripening stage and cultivar on physicochemical properties, sugar and organic acid profiles, and antioxidant compositions of strawberries. *Food Sci. Biotechnol.* **2019**, *28*, 1659–1667. [CrossRef]
25. Ornelas-Paz, J.d.J.; Yahia, E.M.; Ramírez-Bustamante, N.; Pérez-Martínez, J.D.; Escalante-Minakata, M.D.P.; Ibarra-Junquera, V.; Acosta-Muñiz, C.; Guerrero-Prieto, V.; Ochoa-Reyes, E. Physical attributes and chemical composition of organic strawberry fruit (*Fragaria x ananassa* duch, cv. Albion) at six stages of ripening. *Food Chem.* **2013**, *138*, 372–381. [CrossRef]
26. Liu, Y.; Zhang, Y.; Jiang, X.; Liu, H. Detection of the quality of juicy peach during storage by visible/near infrared spectroscopy. *Vib. Spectrosc.* **2020**, *111*, 103152. [CrossRef]
27. Wang, X.; Wang, S.; Wang, W.; Ge, Z.; Zhang, L.; Li, C.; Zhang, B.; Zong, W. Comparison of the effects of dynamic high-pressure microfluidization and conventional homogenization on the quality of peach juice. *J. Sci. Food Agric.* **2019**, *99*, 5994–6000. [CrossRef]

28. Aaby, K.; Grimsbo, I.H.; Hovda, M.B.; Rode, T.M. Effect of high pressure and thermal processing on shelf life and quality of strawberry purée and juice. *Food Chem.* **2018**, *260*, 115–123. [CrossRef]
29. Mtaoua, H.; Sánchez-Vega, R.; Ferchichi, A.; Martín-Belloso, O. Impact of high-intensity pulsed electric fields or thermal treatment on the quality attributes of date juice through storage. *J. Food Processing Preserv.* **2017**, *41*, e13052. [CrossRef]
30. Geveke, D.J.; Aubuchon, I.; Zhang, H.Q.; Boyd, G.; Sites, J.E.; Bigley, A.B.W. Validation of a pulsed electric field process to pasteurize strawberry purée. *J. Food Eng.* **2015**, *166*, 384–389. [CrossRef]
31. Vallverdú-Queralt, A.; Odriozola-Serrano, I.; Oms-Oliu, G.; Lamuela-Raventós, R.M.; Elez-Martínez, P.; Martín-Belloso, O. Changes in the polyphenol profile of tomato juices processed by pulsed electric fields. *J. Agric. Food Chem.* **2012**, *60*, 9667–9672. [CrossRef] [PubMed]
32. Vallverdú-Queralt, A.; Odriozola-Serrano, I.; Oms-Oliu, G.; Lamuela-Raventós, R.M.; Elez-Martínez, P.; Martín-Belloso, O. Impact of high-intensity pulsed electric fields on carotenoids profile of tomato juice made of moderate-intensity pulsed electric field-treated tomatoes. *Food Chem.* **2013**, *141*, 3131–3138. [CrossRef] [PubMed]
33. Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Phenolic acids, flavonoids, vitamin c and antioxidant capacity of strawberry juices processed by high-intensity pulsed electric fields or heat treatments. *Eur. Food Res. Technol.* **2008**, *228*, 239–248. [CrossRef]
34. Pradas, I.; Medina, J.J.; Ortiz, V.; Moreno-Rojas, J.M. 'Fuentepina' and 'amiga', two new strawberry cultivars: Evaluation of genotype, ripening and seasonal effects on quality characteristics and health-promoting compounds. *J. Berry Res.* **2015**, *5*, 157–171. [CrossRef]
35. Fait, A.; Hanhineva, K.; Beleggia, R.; Dai, N.; Rogachev, I.; Nikiforova, V.J.; Fernie, A.R.; Aharoni, A. Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. *Plant Physiol.* **2008**, *148*, 730–750. [CrossRef]
36. Aubert, C.; Bruaut, M.; Chalot, G.; Cottet, V. Impact of maturity stage at harvest on the main physicochemical characteristics, the levels of vitamin c, polyphenols and volatiles and the sensory quality of gariguette strawberry. *Eur. Food Res. Technol.* **2020**, *247*, 37–49. [CrossRef]
37. Wei, M.; Wang, H.; Ma, T.; Ge, Q.; Fang, Y.; Sun, X. Comprehensive utilization of thinned unripe fruits from horticultural crops. *Foods* **2021**, *10*, 2043. [CrossRef]
38. Luengo, E.; Álvarez, I.; Raso, J. Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. *Innov. Food Sci. Emerg. Technol.* **2013**, *17*, 79–84. [CrossRef]
39. Roobab, U.; Aadil, R.M.; Madni, G.M.; Bekhit, A.E.-D. The impact of nonthermal technologies on the microbiological quality of juices: A review. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 437–457. [CrossRef]
40. Barba, F.J.; Parniakov, O.; Pereira, S.A.; Wiktor, A.; Grimi, N.; Boussetta, N.; Saraiva, J.A.; Raso, J.; Martin-Belloso, O.; Witrowa-Rajchert, D.; et al. Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Res. Int.* **2015**, *77*, 773–798. [CrossRef]
41. Siddeeg, A.; Manzoor, M.F.; Ahmad, M.H.; Ahmad, N.; Ahmed, Z.; Khan, M.K.I.; Maan, A.A.; Mahr Un, N.; Zeng, X.-A.; Ammar, A.-F. Pulsed electric field-assisted ethanolic extraction of date palm fruits: Bioactive compounds, antioxidant activity and physicochemical properties. *Processes* **2019**, *7*, 585. [CrossRef]
42. Stübler, A.-S.; Lesmes, U.; Juadjur, A.; Heinz, V.; Rauh, C.; Shpigelman, A.; Aganovic, K. Impact of pilot-scale processing (thermal, pef, hpp) on the stability and bioaccessibility of polyphenols and proteins in mixed protein- and polyphenol-rich juice systems. *Innov. Food Sci. Emerg. Technol.* **2020**, *64*, 102426. [CrossRef]
43. Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Impact of high-intensity pulsed electric fields variables on vitamin c, anthocyanins and antioxidant capacity of strawberry juice. *LWT Food Sci. Technol.* **2009**, *42*, 93–100. [CrossRef]
44. Galani, J.H.Y.; Patel, J.S.; Patel, N.J.; Talati, J.G. Storage of fruits and vegetables in refrigerator increases their phenolic acids but decreases the total phenolics, anthocyanins and vitamin c with subsequent loss of their antioxidant capacity. *Antioxidants* **2017**, *6*, 59. [CrossRef] [PubMed]
45. Odriozola-Serrano, I.; Soliva-Fortuny, R.; Hernández-Jover, T.; Martín-Belloso, O. Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments. *Food Chem.* **2009**, *112*, 258–266. [CrossRef]
46. Mukherjee, P.K. Qualitative analysis for evaluation of herbal drugs. In *Quality Control and Evaluation of Herbal Drugs*; Elsevier: London, UK, 2019; pp. 79–149.
47. Manzoor, M.F.; Zeng, X.-A.; Rahaman, A.; Siddeeg, A.; Aadil, R.M.; Ahmed, Z.; Li, J.; Niu, D. Combined impact of pulsed electric field and ultrasound on bioactive compounds and ft-ir analysis of almond extract. *J. Food Sci. Technol.* **2019**, *56*, 2355–2364. [CrossRef]
48. Martín-García, B.; Tylewicz, U.; Verardo, V.; Pasini, F.; Gómez-Caravaca, A.M.; Caboni, M.F.; Rosa, M.D. Pulsed electric field (pef) as pre-treatment to improve the phenolic compounds recovery from brewers' spent grains. *Innov. Food Sci. Emerg. Technol.* **2020**, *64*, 102402. [CrossRef]
49. Zhang, Y.; Liao, X.; Ni, Y.; Wu, J.; Hu, X.; Wang, Z.; Chen, F. Kinetic analysis of the degradations and its color change of cyaniding-3-glucoside exposed to pulsed electric field. *Eur. Food Res. Technol.* **2007**, *224*, 597–603. [CrossRef]
50. Putnik, P.; Pavlić, B.; Šojić, B.; Zavadlav, S.; Žuntar, I.; Kao, L.; Kitonić, D.; Kovačević, D.B. Innovative hurdle technologies for the preservation of functional fruit juices. *Foods* **2020**, *9*, 699. [CrossRef]

51. Poojary, M.M.; Lund, M.N.; Barba, F.J. Pulsed electric field (pef) as an efficient technology for food additives and nutraceuticals development. In *Pulsed Electric Fields to Obtain Healthier and Sustainable Food for Tomorrow*; Elsevier: London, UK, 2020; pp. 65–99.
52. Odriozola-Serrano, I.; Soliva-Fortuny, R.; Gimeno-Añó, V.; Martín-Belloso, O. Kinetic study of anthocyanins, vitamin c, and antioxidant capacity in strawberry juices treated by high-intensity pulsed electric fields. *J. Agric. Food Chem.* **2008**, *56*, 8387–8393. [CrossRef]
53. Guderjan, M.; Töpfl, S.; Angersbach, A.; Knorr, D. Impact of pulsed electric field treatment on the recovery and quality of plant oils. *J. Food Eng.* **2005**, *67*, 281–287. [CrossRef]
54. Puértolas, E.; Cregenzán, O.; Luengo, E.; Álvarez, I.; Raso, J. Pulsed-electric-field-assisted extraction of anthocyanins from purple-fleshed potato. *Food Chem.* **2013**, *136*, 1330–1336. [CrossRef] [PubMed]
55. Asavasanti, S.; Ristenpart, W.; Stroeve, P.; Barrett, D.M. Permeabilization of plant tissues by monopolar pulsed electric fields: Effect of frequency. *J. Food Sci.* **2011**, *76*, E98–E111. [CrossRef] [PubMed]
56. Ersus, S.; Oztop, M.H.; McCarthy, M.J.; Barrett, D.M. Disintegration efficiency of pulsed electric field induced effects on onion (*Allium cepa* L.) tissues as a function of pulse protocol and determination of cell integrity by 1h-nmr relaxometry. *J. Food Sci.* **2010**, *75*, E444–E452. [CrossRef]

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A Chemometric Investigation on the Functional Potential in High Power Ultrasound (HPU) Processed Strawberry Juice Made from Fruits Harvested at two Stages of Ripeness

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Abstract: This work aimed to investigate the influence of high-power ultrasound (HPU) technology on the stability of bioactive compounds in strawberry juices obtained from fruits with different stages of ripeness (75% vs. 100%) and stored at 4 °C for 7 days. HPU parameters were amplitude (25, 50, 75, and 100%), pulses (50 vs. 100%) and treatment time (5 vs. 10 min). Amplitude and pulse had a significant effect ($p \leq 0.05$) on all bioactive compounds except flavonols and hydroxycinnamic acids. The treatment duration of 5 min vs. 10 min had a significant positive impact on the content of anthocyanins, flavonols and condensed tannins, while the opposite was observed for total phenols, whereas no statistically significant effect was observed for hydroxycinnamic acids. The temperature changes during HPU treatment correlated positively with almost all HPU treatment parameters (amplitude, pulse, energy, power, frequency). Optimal parameters of HPU were obtained for temperature changes, where the highest content of a particular group of bioactive compounds was obtained. Results showed that by combining fruits with a certain ripeness and optimal HPU treatment, it would be possible to produce juices with highly preserved bioactive compounds, while HPU technology has prospects for application in functional food products.

Keywords: strawberry juice; maturity; ultrasound; polyphenols; anthocyanins; processing; functional food

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1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a seasonal fruit that is very popular among consumers, as well as its products such as jams, purees and juices [1]. This fruit is rich in various bioactive compounds, especially anthocyanins, phenolic acids and flavonoids, which have many health-promoting properties due to their antioxidant effects [2–4]. On the other hand, the increased incidence of certain diseases (e.g., obesity, cardiovascular and neurological diseases, cancer) associated with poor diets and unhealthy lifestyles has made consumers in today's world more aware of healthy diets. Functional foods such as juices [5] are particularly important as part of a healthy diet [6]. Considering their chemical composition and biological potential, strawberries can be considered a functional food [7]. Therefore, strawberry fruits are used in the production of various functional products such as functional dairy products [8,9], functional gluten-free products [10], functional

fermented beverages [11] and products [12], functional marmalade [13], functional jelly candies [14], functional fruit juices [15], functional fruit drinks [16] and nectars [17].

Functional strawberry juices are of particular interest as they have been shown to possess anti-inflammatory, cardioprotective, anticancer, antioxidant, genoprotective, and neuroprotective properties [7]. Strawberries also showed promising antiviral properties against various pathogenic viruses [18], which is especially important in the current era of global pandemics and crises. Moreover, the global fruit and vegetable juices market was estimated to reach USD 131.62 billion in 2021 and is projected to grow at a compound annual growth rate (CAGR) of 6.3% from 2022 to 2030. Therefore, the interest in producing and consuming juices is steadily increasing [19].

The most critical parameter for successful strawberry juice production is the selection of the cultivar that best suits the cultivation method and ecological conditions [20,21]. Strawberries are susceptible to transportation, which significantly affects their quality, i.e., physical and chemical parameters, bioactive composition and sensory properties [22]. A recent study has confirmed that strawberries harvested at a lower stage of ripeness (75% red fruits) have better structural characteristics for transport and storage, while their processing into juice has also been shown to be good in terms of maintaining quality compared to strawberry juices obtained from 100% ripe strawberries [23]. When processed into juice, fruits with 75% ripeness showed less color change than fruits with 100% ripeness, and sensory characteristics were similar to fully ripe fruits [23]. Thus, by selecting an appropriate cultivar and fruit ripeness level, as well as a strategy for sustainable processing, strawberries could be an excellent raw material for producing high-quality functional juices and serving as fruit meals for consumers while meeting recommendations for higher daily fruit intake [24].

In industrial production, juices are usually preserved by the conventional pasteurization process to extend their shelf-life. Besides the thermolabile bioactive compounds, high temperatures during pasteurization generally cause chemical alterations in volatile fractions and, consequently, affect strawberry products' nutritional, biological, and sensory quality [25,26]. Hartmann et al. [27] found a significant loss of vitamin C, anthocyanins, total phenols and antioxidant capacity by heating strawberry juices at 85 °C for 5 s and 15 min. A longer processing time at the same temperature (85 °C) resulted in significantly higher losses of polyphenols and anthocyanins. In addition, heating causes reactions between hexoses with amino acids and the formation of Maillard reaction products, which cause browning of the juice and negatively affect the product's appearance and overall sensory impression [28]. Therefore, the application of innovative non-thermal technologies has been increasingly explored recently. Chemat et al. [29,30] emphasized that when selecting new process technologies, special attention should be paid to environmentally friendly and sustainable technologies that allow for saving both energy and water while ensuring a safe and high-quality product. One of these new technologies is ultrasound-assisted processing.

The main phenomenon responsible for the effects of ultrasonic processing on liquid media is acoustic cavitation [31], which causes various physical and chemical changes [30]. Compared to thermal pasteurization, ultrasonic processing of juices has the following main advantages: control of enzymatic browning, better preservation of bioactive compounds, prolonged shelf life and improved physicochemical properties of fruit juices [32]. Studies have shown that High Power Ultrasound (HPU) treatment does not cause significant changes in physicochemical and color parameters of strawberry juice and that HPU treatment contributes to significant improvements in bioactive compounds' contents (i.e., total phenolic content, ascorbic acid, total anthocyanins) and antioxidant activity of the product depending on the adjusted process parameters [33–36].

Since HPU technology has not yet been used to process fruit of different ripeness levels, it is unknown whether this technology would be suitable for processing strawberry juices from two ripeness levels. A recent study applying High Intensity Pulsed Electric Field (HIPEF) has shown that this technology is suitable for processing strawberry juices

from different ripening stages, as it improves the stability of bioactive compounds during shelf life [37].

In summary, this study aimed to investigate the influence of HPU treatment parameters (amplitude, pulse, and treatment duration) on the stability of bioactive compounds in strawberry juices from fruits of different ripening stages (75% vs. 100%) during storage at 4 °C for 7 days. The application of chemometrics to evaluate the influence of ultrasonic processing parameters was utilized to find optimal HPU processing conditions in terms of the highest stability of bioactive compounds.

2. Results and Discussion

2.1. The Use of Chemometrics for the Evaluation of HPU Processing

Untreated juices (control samples) from both ripening stages were compared in terms of soluble solids content (SSC) and pH, as well as total phenolic compounds (TPC), monomeric anthocyanins (ANT), hydroxycinnamic acids (HCA), flavonols (FL) and condensed tannins (CT). The levels of all studied bioactive compounds in the control samples were higher in juice from 100% ripe fruit than in 75% (Table 1). Aubert et al. [38] recorded a 1.6 times higher value of total phenols in fully ripe strawberry fruit than in 75% ripe strawberry fruit. The most represented group of bioactive compounds was CT (81.05%), followed by HCA (14.74%), ANT (13.02%) and finally, FL (3.26%). The average values of the bioactive compounds of the control samples are shown in Table 1.

Table 1. Average values for analytical parameters in untreated samples (control).

Maturity	TPC	ANT	HCA	FL	CT
75%	93.62 ± 1.99	8.99 ± 0.13	10.56 ± 0.68	1.77 ± 0.68	70.65 ± 1.53
100%	96.18 ± 3.81	17.84 ± 0.31	16.66 ± 0.71	2.44 ± 0.82	88.75 ± 1.60

Results are expressed as the mean ± STD for control samples in mg 100 mL^{-1}; TPC—total phenolic compounds; ANT—monomeric anthocyanins; HCA—hydroxycinnamic acids; FL—flavonols; CT—condensed tannins.

Data analysis initially examined relationships between the samples by an exploratory hierarchical Ward's cluster analysis. When samples were analyzed for standardized similarities for various types of treatments (controls vs. HPU), maturity (%), HPU pulse (%), amplitude (%), treatment time (min), content of TPC (mg 100 mL^{-1}), ANT (mg 100 mL^{-1}), HCA (mg 100 mL^{-1}), FL (mg 100 mL^{-1}), CT (mg 100 mL^{-1}), SSC (%), and pH, it was revealed that the most similar samples to controls were those treated with a maturity of 100%, a pulse of 50%, an amplitude of 25%, and treatment durations of 5 and 10 min. They were similar to controls and clustered together regardless of maturity (Figure 1). Other samples similar to controls were those with a maturity of 100%, a pulse of 100%, an amplitude of 25%, and a treatment duration of 5 min.

More detailed examination with Kruskal–Wallis analysis revealed that the chemical profile of the control samples compared with the HPU-treated samples was similar for all polyphenolic groups, except for CT. This group of compounds was found in higher amounts in the HPU samples (Table 2). These results could be a consequence of the effect of cavitation under the influence of HPU [39], which degrades the cellular structure and allows easier CT extraction into the surrounding juice [40,41]. In their research, Bautista-Ortin et al. [42] concluded that HPU treatment is the most effective method to preserve and extract CT during red winemaking, regardless of the different maceration times. Similar results were confirmed by the research of Plaza et al. [43].

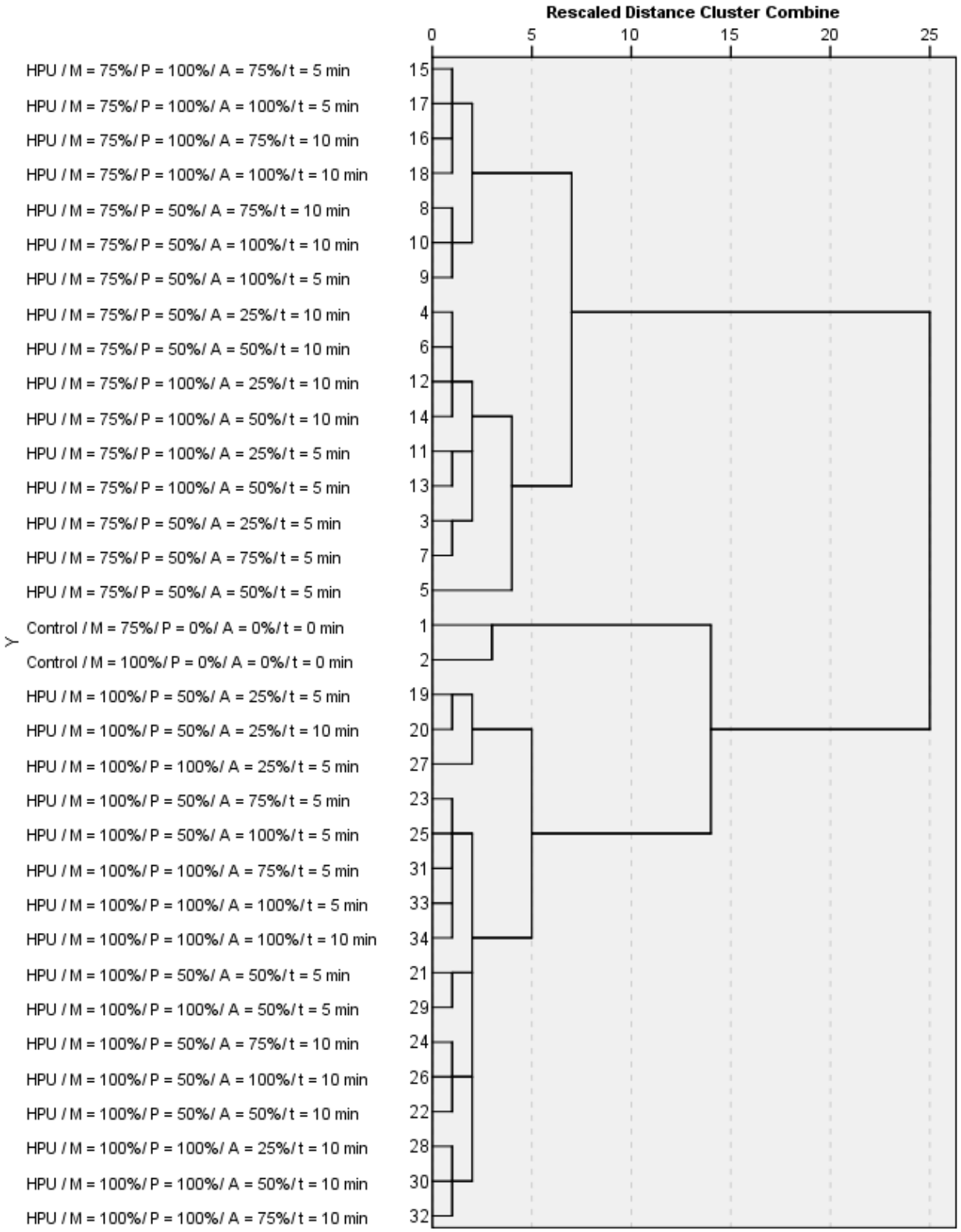

Figure 1. Results of the hierarchical cluster analysis of averaged and standardized samples.

Table 2. Kruskal–Wallis test statistics for the HPU vs. control samples.

Bioactive Compound	Treatment	Mean Rank	Chi-Square	Significance
TPC	Control HPU	2.50 32.5	2.528	0.11
ANT	Control HPU	2.50 32.5	1.838	0.18
HCA	Control HPU	2.50 32.5	0.633	0.43
FL	Control HPU	2.50 32.5	1.416	0.23
CT	Control HPU	2.50 32.5	4.134	0.04

Results are expressed as the mean rank for control vs. HPU samples mg 100 mL^{-1}; TPC—total phenolic compounds; ANT—monomeric anthocyanins; HCA—hydroxycinnamic acids; FL—flavonols; CT—condensed tannins.

2.2. The Changes of Bioactive Compounds in Strawberry Juices under HPU Processing and Storage

Table 3 shows the influence of ripeness and storage on the control juice samples.

Table 3. Changes in the bioactive compounds in control juice samples during storage.

Variable	n	TPC	ANT	HCA	FL	CT
Maturity	4	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.06$ ‡	$p \leq 0.01$ †
75%	4	103.1 ± 1.26^{a}	8.84 ± 0.09^{b}	11.71 ± 0.24^{b}	2.25 ± 0.55^{a}	75.56 ± 0.91^{b}
100%	4	90.73 ± 1.26^{b}	17.21 ± 0.09^{a}	17.76 ± 0.24^{a}	4.27 ± 0.55^{a}	86.54 ± 0.91^{a}
Storage		$p = 0.09$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.02$ †	$p = 0.10$ ‡
0 days	4	94.90 ± 1.26^{a}	13.42 ± 0.09^{a}	13.61 ± 0.24^{b}	2.11 ± 0.55^{b}	79.70 ± 0.91^{a}
7 days	4	98.94 ± 1.26^{a}	12.63 ± 0.09^{b}	15.86 ± 0.24^{a}	4.41 ± 0.55^{a}	82.39 ± 0.91^{a}
Dataset average	8	96.92 ± 0.89	13.023 ± 0.09	14.74 ± 0.17	3.26 ± 0.39	81.05 ± 0.64

The results are expressed as the mean ± standard error in mg 100 mL^{-1}. Values represented with different letters in a column are statistically different at $p \leq 0.05$. † significant factor in multifactor analysis. ‡ not significant factor in multifactor analysis. TPC—total phenolic content; ANT—monomeric anthocyanins; HCA—total hydroxycinnamic acids; FL—flavonols; CT—condensed tannins.

The levels of all studied bioactive compounds in the control samples, except for total phenolic compounds and FL, were significantly higher ($p \leq 0.01$) in juices made from 100% ripe fruit than those of 75% ripeness, this is largely consistent with our previous findings [37]. As ripeness increased, TPC decreased in control samples, which is consistent with other literature [44]. Indeed, phenolic compounds are synthesized in the skin of fruits, and considering the lower mass of unripe fruits, their proportion is higher in unripe (smaller) fruits than in larger (riper) fruits [44]. Anthocyanins, the compounds responsible for the red color of strawberries, were almost twice as abundant in juices made of 100% ripe strawberries vs. 75%. Considering that the intensity of the red color increases as strawberries ripen, it was expected that the riper fruits and their products would have higher levels of anthocyanins, which is consistent with the results of Pradas et al. [45]. The HCA content in juices from fully ripe strawberries is almost 52% higher compared to juices from 75% ripe fruits. The higher HCA concentration in the juice of fully ripe strawberries can be explained by the accumulation of phenolic acids [45]. FL was the only exception that was not affected by ripeness. According to studies by Pradas et al. [45], the degree of ripeness had no statistically significant effect on the proportion of flavonols, mainly determined by the cultivar.

Storage positively influenced the contents of HCA and FL in control samples, while the contents of ANT decreased (Table 3). The value of HCA was 6.2% higher after 7 days of

storage than after 0 days, which is in agreement with other literature reports [37,38]. The previous study confirmed that total and individual anthocyanins were degraded during storage according to first-order reaction kinetics, and the rate was strongly dependent on temperature [46]. However, storage of the juices for 7 days did not affect the content of TPC and CT. Similarly, the TPC content of the lychee juices after storage at 4 °C for 168 h was not significantly lower than that of the different lychee juices before storage [47].

The changes in bioactive compounds in HPU-treated juice samples during storage are shown in Table 4.

Table 4. Changes in the bioactive compounds in juice samples under HPU during storage.

Variable	n	TPC	ANT	HCA	FL	CT
Maturity		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
75%	64	92.61 ± 0.49 ^{a}	8.22 ± 0.03 ^{b}	10.66 ± 0.08 ^{b}	2.06 ± 0.11 ^{b}	67.23 ± 0.21 ^{b}
100%	64	78.59 ± 0.49 ^{b}	15.02 ± 0.03 ^{a}	14.92 ± 0.08 ^{a}	2.94 ± 0.11 ^{a}	70.48 ± 0.21 ^{a}
Amplitude		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.91$ ‡	$p \leq 0.01$ †
25%	32	89.08 ± 0.69 ^{a}	12.14 ± 0.04 ^{a}	13.24 ± 0.12 ^{a}	2.57 ± 0.16 ^{a}	73.56 ± 0.30 ^{a}
50%	32	86.22 ± 0.69 ^{b}	11.48 ± 0.04 ^{b}	12.83 ± 0.12 ^{b}	2.54 ± 0.16 ^{a}	68.87 ± 0.30 ^{b}
75%	32	82.16 ± 0.69 ^{c}	11.60 ± 0.04 ^{c}	12.66 ± 0.12 ^{b,c}	2.42 ± 0.16 ^{a}	67.76 ± 0.30 ^{c}
100%	32	84.94 ± 0.69 ^{b}	11.27 ± 0.04 ^{d}	12.44 ± 0.12 ^{c}	2.47 ± 0.16 ^{a}	65.23 ± 0.30 ^{d}
Pulse		$p = 0.02$ †	$p \leq 0.01$ †	$p = 0.19$ ‡	$p = 0.05$ †	$p \leq 0.01$ †
50%	64	84.88 ± 0.49 ^{b}	11.71 ± 0.03 ^{a}	12.86 ± 0.08 ^{a}	2.66 ± 0.12 ^{a}	69.30 ± 0.21 ^{a}
100%	64	86.32 ± 0.49 ^{a}	11.53 ± 0.03 ^{b}	12.72 ± 0.08 ^{a}	2.34 ± 0.12 ^{b}	68.41 ± 0.21 ^{b}
Treatment time		$p = 0.09$ ‡	$p \leq 0.01$ †	$p = 0.49$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †
5 min	64	84.97 ± 0.49 ^{a}	11.75 ± 0.03 ^{a}	12.75 ± 0.08 ^{a}	2.75 ± 0.12 ^{a}	69.38 ± 0.21 ^{a}
10 min	64	86.23 ± 0.49 ^{a}	11.50 ± 0.03 ^{b}	12.83 ± 0.08 ^{a}	2.26 ± 0.12 ^{b}	68.33 ± 0.21 ^{b}
Storage		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.54$ ‡
0 days	64	87.25 ± 0.49 ^{a}	12.03 ± 0.03 ^{a}	12.41 ± 0.08 ^{b}	1.81 ± 0.11 ^{b}	68.76 ± 0.21 ^{a}
7 days	64	83.95 ± 0.49 ^{b}	11.21 ± 0.03 ^{b}	13.18 ± 0.08 ^{a}	3.20 ± 0.11 ^{a}	68.95 ± 0.21 ^{a}
Dataset average	128	85.60 ± 0.34	11.62 ± 0.02	12.79 ± 0.05	2.50 ± 0.08	68.55 ± 0.15

The results are expressed as the mean ± standard error in mg 100 mL^{-1}. Values represented with different letters in a column are statistically different at $p \leq 0.05$. † significant factor in multifactor analysis. ‡ not significant factor in multifactor analysis. TPC—total phenolic content; ANT—monomeric anthocyanins; HCA—total hydroxycinnamic acids; FL—flavonols; CT—condensed tannins.

Juices from riper strawberries had higher levels of ANT, HCA, FL, and CT, but the same as in untreated juices, TPC content was higher in the samples from less ripe strawberries (75%). As mentioned earlier, higher TPC contents were found in unripe fruits compared to ripe fruits [44].

To find the optimal parameters for HPU processing of strawberry juices, it is necessary to consider the influence of each HPU process parameter on the stability of bioactive compounds. Thus, increasing the amplitude from 25% to 75% resulted in a statistically significant decrease in the TPC value, while further increasing the amplitude up to 100% significantly increased the TPC value. These results may indicate an optimal amplitude value at which the TPC is best preserved and emphasizes the need to optimize HPU parameters. In the study by Bursać Kovačević et al. [48], increasing the amplitude from 40 to 80% decreased ($p \leq 0.05$) the TPC value, which is in agreement with our study. On the other hand, Pala et al. [49] reported that amplitude (50%, 75%, and 100%) did not statistically significantly affect the TPC value in HPU-treated pomegranate juice.

Increasing the amplitude from 25 to 100% significantly decreased the content of ANT. Tiwari et al. [50] found the same trend where increasing the amplitude caused a statistically significant decrease in ANT content in HPU-treated strawberry juice (1500 W, 20 kHz). A significant decrease in ANT content at higher amplitudes (75 and 100%) in HPU-treated pomegranate juice was found by Pala et al. [49]. A possible reason for the observed trend could be explained by cavitational collapse and free radical formation [51,52]. Moreover,

the increase in amplitude significantly decreased HCA and CT. These results are consistent with the studies of Lukić et al. [53], who found that higher frequencies negatively affected the concentrations of total tannins in red wine. The studies performed by Celotti et al. [54] are in some agreement with the results obtained, as they found the same trend of decreasing CT value with increasing amplitude from 41 to 81% in red wine treated with HPU (200 W, 20 kHz) at 15 and 30 day storage.

Unlike the other groups of compounds, FL was the only exception and was not affected by the variations in amplitude. A similar pattern was found in the research of Bursać Kovačević et al. [48], where variations of amplitude (40 and 80%) did not significantly affect the content of total flavan-3-ols in HPU-treated cloudy apple juice.

As for the pulse, there are two basic types of ultrasound generation, continuous and pulsed. In pulsed generation, the ultrasound output of the generator is switched on and off for a short time and the process is repeated. However, the effect of pulse modulation on the quality characteristics of fruit juices is still not thoroughly explored [32]. Therefore, in this study, we aim to investigate how two pulse modes affect bioactive juice quality. To that end, a higher pulse duration (100%) positively affected the stability of TPC, while higher values of ANT, FL and CT were observed when treated with a lower pulse duration (50%). This influence was not related to the HCA content. In the literature, it is not possible to find a reason for this trend. Still, the 100% pulse may be too invasive for the stability of the studied subgroups of polyphenolic compounds; therefore, for their preservation, HPU treatment with a lower pulse is suggested.

When considering the influence of treatment duration, it appears that a longer duration of sonication (10 min vs. 5 min) has either no effect (TPC and HCA) or a negative effect (ANT, FL, CT). Studies by other authors also confirmed the insignificant effect of treatment time on TPC [48,49,55,56]. Moreover, increasing the pulse from 50 to 100% and the treatment time from 5 to 10 min decreased ANT content. Studies by Tiwari et al. [50] confirmed the significant effect of treatment time on the reduction of ANT in HPU-treated strawberry juices. Wang et al. [35] found that total phenolics increased significantly with the increasing duration of ultrasound treatment. The authors explained this by adding sonochemically generated hydroxyl radicals ($OH^{\cdot}$) to the aromatic ring of the phenolic compounds in the ortho-, meta- and para-positions. In addition, they suggested that during ultrasound treatment, the increase in mass transfer rates and the possible disruption of the cell wall of strawberry tissue due to the formation of microcavities could also lead to the release of more phenolic constituents into the juice. Another important effect when strawberry juices are sonicated is the change in the microstructure of the pulp tissue of the juice. The change in cell structures caused by ultrasound treatment increased with increasing treatment time. Therefore, variations regarding the different influence of processing time on different subgroups of polyphenolic compounds are also possible, considering that not all are located at the same positions in the cellular structures [35].

Storage had a statistically significant effect on the reduction of TPC in treated juice after 7 days of storage. These results are in agreement with those of Bursać Kovačević et al. [48], who found the same trend in HPU-treated cloudy apple juice (100 W, 30 kHz frequency) when stored at 4 °C for 7 days.

The 7 day storage had a statistical effect on the decrease of ANT content in the treated juices, which agrees with the results of Tiwari et al. [51], who found a 10% loss of ANT in strawberry juice during 10 day storage at 4 °C. Decreasing ANT levels during the storage of HPU juices has also been reported in other studies [57].

In contrast to TPC and ANT, whose levels decreased after 7 days of storage, HCA and FL increased and the level of CT remained constant. This may indicate that condensation of ANT increases the level of CT while HCA and FL are released into the juices. Similar patterns were previously observed in control samples (Table 3), so it is fairly safe to assume that these changes are due to certain natural processes that occur in strawberry fruit during ripening and storage. In any case, this transformation is not efficient enough to keep TPC constant, so it decreases during storage. In other words, the influences from

the HPU samples can be well detected and separated from those of natural occurrences. Tomadoni et al. [34] observed that both control and ultrasound-treated strawberry juices increased phenolic compounds during storage in the refrigerator (0, 3, 7, and 10 days). The authors concluded that the changes that caused senescence and decomposition of the cell structure and, consequently, the release of free phenolic acids and free amino acids, may contribute to the increase in polyphenol contents [34]. Finally, the results on the influence of storage on CT are consistent with the results of Lukić et al. [53], who found no statistically significant influence of storage times of 3 and 6 months on the content of total tannins in HPU-treated red wine (700 W, 20 kHz).

2.3. Optimization of HPU Parameters for Strawberry Juice Treatment

Strawberry is an important fruit used for juicing due to its health-promoting bioactive constituents and potential health effects [2]. However, processing and storage conditions may play an important role in the bioavailability of these health-promoting compounds [58]. A previous study suggests that strawberry juices have increased levels of anthocyanins and other phenolic components from strawberry after processing by means of innovative technologies, exhibiting larger amounts compared to the untreated samples. This is suggested to be related to increased matrix disruption and extractability from the matrix, thus liberating bioactive compounds and making them more available for digestion [59]. Therefore, processing parameters and storage conditions must be optimized to ensure the highest quality of stored juices to provide potential health-benefits.

Numerous studies have already been conducted showing the negative influence of temperature on bioactive compounds such as total phenols, anthocyanins, flavonoids, vitamin C and others [27,60,61]. Since temperature changes are crucial for maintaining nutrient content and bioactive value in juices, we wanted to find out how temperature change (ΔT) relates to other HPU parameters. As shown in Table 5, ΔT ranged from 4 to even 54 °C during HPU processing. It is evident that this is a wide temperature range that certainly has an impact on the quality of the treated juices.

Table 5. HPU treatment parameters.

Amplitudes (%)	Pulse (%)	Treatment Time (min)	Energy (Wh)	Power (W)	Frequency (kHz)	ΔT (°C)
25.0	50.0	10.0	2.9	40.0	23.7	4.0
25.0	50.0	5.0	1.5	42.0	23.7	8.0
25.0	50.0	10.0	3.0	42.0	23.6	12.0
50.0	50.0	10.0	3.5	78.0	23.7	14.0
50.0	50.0	5.0	1.9	71.0	23.7	15.0
50.0	50.0	10.0	3.7	71.0	23.8	16.0
25.0	50.0	5.0	1.5	45.0	23.6	17.0
25.0	100.0	5.0	3.5	44.0	23.8	18.0
66.7	66.7	6.7	3.7	78.7	23.7	19.0
50.0	100.0	5.0	6.6	90.0	23.8	21.0
37.5	75.0	7.5	4.4	52.5	23.7	22.0
25.0	100.0	5.0	3.8	38.0	23.6	23.0
75.0	50.0	5.0	2.2	85.0	23.8	25.0
100.0	50.0	7.5	3.1	88.0	23.8	26.0
75.0	50.0	10.0	4.2	85.0	23.8	29.0

Table 5. *Cont.*

Amplitudes (%)	Pulse (%)	Treatment Time (min)	Energy (Wh)	Power (W)	Frequency (kHz)	ΔT (°C)
75.0	100.0	5.0	10.0	133.0	23.8	31.0
75.0	50.0	10.0	4.4	74.0	23.8	33.0
75.0	75.0	10.0	7.9	76.0	23.8	34.0
100.0	100.0	5.0	11.5	177.0	23.8	35.0
50.0	100.0	5.0	6.6	93.0	23.8	36.0
100.0	100.0	5.0	11.1	166.0	23.8	37.0
50.0	100.0	10.0	12.8	90.0	23.8	46.0
75.0	100.0	7.5	13.5	129.0	23.8	49.0
100.0	100.0	10.0	20.8	177.0	23.8	50.0
75.0	100.0	10.0	16.4	128.0	23.8	51.0
100.0	100.0	10.0	19.9	176.0	23.8	54.0

From Table 6, it can be seen that ΔT was strongly positively associated with all HPU parameters, namely: energy, power, pulse, amplitudes, and frequency. The only exception was the length of treatment time, which showed the same pattern as the other HPU parameters, except that the correlation was slightly weaker. The increase in amplitude correlated positively (0.59) with temperature change. All other relationships are listed in the same table. In the study of Margean et al. [62], by changing the amplitude parameter from 50 to 70%, an increase in temperature was observed in red grape juice during HPU treatment (750 W, 20 kHz).

Table 6. Pearson Correlations for HPU parameters.

	ΔT (°C)	Amplitudes (%)	Pulse (%)	Treatment Time (min)	Energy (Wh)	Power (W)	Frequency (kHz)
ΔT (°C)	1	0.59 †	0.61 †	0.22 ‡	0.85 †	0.76 †	0.52 †
Amplitudes (%)		1	0	0	0.39 †	0.79 †	0.65 †
Pulse (%)			1	0	0.73 †	0.44 †	0.13 ‡
Treatment time (min)				1	0.37 †	−0.02 ‡	−0.10 ‡
Energy (Wh)					1	0.76 †	0.31 ‡
Power (W)						1	0.58 †
Frequency (kHz)							1

Values represented are Pearson Correlations that are statistically significant at $p \leq 0.05$. † significant correlations; ‡ not significant correlations.

Considering that temperature changes correlated with HPU parameters, Table 7 shows the optimal temperature changes (ΔT) and the optimal degree of ripeness at which the maximum content of bioactive compounds is reached. Thus, the highest content of total phenolic compounds (TPC) and FL, respectively, 102.96 mg 100 mL^{-1} and 2.68 mg 100 mL^{-1}, can be obtained from fruits with a ripening degree of 75% and a temperature change of 4 °C.

Table 7. Optimal HPU parameters for lowest ΔT and maturity with maximum mg 100 mL^{-1} of polyphenols in samples.

Analytical Variable	TPC	ANT	HCA	FL	CT
Content (mg 100 mL^{-1})	102.96	15.58	14.14	2.68	75.28
Maturity (%)	75	100	100	75	100
ΔT (°C)	4.0	35.41	36.77	4.0	4.0

TPC—total phenolic content; ANT—monomeric anthocyanins; HCA—total hydroxycinnamic acids; FL—flavonols; CT—condensed tannins.

Relating the optimal temperature to the applied HPU treatment parameters (Table 5), it can be observed that a temperature change of 4 °C corresponds to the HPU parameters amplitude 25%, pulse 50%, and treatment duration 10 min, at which the highest values of TPC, FL, and CT were obtained. From this it is clear that higher temperatures do not favor the content of TPC, FL and CT. These results agree with the study of Jabbar et al. [63], where with an increase in temperature from 20 to 60 °C during thermosonication, a significant decrease in the content of TPC, FL and CT in carrot juice was observed. Similar results were obtained by Dundar et al. [64], in which increasing the temperature from 25 to 75 °C during ultrasonication negatively affected the content of total phenols in cloudy strawberry nectar, more specifically, the highest TPC yield of 779.8 mg L^{-1} was obtained at a temperature of 25 °C.

Wahia et al. [65] optimized the thermosonication of orange juice in the temperature range of 45–70 °C and found that the optimal parameters with the highest content of total phenols were 495.34 mg 100 mL^{-1} at a temperature of 49.53 °C, a treatment time of 28.87 min and a frequency of 20.85 kHz. On the other hand, Pokhrel et al. [66] did not observe any statistically significant effect of temperature (50–58 °C) during ultrasound treatment on the content of total phenols in carrot juice.

In contrast to TPC, FL, and CT, the maximum contents of ANT and HCA, 15.58 mg 100 mL^{-1} and 14.14 mg 100 mL^{-1}, respectively, were measured at significantly higher temperature changes, 35.41 °C and 36.77 °C, of fully ripe fruit (Table 7). Again, comparing the temperature change with the applied HPU parameters from Table 5 and interpolating, we obtained that the highest values of ANT and HCA were obtained at a pulse of 100% and a treatment time of 5 min with different amplitudes of 70.5% and 88.5%, respectively.

Margean et al. [62] confirmed that the HCA content in red grape juice increases with an increase in amplitude from 50 to 70% as well as temperature. As can be seen from the results, HCA and ANT gave higher yields at higher temperatures. This is confirmed by the studies of Dundar et al. [64] who observed the highest ANT content in cloudy strawberry nectar during ultrasonic treatment at 50 °C.

3. Materials and Methods

3.1. Chemicals and Standards

HPLC 99% pure methanol obtained from Honeywell (Honeywell, Paris, France) was used as an extraction solvent, while Folin–Ciocalteau reagent obtained from Fisher Scientific (Fisher Scientific, Loughborough, UK) was used for spectrophotometric determination of total phenols. Hydrochloric acid (37%, *w*/*w*), sulfuric acid (96%, p.a.), sodium carbonate, anhydrous (99.5–100.5%), and formic acid (98%, p.a.) were obtained from Lachner (Lachner s.r.o., Neratovice, Czech Republic). Ethanol (96% pure) was obtained from Gram-mol (Gram-mol d.o.o., Zagreb, Croatia). Quercetin (95%) and gallic acid standard (97.5–102.5%) were purchased from Acros Organics (Acros Organics, Guangzhou, China) and Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA). Vanillin (99%), potassium chloride (99.0–100.5%), sodium acetate anhydride (99%), and chlorogenic acid (min. 95%) were purchased from Thermo Fisher (Thermo Fisher GmbH, Kandel, Germany).

3.2. Production of Strawberry Juice

Strawberry fruits (*Fragaria x ananassa* Duch, cv. 'Albion') were grown and harvested in 2021 at Jagodar HB, Donja Lomnica, Croatia. Fruits were harvested at two different ripening stages: (i) 75% ripe fruits, i.e., technological ripening (F1), and (ii) 100% ripe fruits, i.e., consumption ripening stage (F2). After harvest, fruits were delivered to the laboratory, cleaned (stems were removed, washed with tap water and dried with cellulose) and stored in plastic containers at −18 °C until processing. Strawberries were thawed overnight in the refrigerator before processing into juices. Juices (J1 and J2) were prepared from fruits of the corresponding ripeness level (F1 and F2) by cold pressing in a Kuvings B6000 Slow Juicer (VerVita d.o.o., Zagreb, Croatia). The prepared juices were immediately subjected to HPU treatment as described in Section 3.3. All juices were filled into hermetically sealed sterile glass bottles.

3.3. High Power Ultrasound (HPU) Processing of Strawberry Juice

Strawberry juice samples were treated with high-power ultrasound on a Hielscher UP400St device (Hielscher Ultrasonics GmbH, Teltow, Germany). The UP400St device consists of a digital ultrasound processor, titanium DN22 (546 mm^2) sonotrode, a stainless-steel base, and an acrylic glass soundproof box. The maximum power of the UP400St ultrasonic processor is 400 W, the amplitude is adjustable from 20 to 100%, the pulse from 10 to 100%, and the treatment time is set manually in the range from 0.1 s to 99 days. The device also includes a digital thermometer with a range of −50 to 200 °C to measure the temperature of the sample before, during and after treatment.

The control samples were untreated juices, while the HPU samples were treated by varying the HPU parameters: amplitude (25, 50, 75 and 100%), pulse (50 and 100%), and treatment duration (5 and 10 min), according to the experimental design (Table 8). Juices were analyzed immediately after HPU treatment, and after storage at 4 °C for 7 days.

Table 8. Experimental design.

Sample	Juice	Storage (Days)	Treatment	Amplitude (%)	Pulse (%)	Treatment Time (min)
1	J1	0	Control	/	/	/
2	J1	0	HPU	25	50	5
3	J1	0	HPU			10
4	J1	0	HPU		100	5
5	J1	0	HPU			10
6	J1	0	HPU	50	50	5
7	J1	0	HPU			10
8	J1	0	HPU		100	5
9	J1	0	HPU			10
10	J1	0	HPU	75	50	5
11	J1	0	HPU			10
12	J1	0	HPU		100	5
13	J1	0	HPU			10
14	J1	0	HPU	100	50	5
15	J1	0	HPU			10

Table 8. *Cont.*

Sample	Juice	Storage (Days)	Treatment	Amplitude (%)	Pulse (%)	Treatment Time (min)
16	J1	0	HPU		100	5
17	J1	0	HPU			10
18	J2	0	Control	/	/	/
19	J2	0	HPU	25	50	5
20	J2	0	HPU			10
21	J2	0	HPU		100	5
22	J2	0	HPU			10
23	J2	0	HPU	50	50	5
24	J2	0	HPU			10
25	J2	0	HPU		100	5
26	J2	0	HPU			10
27	J2	0	HPU	75	50	5
28	J2	0	HPU			10
29	J2	0	HPU		100	5
30	J2	0	HPU			10
31	J2	0	HPU	100	50	5
32	J2	0	HPU			10
33	J2	0	HPU		100	5
34	J2	0	HPU			10
35	J1	7	Control	/	/	/
36	J1	7	HPU	25	50	5
37	J1	7	HPU			10
38	J1	7	HPU		100	5
39	J1	7	HPU			10
40	J1	7	HPU	50	50	5
41	J1	7	HPU			10
42	J1	7	HPU		100	5
43	J1	7	HPU			10
44	J1	7	HPU	75	50	5
45	J1	7	HPU			10
46	J1	7	HPU		100	5
47	J1	7	HPU			10
48	J1	7	HPU	100	50	5
49	J1	7	HPU			10
50	J1	7	HPU		100	5
51	J1	7	HPU			10
52	J2	7	Control	/	/	/
53	J2	7	HPU	25	50	5

Table 8. *Cont.*

Sample	Juice	Storage (Days)	Treatment	Amplitude (%)	Pulse (%)	Treatment Time (min)
54	J2	7	HPU			10
55	J2	7	HPU		100	5
56	J2	7	HPU			10
57	J2	7	HPU	50	50	5
58	J2	7	HPU			10
59	J2	7	HPU		100	5
60	J2	7	HPU			10
61	J2	7	HPU	75	50	5
62	J2	7	HPU			10
63	J2	7	HPU		100	5
64	J2	7	HPU			10
65	J2	7	HPU	100	50	5
66	J2	7	HPU			10
67	J2	7	HPU		100	5
68	J2	7	HPU			10

J1—strawberry juice prepared from 75% ripe strawberries; J2—strawberry juice prepared from 100% ripe strawberries; Control—untreated samples.

3.4. Extraction of Bioactive Compounds

The extraction of bioactive compounds from juices J1 and J2 was prepared according to a modified protocol from the literature [67]. Immediately before extraction, the juice samples were briefly homogenized using a vortex shaker (Grant Instruments Ltd., Cambs, UK). The extraction procedure was performed by pipetting 5 mL of a homogenized sample of strawberry juice into an Erlenmeyer flask and adding 20 mL of the extraction solvent (1% formic acid in 80% methanol, v/v). Then, the prepared mixture was extracted in an ultrasonic bath DT 514 H Sonorex Digitec 13.5 L (Bandelin electronic GmbH, Berlin, Germany) at 50 °C for 15 min. After extraction, the supernatants were filtered into 25 mL volumetric flasks and made up to the mark with extraction solvent and stored at 4 °C until analysis. All extracts were prepared in duplicates.

3.5. Determination of Total Phenolic Content (TPC)

A modified Follin–Ciocalteu method from the literature was used to determine the TPC [68]. Total phenols were determined by pipetting 400 µL of the extract (previously diluted 1:1 with the extraction solvent), 400 µL of the F.C. reagent (previously diluted 5× with distilled water), and 4 mL of a 7.5% sodium carbonate solution. The reaction mixture was allowed to stand at room temperature for 20 min and then the absorbance was measured at 725 nm using a LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany). The determination for each sample was prepared in parallel. The TPC was calculated from a calibration curve prepared with different concentrations of gallic acid solutions (10–250 mg L^{-1}), and results were expressed as mg gallic acid equivalent (GAE) per 100 g or 100 mL of sample.

3.6. Determination of Total Monomeric Anthocyanins (ANT)

The spectrophotometric pH differential method has been used to determine ANT [69]. Briefly, 1 mL of the extract was mixed with 4 mL of 0.4 M buffer pH 4.5 (sodium acetate buffer) and separated with 4 mL of 0.025 M buffer pH 1.0 (potassium chloride buffer). After standing for 20 min at room temperature, the absorbance of the reaction mixture was

measured at 520 and 700 nm on a LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany). Determination was prepared in parallel for each sample, and deionized water was used as a blank. According to the equation from the literature [69], the concentration of monomeric anthocyanins is expressed as pelargonidin-3-glucoside equivalent (Pg-3-G) (mg 100 mL^{-1}).

3.7. Determination of Total Hydroxycinnamic Acids (HCA)

A modified spectrophotometric method described in the literature was used to determine HCA [70]. Briefly, 250 µL of solution 1 (1 g L^{-1} solution of HCl dissolved in 96% ethanol) and 4.55 mL of solution 2 (2 g L^{-1} HCl dissolved in distilled water) were added to 250 µL of the extract. After homogenization for 1 min with a vortex shaker (Grant Instruments Ltd., Cambs, UK), the reaction mixture was allowed to stand in the dark at room temperature for 30 min. Then, the color reaction was measured at 320 nm using a LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany). For the blank, the determination procedure was identical, except that the extraction solvent was used instead of the extract. For each sample, the measurements were performed in parallel. A calibration curve was prepared from different concentrations of chlorogenic acid solutions (10–600 mg L^{-1}), which was used to determine the HCA content in the extracts. The results were expressed as mg chlorogenic acid equivalent (CAE) per 100 g or 100 mL of the sample.

3.8. Determination of Total Flavonols (TF)

A modified spectrophotometric method described in the literature was used for the determination of TF [70]. Briefly, 250 µL of solution 1 (1 g L^{-1} HCl dissolved in 96% ethanol) and 4.55 mL of solution 2 (2 g L^{-1} HCl dissolved in distilled water) were added to 250 µL of the extract. After homogenization for 1 min with a vortex shaker (Grant Instruments Ltd., Cambs, UK), the reaction mixture was allowed to stand in the dark at room temperature for 30 min. Then, the color reaction was measured at 360 nm on a LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany). For the blank, the determination procedure was identical, except that the extraction solvent was used instead of the extract. For each sample, the measurements were performed in parallel. A calibration curve was prepared from different concentrations of the quercetin solution (10–600 mg L^{-1}) from which the content of FL in the extracts was determined. The results were expressed as mg quercetin equivalent (QE) per 100 g or 100 mL of the sample.

3.9. Determination of Condensed Tannins (CT)

A modified spectrophotometric method described in the literature was used to determine CT [71]. Briefly, 2.5 mL of reagent 1 (25% H_2SO_4 solution in methanol) and 1 mL of extract were added to 2.5 mL of reagent 2 (1% vanillin solution in methanol). After homogenization for 1 min with a vortex shaker (Grant Instruments Ltd., Cambs, UK), the reaction mixture was allowed to stand at room temperature for 10 min. The color reaction was then measured at 500 nm using a LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany). For the blank, the determination procedure was identical, except that the extraction solvent was used instead of the extract. For each sample, measurements were performed in parallel. A calibration curve was generated from different concentrations of catechin solution (10–120 mg L^{-1}) and the results were expressed as mg catechin equivalent (CA) per 100 g or 100 mL of the sample.

3.10. Statistical Analysis

Descriptive statistics were used for the characterization of the sample. Discrete variables were tested by MANOVA. Exploratory hierarchical Ward's cluster analysis was used for measuring standardized similarities in samples. Nonparametric analysis employed the Kruskal–Wallis test. Pearson's linear correlation tested the relation between the pairs of continuous variables. Linear regression was employed to build and compare mathematical

models. The level of significance for all tests was $\alpha \leq 0.05$, and results were analyzed using SPSS software (v.22). Statgraphics Centurion XVII was used to build and compare mathematical models (Statpoint Technologies Inc., Warrenton, VI, USA).

4. Conclusions

HPU technology was used for the first time in producing functional strawberry juices from fruits with different degrees of ripeness. The results showed that strawberry fruit juices treated with HPU from fruits with 100% ripeness had higher contents of ANT, HCA, FL, and CT than juice samples from fruits with 75% ripeness, whereas the opposite trend was observed for TPC. Therefore, strawberry juices from both ripening stages are suitable for HPU treatment because they have a solid bioactive value.

When considering the influence of HPU treatment parameters, it was found that increasing all processing parameters (e.g., amplitude, pulse, treatment duration) generally negatively affected the examined bioactive compounds. A 7 day storage had a statistically positive effect on the content of HCA and FL in HPU-treated juice samples, a negative effect on TPC and ANT, while it did not affect CT. The same trend was observed in the untreated samples, except for TPC, where 7 days of storage had a positive effect on the content.

Since a wide range of temperature changes was observed during HPU treatment of strawberry juices, the analysis showed that they were significantly correlated with almost all HPU treatment parameters (e.g., amplitude, pulses, energy, power, and frequency). Consequently, the optimal HPU parameters for these compounds were an amplitude of 25%, a pulse of 50%, and a treatment duration of 10 min. Thus, combining a suitable fruit ripening stage and HPU treatment parameters with optimal temperature variations during the treatment would be possible to obtain a high content of bioactive compounds in the juices. Therefore, HPU technology has great potential for producing functional foods based on strawberry juices.

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References

1. Basu, A.; Nguyen, A.; Betts, N.M.; Lyons, T.J. Strawberry as a functional food: An evidence-based review. *Crit. Rev. Food Sci. Nutr.* **2013**, *54*, 790–806. [CrossRef] [PubMed]
2. Miller, K.; Feucht, W.; Schmid, M. Bioactive compounds of strawberry and blueberry and their potential health effects based on human intervention studies: A brief overview. *Nutrients* **2019**, *11*, 1510. [CrossRef] [PubMed]
3. Baby, B.; Antony, P.; Vijayan, R. Antioxidant and anticancer properties of berries. *Crit. Rev. Food Sci. Nutr.* **2017**, *58*, 2491–2507. [CrossRef] [PubMed]

4. Tomašević, I.; Putnik, P.; Valjak, F.; Pavlić, B.; Šojić, B.; Bebek Markovinović, A.; Bursać Kovačević, D. 3d printing as novel tool for fruit-based functional food production. *Curr. Opin. Food Sci.* **2021**, *41*, 138–145. [CrossRef]
5. Žuntar, I.; Petric, Z.; Bursać Kovačević, D.; Putnik, P. Safety of probiotics: Functional fruit beverages and nutraceuticals. *Foods* **2020**, *9*, 947. [CrossRef]
6. Granato, D.; Barba, F.J.; Bursać Kovačević, D.; Lorenzo, J.M.; Cruz, A.G.; Putnik, P. Functional foods: Product development, technological trends, efficacy testing, and safety. *Annu. Rev. Food Sci. Technol.* **2020**, *11*, 93–118. [CrossRef]
7. Giampieri, F.; Forbes-Hernandez, T.Y.; Gasparrini, M.; Alvarez-Suarez, J.M.; Afrin, S.; Bompadre, S.; Quiles, J.L.; Mezzetti, B.; Battino, M. Strawberry as a health promoter: An evidence-based review. *Food Funct.* **2015**, *6*, 1386–1398. [CrossRef]
8. Kowaleski, J.; Quast, L.B.; Steffens, J.; Lovato, F.; Rodrigues dos Santos, L.; Zambiazi da Silva, S.; Maschio de Souza, D.; Felicetti, M.A. Functional yogurt with strawberries and chia seeds. *Food Biosci.* **2020**, *37*, 100726. [CrossRef]
9. Balthazar, C.F.; Santillo, A.; Guimarães, J.T.; Capozzi, V.; Russo, P.; Caroprese, M.; Marino, R.; Esmerino, E.A.; Raices, R.S.L.; Silva, M.C.; et al. Novel milk–juice beverage with fermented sheep milk and strawberry (*fragaria* × *ananassa*): Nutritional and functional characterization. *J. Dairy Sci.* **2019**, *102*, 10724–10736. [CrossRef]
10. Korus, J.; Juszczak, L.; Ziobro, R.; Witczak, M.; Grzelak, K.; SÓJka, M. Defatted strawberry and blackcurrant seeds as functional ingredients of gluten-free bread. *J. Texture Stud.* **2012**, *43*, 29–39. [CrossRef]
11. Zhao, Z.; Wu, X.; Chen, H.; Liu, Y.; Xiao, Y.; Chen, H.; Tang, Z.; Li, Q.; Yao, H. Evaluation of a strawberry fermented beverage with potential health benefits. *PeerJ* **2021**, *9*, e11974. [CrossRef]
12. Guo, L.; Li, K.; Kang, J.S.; Kang, N.J.; Son, B.G.; Choi, Y.W. Strawberry fermentation with *Cordyceps militaris* has anti-adipogenesis activity. *Food Biosci.* **2020**, *35*, 100576. [CrossRef]
13. Özbek, T.; Şahin-Yeşilçubuk, N.; Demirel, B. Quality and nutritional value of functional strawberry marmalade enriched with chia seed (*Salvia hispanica* L.). *J. Food Qual.* **2019**, *2019*, 1–8. [CrossRef]
14. Ali, M.R.; Mohamed, R.M.; Abedelmaksoud, T.G. Functional strawberry and red beetroot jelly candies rich in fibers and phenolic compounds. *Food Syst.* **2021**, *4*, 82–88. [CrossRef]
15. Cassani, L.; Tomadoni, B.; Moreira, M.R.; Agüero, M.V. Improving quality parameters of functional strawberry juices: Optimization of prebiotic fiber enrichment and geraniol treatment. *Food Bioprocess Technol.* **2018**, *11*, 2110–2124. [CrossRef]
16. Soliman, Z.; Mekky, T.M.; Zeid, M.H. Production of functional drinks from blood orange and strawberry. *J. Home Econ.* **2015**, *25*, 131–148.
17. Moscovici Joubran, A.; Katz, I.H.; Okun, Z.; Davidovich-Pinhas, M.; Shpigelman, A. The effect of pressure level and cycling in high-pressure homogenization on physicochemical, structural and functional properties of filtered and non-filtered strawberry nectar. *Innov. Food Sci. Emerg. Technol.* **2019**, *57*, 102203. [CrossRef]
18. Santhi, V.P.; Sriramavaratharajan, V.; Murugan, R.; Masilamani, P.; Gurav, S.S.; Sarasu, V.P.; Parthiban, S.; Ayyanar, M. Edible fruit extracts and fruit juices as potential source of antiviral agents: A review. *J. Food Meas. Charact.* **2021**, *15*, 5181–5190. [CrossRef]
19. Available online: https://www.grandviewresearch.com/industry-analysis/fruit-vegetable-juice-market (accessed on 14 September 2022).
20. Šamec, D.; Maretić, M.; Lugarić, I.; Mešić, A.; Salopek-Sondi, B.; Duralija, B. Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis. *Food Chem.* **2016**, *194*, 828–834. [CrossRef]
21. Voća, S.; Duralija, B.; Družić, J.; Skendrović-Babojelić, M.; Dobričević, N.; Čmelik, Z. Influence of cultivation systems on physical and chemical composition of strawberry fruits cv. Elsanta. *Agric. Conspec. Sci.* **2006**, *71*, 171–174.
22. Azam, M.; Ejaz, S.; Naveed Ur Rehman, R.; Khan, M.; Qadri, R. Postharvest quality management of strawberries. In *Strawberry—Pre- and Post-Harvest Management Techniques for Higher Fruit Quality*; Toshiki, A., Md, A., Eds.; IntechOpen: London, UK, 2019; pp. 1–21. [CrossRef]
23. Bebek Markovinović, A.; Putnik, P.; Duralija, B.; Krivohlavek, A.; Ivešić, M.; Mandić Andačić, I.; Palac Bešlić, I.; Pavlić, B.; Lorenzo, J.M.; Bursać Kovačević, D. Chemometric valorization of strawberry (*fragaria* × *ananassa* duch.) cv. 'Albion' for the production of functional juice: The impact of physicochemical, toxicological, sensory, and bioactive value. *Foods* **2022**, *11*, 640. [CrossRef] [PubMed]
24. Bursać Kovačević, D.; Brdar, D.; Fabečić, P.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. Strategies to achieve a healthy and balanced diet: Fruits and vegetables as a natural source of bioactive compounds. In *Agri-Food Industry Strategies for Healthy Diets and Sustainability*; Barba, F.J., Putnik, P., Bursać Kovačević, D., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 51–88.
25. Teribia, N.; Buvé, C.; Bonerz, D.; Aschoff, J.; Hendrickx, M.; Loey, A.V. Effect of cultivar, pasteurization and storage on the volatile and taste compounds of strawberry puree. *LWT* **2021**, *150*, 112007. [CrossRef]
26. Bursać Kovačević, D.; Putnik, P.; Dragović-Uzelac, V.; Vahčić, N.; Babojelić, M.S.; Levaj, B. Influences of organically and conventionally grown strawberry cultivars on anthocyanins content and color in purees and low-sugar jams. *Food Chem.* **2015**, *181*, 94–100. [CrossRef] [PubMed]
27. Hartmann, A.; Patz, C.-D.; Andlauer, W.; Dietrich, H.; Ludwig, M. Influence of processing on quality parameters of strawberries. *J. Agric. Food Chem.* **2008**, *56*, 9484–9489. [CrossRef] [PubMed]
28. Aguiló-Aguayo, I.; Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. *LWT—Food Sci. Technol.* **2009**, *42*, 813–818. [CrossRef]

29. Chemat, F.; Rombaut, N.; Sicaire, A.-G.; Meullemiestre, A.; Fabiano-Tixier, A.-S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.* **2017**, *34*, 540–560. [CrossRef]
30. Chemat, F.; Zille, H.; Khan, M.K. Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. Sonochem.* **2011**, *18*, 813–835. [CrossRef]
31. Dimić, I.; Teslić, N.; Putnik, P.; Bursać Kovačević, D.; Zeković, Z.; Šojić, B.; Mrkonjić, Ž.; Čolović, D.; Montesano, D.; Pavlić, B. Innovative and conventional valorizations of grape seeds from winery by-products as sustainable source of lipophilic antioxidants. *Antioxidants* **2020**, *9*, 568. [CrossRef]
32. Dolas, R.; Saravanan, C.; Kaur, B.P. Emergence and era of ultrasonic's in fruit juice preservation: A review. *Ultrason. Sonochem.* **2019**, *58*, 104609. [CrossRef]
33. Bhat, R.; Goh, K.M. Sonication treatment convalesce the overall quality of hand-pressed strawberry juice. *Food Chem.* **2017**, *215*, 470–476. [CrossRef]
34. Tomadoni, B.; Cassani, L.; Viacava, G.; Moreira, M.D.R.; Ponce, A. Effect of ultrasound and storage time on quality attributes of strawberry juice. *J. Food Process Eng.* **2017**, *40*, e12533. [CrossRef]
35. Wang, J.; Wang, J.; Ye, J.; Vanga, S.K.; Raghavan, V. Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control* **2019**, *96*, 128–136. [CrossRef]
36. Yildiz, G.; Aadil, R.M. Comparison of high temperature-short time and sonication on selected parameters of strawberry juice during room temperature storage. *J. Food Sci. Technol.* **2019**, *57*, 1462–1468. [CrossRef]
37. Bebek Markovinović, A.; Putnik, P.; Stulić, V.; Batur, L.; Duralija, B.; Pavlić, B.; Vukušić Pavičić, T.; Herceg, Z.; Bursać Kovačević, D. The application and optimization of hipef technology in the processing of juice from strawberries harvested at two stages of ripeness. *Foods* **2022**, *11*, 1997. [CrossRef]
38. Aubert, C.; Bruaut, M.; Chalot, G.; Cottet, V. Impact of maturity stage at harvest on the main physicochemical characteristics, the levels of vitamin c, polyphenols and volatiles and the sensory quality of gariguette strawberry. *Eur. Food Res. Technol.* **2020**, *247*, 37–49. [CrossRef]
39. Cruz-Cansino, N.D.S.; Ramírez-Moreno, E.; León-Rivera, J.E.; Delgado-Olivares, L.; Alanis-Garcia, E.; Ariza-Ortega, J.A.; Manriquez-Torres, J.D.J.; Jaramillo-Bustor, D.P. Shelf life, physicochemical, microbiological and antioxidant properties of purple cactus pear (*opuntia ficus indica*) juice after thermoultrasound treatment. *Ultrason. Sonochem.* **2015**, *27*, 277–286. [CrossRef]
40. Abid, M.; Jabbar, S.; Wu, T.; Hashim, M.M.; Hu, B.; Lei, S.; Zhang, X.; Zeng, X. Effect of ultrasound on different quality parameters of apple juice. *Ultrason. Sonochem.* **2013**, *20*, 1182–1187. [CrossRef]
41. Kataoka, H. New trends in sample preparation for analysis of plant-derived medicines. *Curr. Org. Chem.* **2010**, *14*, 1698–1713. [CrossRef]
42. Bautista-Ortín, A.B.; Jiménez-Martínez, M.D.; Jurado, R.; Iniesta, J.A.; Terrades, S.; Andrés, A.; Gómez-Plaza, E. Application of high-power ultrasounds during red wine vinification. *Int. J. Food Sci. Technol.* **2017**, *52*, 1314–1323. [CrossRef]
43. Plaza, E.G.; Jurado, R.; Iniesta, J.A.; Bautista-Ortín, A.B. High power ultrasounds: A powerful, non-thermal and green technique for improving the phenolic extraction from grapes to must during red wine vinification. *BIO Web Conf.* **2019**, *12*, 02001. [CrossRef]
44. Wei, M.; Wang, H.; Ma, T.; Ge, Q.; Fang, Y.; Sun, X. Comprehensive utilization of thinned unripe fruits from horticultural crops. *Foods* **2021**, *10*, 2043. [CrossRef] [PubMed]
45. Pradas, I.; Medina, J.J.; Ortiz, V.; Moreno-Rojas, J.M. 'Fuentepina' and 'amiga', two new strawberry cultivars: Evaluation of genotype, ripening and seasonal effects on quality characteristics and health-promoting compounds. *J. Berry Res.* **2015**, *5*, 157–171. [CrossRef]
46. Muche, B.M.; Speers, R.A.; Rupasinghe, H.P.V. Storage temperature impacts on anthocyanins degradation, color changes and haze development in juice of "merlot" and "ruby" grapes (*Vitis vinifera*). *Front. Nutr.* **2018**, *5*, 100. [CrossRef] [PubMed]
47. Su, D.; Wang, Z.; Dong, L.; Huang, F.; Zhang, R.; Jia, X.; Wu, G.; Zhang, M. Impact of thermal processing and storage temperature on the phenolic profile and antioxidant activity of different varieties of lychee juice. *Lwt* **2019**, *116*, 108578. [CrossRef]
48. Bursać Kovačević, D.; Bilobrk, J.; Buntić, B.; Bosiljkov, T.; Karlović, S.; Rocchetti, G.; Lucini, L.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. High-power ultrasound altered the polyphenolic content and antioxidant capacity in cloudy apple juice during storage. *J. Food Process. Preserv.* **2019**, *43*, e14023. [CrossRef]
49. Pala, Ç.U.; Zorba, N.N.D.; ÖZcan, G. Microbial inactivation and physicochemical properties of ultrasound processed pomegranate juice. *J. Food Prot.* **2015**, *78*, 531–539. [CrossRef]
50. Tiwari, B.K.; O'Donnell, C.P.; Patras, A.; Cullen, P.J. Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. *J. Agric. Food Chem.* **2008**, *56*, 10071–10077. [CrossRef]
51. Tiwari, B.K.; O'Donnell, C.P.; Patras, A.; Brunton, N.; Cullen, P.J. Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage. *Eur. Food Res. Technol.* **2008**, *228*, 717–724. [CrossRef]
52. Suslick, K.S. The chemical effects of ultrasound. *Sci. Am.* **1989**, *260*, 80–86. [CrossRef]
53. Lukić, K.; Brnčić, M.; Ćurko, N.; Tomašević, M.; Valinger, D.; Denoya, G.I.; Barba, F.J.; Ganić, K.K. Effects of high power ultrasound treatments on the phenolic, chromatic and aroma composition of young and aged red wine. *Ultrason. Sonochem.* **2019**, *59*, 104725. [CrossRef]
54. Celotti, E.; Stante, S.; Ferraretto, P.; Román, T.; Nicolini, G.; Natolino, A. High power ultrasound treatments of red young wines: Effect on anthocyanins and phenolic stability indices. *Foods* **2020**, *9*, 1344. [CrossRef]

55. Fonteles, T.V.; Costa, M.G.M.; de Jesus, A.L.T.; de Miranda, M.R.A.; Fernandes, F.A.N.; Rodrigues, S. Power ultrasound processing of cantaloupe melon juice: Effects on quality parameters. *Food Res. Int.* **2012**, *48*, 41–48. [CrossRef]
56. Costa, M.G.M.; Fonteles, T.V.; de Jesus, A.L.T.; Almeida, F.D.L.; de Miranda, M.R.A.; Fernandes, F.A.N.; Rodrigues, S. High-intensity ultrasound processing of pineapple juice. *Food Bioprocess Technol.* **2011**, *6*, 997–1006. [CrossRef]
57. Jiang, B.; Mantri, N.; Hu, Y.; Lu, J.; Jiang, W.; Lu, H. Evaluation of bioactive compounds of black mulberry juice after thermal, microwave, ultrasonic processing, and storage at different temperatures. *Food Sci. Technol. Int.* **2014**, *21*, 392–399. [CrossRef]
58. Cassani, L.; Gerbino, E.; Moreira, M.d.R.; Gómez-Zavaglia, A. Influence of non-thermal processing and storage conditions on the release of health-related compounds after in vitro gastrointestinal digestion of fiber-enriched strawberry juices. *J. Funct. Foods* **2018**, *40*, 128–136. [CrossRef]
59. Stübler, A.-S.; Lesmes, U.; Juadjur, A.; Heinz, V.; Rauh, C.; Shpigelman, A.; Aganovic, K. Impact of pilot-scale processing (thermal, PEF, HPP) on the stability and bioaccessibility of polyphenols and proteins in mixed protein- and polyphenol-rich juice systems. *Innov. Food Sci. Emerg. Technol.* **2020**, *64*, 102426. [CrossRef]
60. Nadeem, M.; Ubaid, N.; Qureshi, T.M.; Munir, M.; Mehmood, A. Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrason. Sonochem.* **2018**, *45*, 1–6. [CrossRef]
61. Igual, M.; García-Martínez, E.; Camacho, M.M.; Martínez-Navarrete, N. Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Food Chem.* **2010**, *118*, 291–299. [CrossRef]
62. Margean, A.; Lupu, M.I.; Alexa, E.; Padureanu, V.; Canja, C.M.; Cocan, I.; Negrea, M.; Calefariu, G.; Poiana, M.-A. An overview of effects induced by pasteurization and high-power ultrasound treatment on the quality of red grape juice. *Molecules* **2020**, *25*, 1669. [CrossRef]
63. Jabbar, S.; Abid, M.; Hu, B.; Hashim, M.M.; Lei, S.; Wu, T.; Zeng, X. Exploring the potential of thermosonication in carrot juice processing. *J. Food Sci. Technol.* **2015**, *52*, 7002–7013. [CrossRef]
64. Dündar, B.; Ağçam, E.; Akyıldız, A. Optimization of thermosonication conditions for cloudy strawberry nectar with using of critical quality parameters. *Food Chem.* **2019**, *276*, 494–502. [CrossRef] [PubMed]
65. Wahia, H.; Zhou, C.; Sarpong, F.; Mustapha, A.T.; Liu, S.; Yu, X.; Li, C. Simultaneous optimization of *Alicyclobacillus acidoterrestris* reduction, pectin methylesterase inactivation, and bioactive compounds enhancement affected by thermosonication in orange juice. *J. Food Process. Preserv.* **2019**, *43*, e14180. [CrossRef]
66. Pokhrel, P.R.; Bermúdez-Aguirre, D.; Martínez-Flores, H.E.; Garnica-Romo, M.G.; Sablani, S.; Tang, J.; Barbosa-Cánovas, G.V. Combined effect of ultrasound and mild temperatures on the inactivation of *E. coli* in fresh carrot juice and changes on its physicochemical characteristics. *J. Food Sci.* **2017**, *82*, 2343–2350. [CrossRef] [PubMed]
67. Bursać Kovačević, D.; Putnik, P.; Dragović-Uzelac, V.; Pedisić, S.; Režek Jambrak, A.; Herceg, Z. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chem.* **2016**, *190*, 317–323. [CrossRef]
68. Yuan, B.; Danao, M.-G.C.; Stratton, J.E.; Weier, S.A.; Weller, C.L.; Lu, M. High pressure processing (hpp) of aronia berry purée: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. *Innov. Food Sci. Emerg. Technol.* **2018**, *47*, 249–255. [CrossRef]
69. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the ph differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278. [CrossRef]
70. Howard, L.R.; Clark, J.R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238–1247. [CrossRef]
71. Sun, B.; Ricardo-da-Silva, J.M.; Spranger, I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. [CrossRef]

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Pulsed Electric Field (PEF) and High-Power Ultrasound (HPU) in the Hurdle Concept for the Preservation of Antioxidant Bioactive Compounds of Strawberry Juice—A Chemometric Evaluation—Part I

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Abstract: This work investigated the influence of pulsed electric field (PEF) and high-power ultrasound (HPU) combined with hurdle technology to preserve the bioactive compounds (BACs) content and antioxidant activity in stored strawberry juices. PEF was performed at 30 kV cm^{-1}, 100 Hz during 1.5, 3, and 4.5 min, while HPU was performed at 25% amplitude and 50% pulse during 2.5, 5.0, and 7.5 min. Total phenols and hydroxycinnamic acids were the most stable BACs during the hurdle treatment without influence of the duration of both treatments, while flavonols and condensed tannins showed a significant stability dependence with respect to the duration of both treatments. Total phenols were also stable during storage, in contrast to the individual groups of BACs studied. A chemometric approach was used to optimize the parameters of the hurdle treatments with respect to the highest level of BACs and the antioxidant activity of the treated juices. In general, shorter treatment times in the hurdle approach resulted in better stability of BACs and antioxidant activity. The hurdle technology investigated in this study has the strong potential to be an excellent concept for optimizing the operating parameters of PEF and HPU technologies in the preservation of functional foods.

Keywords: hurdle technology; non-thermal technology; functional juice; polyphenolic content; storage

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1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a highly sensitive fruit and, therefore, has a short shelf life after harvest. Due to the rapid loss of quality, strawberries quickly lose value in the market [1]. To avoid potential losses and use high-quality raw material, ripe strawberry fruit should be processed appropriately as soon as possible. Strawberry products, just like fresh fruits, contain significant bioactive potential that can provide numerous health benefits, as shown by numerous studies on their antioxidant, anti-inflammatory, microbial modulatory, and cell-protective activities [2]. The functional properties of strawberry fruit are due to its rich composition of bioactive compounds (BACs). The bioactive potential of strawberries is mainly determined using a large number of different phenolic compounds, most of which have significant antioxidant activity [3].

Among polyphenols present in strawberries, flavonoids and their derivatives, including flavonols, flavonols, and anthocyanins, are the most abundant. After anthocyanins, flavonols dominate and can be present in both monomeric and polymeric forms called tannins [4,5]. Phenolic acids (e.g., derivatives of hydroxycinnamic acid and hydroxybenzoic acid) were also found in strawberries but in smaller amounts than flavonoids [4,6]. Since

a large proportion of BACs transfer from the fresh fruit to the juice during processing, strawberry juice can be considered a functional food just like the fresh fruit [7].

In recent years, consumers are increasingly looking for highly nutritious and high-quality foods that are as close as possible to fresh and/or without chemical additives. Fruit juices are also becoming increasingly popular, mainly because they are consumed relatively quickly and easily and can replace a fresh fruit meal. Therefore, strawberry juice is increasingly in demand by consumers, not only for its appealing sensory properties but also for its nutritional and bioactive composition and health benefits, hence being very important for promoting human health and preventing diseases [8].

Juices are usually preserved using pasteurization because thermal processing ensures food safety and a longer shelf life. However, elevated temperatures during processing can decompose unstable BACs, such as polyphenolic compounds, thus reducing antioxidant activity, which ultimately affects the quality of the final product [9–11]. Accordingly, there has been an increased demand for technologies that not only preserve food but also do not affect its nutritional composition, are energy efficient, and can be used to reuse food industry by-products [12]. Therefore, advanced technologies in food production/processing, such as pulsed electric field (PEF) and high-power ultrasound (HPU), are increasingly becoming the focus of functional food processing [13].

The use of PEF technology has shown good results in maintaining good nutritional and sensory quality in treated fruit juices [14,15]. The use of HPU as a replacement for pasteurization is very promising, as the acoustic energy of ultrasound is directly transferred to the whole juice volume, significantly reducing the operating time. The higher energy savings compared to conventional methods validate this technology for industrial applications [16]. A recent trend in food preservation, known as the hurdle concept, involves the use of a carefully selected sequence of technologies, which can be either thermal and/or non-thermal, with the aim of preserving food quality and extending shelf life. The technologies used in this approach must be optimized in advance and are, therefore, typically operated under lower processing conditions than if they were used independently. In this way, potential negative effects of the technologies are avoided while their synergistic effects are promoted [17].

In juice processing, PEF and HPU technologies are often combined with other hurdles to maintain native quality characteristics [18–21]. To date, various combinations of hurdle technologies have been tested in the preservation of strawberry juices [22–26], but the combination of PEF and HPU as the most promising technologies for processing functional juices has not been investigated yet. Therefore, the aim of this study was to investigate the impact of PEF and HPU processing via the hurdle concept. In previous studies, the treatment parameters for both technologies were optimized [27,28] and adapted to the hurdle concept, so in this study, a further step was taken with the optimal parameters for PEF (30 kV cm^{-1}, 100 Hz) and HPU (amplitude 25%, pulse 50%) selected. To better investigate the synergistic effect of these technologies when treated together, different treatment times were tested for PEF 1.5, 3.0, and 4.5 min and for HPU 2.5, 5.0, and 7.5 min. All strawberry juices were analyzed for their physicochemical properties, BAC content, and antioxidant activity before, immediately after treatment, and after 7 days of cold storage. The results were assessed using chemometric techniques to find and optimize the best combination of PEF and HPU technology for mutual strawberry juice treatment to best preserve the antioxidant bioactive compounds during cold storage.

2. Materials and Methods

2.1. Chemicals and Standards

HPLC 99% pure methanol was purchased from Honeywell (Paris, France). Folin-Ciocalteau (FC) reagent was obtained from Fisher Scientific UK (Loughborough, UK). Sodium carbonate, anhydrous (99.5–100.5%), sulfuric acid (96%, p.a.), hydrochloric acid (37%, *w*/*w*), and formic acid (98%, p.a.) were obtained from Lach-Ner (Neratovice, Czech Republic). Ethanol (96% pure) was purchased from Gram-mol (Zagreb, Croatia). Quercetin

(95%) was purchased from Acros Organics (Guangzhou, China). Vanillin (99%) and chlorogenic acid (min. 95%) were obtained from Thermo Fisher (Kandel, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl radical), gallic acid standard (97.5–102.5%), and TPTZ (2,4,6-tris-2-pyridyl-*s*-triazine) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Iron (III)-chloride hexahydrate and sodium acetate trihydrate resistant to potassium permanganate were obtained from Kemika (Zagreb, Croatia). Glacial acetic acid (≥99.8%) was purchased from Honeywell Fluka™ (Seelze, Germany), and Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) used for FRAP and DPPH assay was purchased from Biosynth (Bratislava, Slovakia).

2.2. Strawberry Juice Preparation

In 2022, strawberries (*Fragaria* × *ananassa* Duch., cultivar 'Albion') were grown at Jagodar HB in Donja Lomnica, Croatia, and picked at full maturity. The strawberries were taken to the laboratory after harvest. The stems were removed, and the fruits were washed with tap water, dried, and kept in hermetically sealed plastic containers at −18 °C until processing. A Kuvings B6000 slow juicer (VerVita d.o.o., Zagreb, Croatia) was used for juice processing using low-speed masticating technology (240 W, speed 60 rpm). The cloudy juices produced were immediately treated using the hurdle approach (Table 1).

Table 1. An experimental research design.

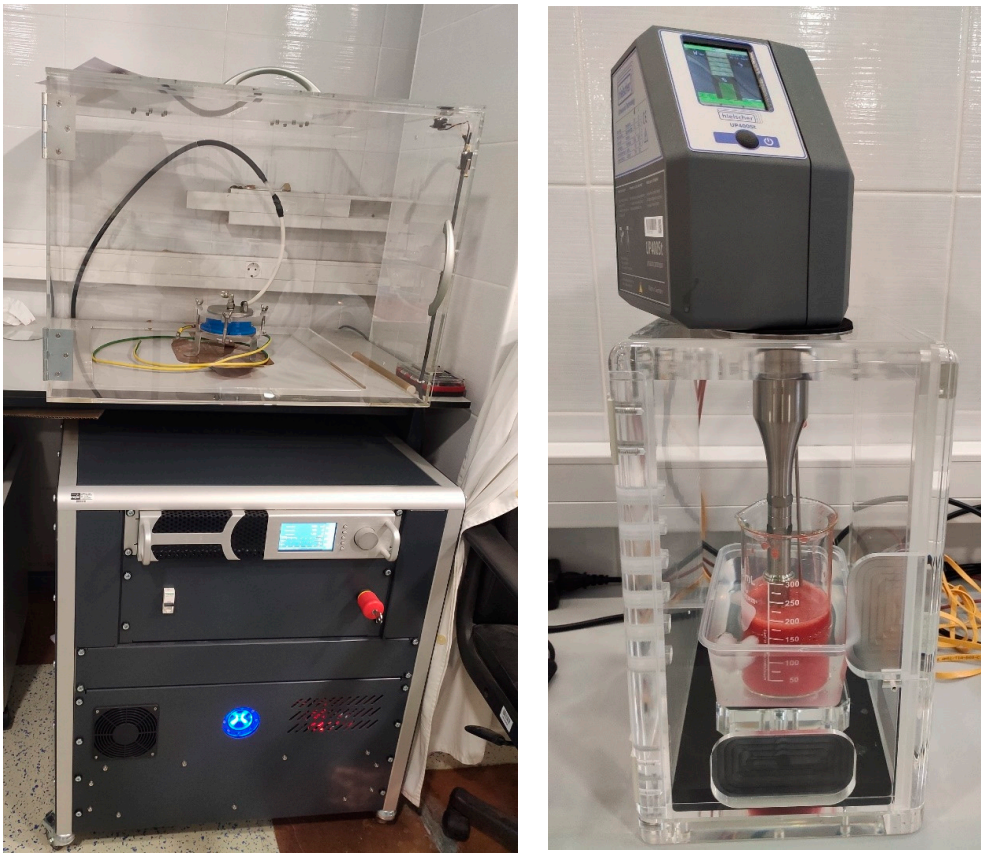
Sample of Juice	Storage (Days)	Processing	PEF Exposure (min)	HPU Exposure (min)
1	0	Control	/	/
2	0	PEF + HPU	1.5	2.5
3	0	PEF + HPU	1.5	5
4	0	PEF + HPU	1.5	7.5
5	0	PEF + HPU	3	2.5
6	0	PEF + HPU	3	5
7	0	PEF + HPU	3	7.5
8	0	PEF + HPU	4.5	2.5
9	0	PEF + HPU	4.5	5
10	0	PEF + HPU	4.5	7.5
11	7	Control	/	/
12	7	PEF + HPU	1.5	2.5
13	7	PEF + HPU	1.5	5
14	7	PEF + HPU	1.5	7.5
15	7	PEF + HPU	3	2.5
16	7	PEF + HPU	3	5
17	7	PEF + HPU	3	7.5
18	7	PEF + HPU	4.5	2.5
19	7	PEF + HPU	4.5	5
20	7	PEF + HPU	4.5	7.5

Control—untreated strawberry juice; PEF + HPU—strawberry juice treated with PEF nad HPU technology in hurdle concept.

2.3. Hurdle Concept Consisting of Pulsed Electric Field (PEF) and High-Power Ultrasound (HPU) for Strawberry Juice Processing

Strawberry juice samples (200 mL) were first treated with PEF and then with HPU technology. The HVG60/1 HIPEF (Impel d.o.o., Zagreb, Croatia) instrument was used for

PEF treatment (Figure 1A), and the Hielscher UP400St instrument (Hielscher Ultrasonics GmbH, Teltow, Germany) was used for HPU treatment (Figure 1B).



(**A**) (**B**)

Figure 1. PEF device HVG60/1 PEF (Impel d.o.o., Zagreb, Croatia) (**A**) and ultrasonic processor Hielscher UP400St, 400 W, 24 Hz (Hielscher Ultrasonics GmbH, Teltow, Germany) (**B**).

The PEF device consisted of three units: a Magna control unit, a high-voltage power source, and a high-voltage pulse generator. The Magna control unit generates control pulses for the high-voltage system. The high-voltage power source converts the input voltage AC of 230 V into DC voltage in the range of 1 to 60 kV. The high-voltage pulse generator passes the input high voltage to the output in the form of pulses with specific parameters. Before starting the treatment, the device was set to the specified parameters. The samples were treated with 100 Hz, a voltage of 30 kV cm^{-1}, a pulse width of 1 µs, and a duration of 1.5, 3.0, and 4.5 min (Table 1). The distance between the ground electrode and the high-voltage electrode was 2.5 cm. Both electrodes were made of stainless steel. During the PEF treatment, the temperature was measured with an infrared thermometer PCE-777 (PCE Instruments, Southampton, UK).

After PEF treatment, strawberry juice samples were subjected to HPU treatment with a titanium horn Ø 22 mm (Figure 1B). The UP400St device has a maximum power of 400 W, amplitude control from 20 to 100%, pulse control from 10 to 100%, and manually adjustable treatment duration. A digital thermometer (50 to 200 °C) was also incorporated into the apparatus for measuring the temperature of the sample prior to, during, and after treatment. Juice samples were treated with HPU parameters of amplitude 25%, pulse 50%, and duration time 2.5, 5.0, and 7.5 min. The control samples were untreated juices. During processing with PEF and HPU technologies, the temperature before treatment and the temperature after treatment were monitored. For PEF technology, the average initial temperature before the start of treatment was 17.16 °C, and after PEF treatment was 17.71 °C, so it can be assumed that there is no temperature difference. For HPU treatment,

the average initial temperature before treatment was 16.61 °C, and after HPU treatment was 19.55 °C, so the influence of temperature can be neglected in this case.

All juice samples were stored in sterilized glass bottles that were securely sealed. After treatment with hurdle technology (PEF + HPU), one batch of juices was subjected to analysis instantly after processing, and another batch was subjected to storage at 4 °C for seven days. The quality indicators associated with physiochemical properties (pH and SSC), stability of BACs, and antioxidant activity were monitored in all juice samples.

2.4. Determination of pH and Soluble Solids Content (SSC)

A Mettler Toledo FiveEasy pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland) was used to measure pH, while a digital ATAGO Pal-3 refractometer (ATAGO Co., Tokyo, Japan), SSC (Brix%) was used to determine SSC. All measurements were performed in duplicates.

2.5. Extraction of Antioxidant Bioactive Compounds

Extraction of BACs from strawberry juice samples was performed using a modified method from the literature [7]. Briefly, 20 mL of 1% formic acid in 80% methanol (v/v) and 5 g of the sample were shaken for 1 min. The mixture was then extracted at 50 °C for 15 min in an ultrasonic bath (DT 514 H Sonorex Digitec 13.5 L, Bandelin electronic, Berlin, Germany). After filtration, the supernatant was diluted to 25 mL in a volumetric flask with extraction solvent. Until analysis, the extracts were stored at 18 °C in an inert gas environment.

2.6. Determination of Total Phenolic Content (TPC)

The modified Folin-Ciocalteau (FC) spectrophotometric assay described in the literature [29] was used to quantify the total phenolic content. 400 µL of the properly diluted extract, 400 µL of the FC reagent previously diluted fivefold with distilled water, and 4 mL of a 7.5% sodium carbonate solution (w/v) were added together. A spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch and Holm, Meckenheim, Germany) was used to measure the absorbance of the colored reaction at 725 nm after the reaction mixture had stood at room temperature for 20 min. Duplicate measurements were performed for each sample. Using a standard calibration curve created with various gallic acid concentrations (10–250 mg L^{-1}), the TPC in the extracts was calculated. Results were expressed as mg gallic acid equivalent (GAE) per 100 g of sample.

2.7. Determination of Total Hydroxycinnamic Acids (HCA) and Total Flavonols (FL)

A modified spectrophotometric method was used to determine HCA and FL [30]. 250 µL of the extract was mixed with 250 µL of solution 1 (1 g L^{-1} HCl solution in 96% ethanol) and 4.55 mL of solution 2 (2 g L^{-1} HCl in distilled water). The reaction was shaken for 10 s and then continued for 30 min at room temperature and in the dark. Subsequently, a LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany) was used to measure the color reaction at 320 nm for the measurement of HCA and the color reaction at 360 nm for the measurement of FL. The same steps were used to prepare the blank sample, but the extract was replaced by an extraction solvent. Parallel measurements were performed for each sample. The HCA content was determined using a calibration curve prepared with different concentrations of chlorogenic acid solution (10–600 mg L^{-1}), while the amount of FL was determined with different concentrations of quercetin solution (10–600 mg L^{-1}). The results of HCA content and FL were expressed as mg chlorogenic acid equivalent (CAE) per 100 g sample and mg quercetin equivalent (QE) per 100 g sample, respectively.

2.8. Determination of Condensed Tannins (CT)

The CT was calculated using a modified spectrophotometric technique that has been reported in the literature [31]. In summary, 2.5 mL of reagent 1 (25% H_2SO_4 solution in

methanol) and 1 mL of extract were added to 2.5 mL of reagent 2 (a solution of 1% vanillin in methanol). The reaction mixture was homogenized for 1 min using a vortex shaker (Grant Instruments Ltd., Cambs, UK) and then left to stand at room temperature for 10 min. A LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany) was then used to measure the color response at 500 nm. The same steps were used to create the blank sample, but the extract was substituted with an extraction solvent. For every sample, parallel measurements were made. Different quantities of catechin solution (10–120 mg L^{-1}) were used to create a calibration curve, and the results were represented as mg of catechin equivalent (CA) per 100 g of the sample.

2.9. In Vitro Measurement of Antioxidant Activity

2.9.1. 2,2-diphenyl-1-picrylhydrazyl Assay (DPPH)

The DPPH spectrophotometric technique described in the literature was used to evaluate the antioxidant activity [32]. In brief, 3 mL of a methanolic 0.5 mM DPPH solution and 1.5 mL of the extract were mixed. At room temperature and in the dark, the reaction mixture was allowed to react for 20 min. 1.5 mL of 100% methanol and 3 mL of a 0.5 mM DPPH solution were mixed as a control. Methanol was used as a blank. The absorbance of the color reaction was then measured at 517 nm. Parallel measurements were performed for each sample. Antioxidant activity was calculated using a calibration curve created using different concentrations of Trolox solution (10–150 μM), and results were expressed as μM Trolox equivalents (TE) per 100 g of sample.

2.9.2. Ferric Reducing Antioxidant Power Assay (FRAP)

The spectrophotometric FRAP approach described in the literature was used to evaluate antioxidant activity [33]. 50 mL of acetate buffer (0.3 M) with a pH 3.6, 5 mL of a 10 mM tripyridyltriazine (TPTZ) solution prepared with HCl (40 mM), and 5 mL of a ferric chloride ($FeCl_3$) solution (20 mM) were combined to prepare the FRAP reagent. Briefly, 4.5 mL of the FRAP reagent and 600 μL of the previously properly diluted extract were added to the glass tubes. Then, the reaction mixture was homogenized on a vortex shaker (Grant Instruments Ltd., Cambridge, UK) for 1 min, then thermostated in a water bath at 37 °C for 10 min. Subsequently, the absorbance of the reaction was determined at 593 nm using an LLG-uniSPEC 2 Spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany). To prepare the blank sample, the same steps were followed, but the extract was replaced by an extraction solvent. A calibration curve was prepared using different concentrations of Trolox solution (10–150 μM). Results were presented as μM Trolox equivalents (TE) per 100 g of sample.

2.10. Statistical Analysis

Experiments were designed as full factorial randomized experimental designs (n = 40) (Table 1). Dependent variables were the contents of (i) total phenols (TPC; mg 100 g^{-1}); (ii) hydroxycinnamic acids (HCA; mg 100 g^{-1}); (iii) flavonols (FL; mg 100 g^{-1}); (iv) condensed tannins (CT; mg 100 g^{-1}); (v) DPPH assay (mg 100 g^{-1}); and (vi) FRAP assay (mg 100 g^{-1}). Independent variables were: (i) exposure to PEF (1.5, 3.0, and 4.5 min); (ii) exposure to HPU (2.5, 5.0, and 7.5 min); and (iii) duration of storage (0 and 7 days). Descriptive statistics were used to assess the basic information on the experimental data set. Differences between treatments (continuous variables) were tested using multivariate analysis of variance (MANOVA). The Pearson coefficient was used to assess correlations between pairs of continuous variables. Exploratory hierarchical Ward's cluster analysis was used to measure standardized similarities in samples. In nonparametric analysis, the Kruskal–Wallis test was employed. The significance levels for rejection of a null hypothesis were $\alpha \leq 0.05$ in all tests. Analyses were performed using IBM SPSS Statistics (v.24), and experimental design was performed using Statgraphics Centurion® (StatPoint Technologies, Inc., Warrenton, VA, USA).

3. Results and Discussion

3.1. Use of Chemometrics to Evaluate Hurdle Processed Samples vs. Control Samples

Chemometric evaluation of data from hurdle-processed samples compared to control (untreated) samples was conducted to investigate the impact of hurdle technology on strawberry juice quality. Ward's hierarchical clustering method showed that in the case where all juice samples were considered for standardized similarities, PEF-treated samples were most similar to controls with 1.5 min and 2.5 min with HPU at the beginning of the storage (0 day). On the 7th day of storage, samples treated with PEF for 1.5 min and HPU for 2.5–7.5 min were most similar to controls. Other samples that were similar to controls were treated with PEF for 3 min and HPU for 2.5 min (Figure 2).

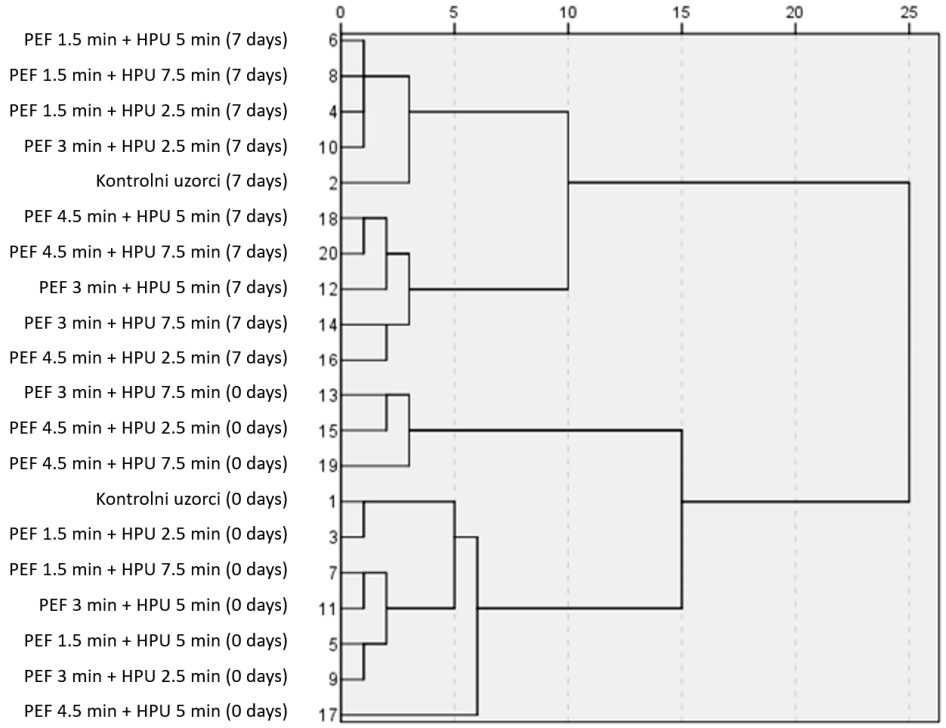


Figure 2. Results of the hierarchical clustering for averaged and standardized juice samples. PEF—Pulsed electric field, HPU—High power ultrasound.

Lower times of both treatments favored the preservation of BACs and antioxidant activity, making the treated samples most similar to the control samples. Previously, it was found that anthocyanin content was largely dependent on the strength and duration of PEF treatment [34]. Thus, it is reasonable to assume that a shorter treatment duration for both technologies favors the preservation of native polyphenol content as found in the untreated samples (i.e., controls). Next, the authors reported the same trend in DPPH antioxidant activity, with values decreasing with increasing HIPEF treatment duration [35]. That control samples of strawberry juices were most similar to HPU-treated samples was previously confirmed for the shortest treatment durations [24,35]. Nevertheless, studies have also shown that longer HPU treatment resulted in better yield of TPC and increased antioxidant activity, such that the longer treated samples had higher levels of BAC and antioxidant activity than the shorter treated samples, while the least treated samples had the most similar levels of BAC and antioxidant activity with the control samples [24,35]. This is because the stability of BACs is cumulatively and synergistically affected by other process parameters of the applied technologies [36].

Overall, the control samples did not differ from the hurdle-treated samples in terms of the BACs assayed, antioxidant activity, and SSC (Figure 3). The only difference was the median pH, where untreated samples were more acidic than the hurdle samples. Although

our results showed a statistically significant change in pH, no changes in pH were detected in the spinach juice samples, neither for the untreated sample nor for the HPU-treated (40 kHz, 200 W, 21 min), the PEF-treated (9 kV cm^{-1}, 1 kHz, 60 mL min^{-1}, 30 ± 2 °C, time: 335 µs), and for the hurdle processed (HPU + PEF) [37]. However, Makroo et al. [38] observed a significant increase in pH after 15 s of ohmic heating of watermelon juice. This could be due to the fact that electrolysis or electrochemical interactions occurred during the treatment [39]. Table 2 shows the numerical values of the Kruskal–Wallis test of the hurdle-treated samples compared to untreated samples, which correlates with the graphical representation in Figure 3.

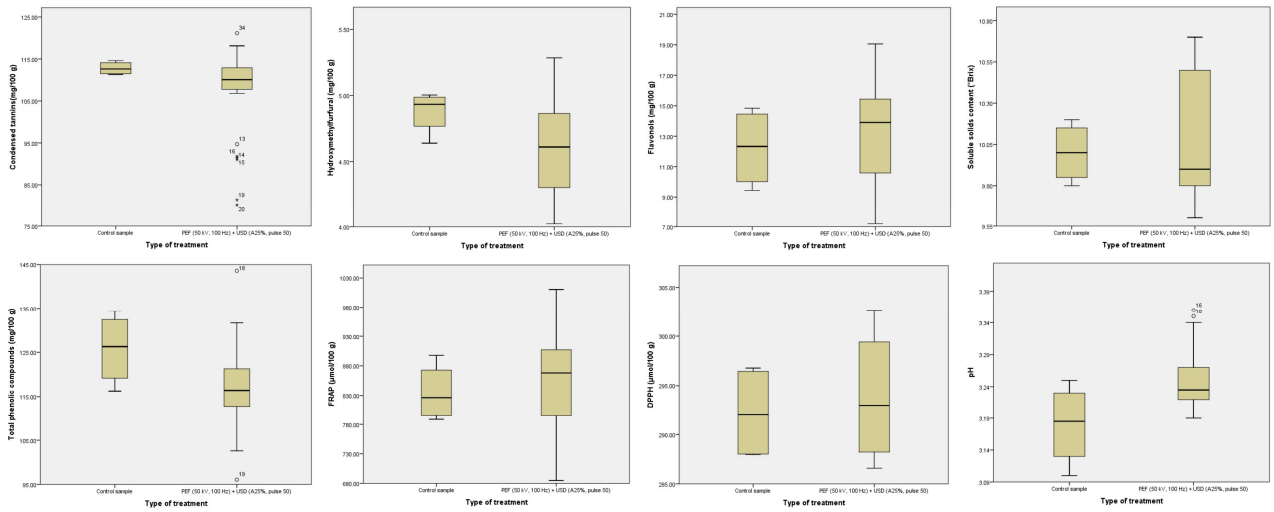


Figure 3. Median values of BAC content, antioxidant activity, SSC, and pH in control vs. hurdle-treated samples. All data are presented as median (25th–75th percentile).

Table 2. Results for Kruskal–Wallis test (hurdle treatment vs. control samples).

	TPC	HCA	FL	CT	DPPH	FRAP	SSC	pH
Chi-Square	3.417	1.829	0.52	2.35	0.813	0.587	0.134	4.64
df	1	1	1	1	1	1	1	1
Sig.	0.07	0.18	0.47	0.13	0.37	0.44	0.71	0.03 *

* Kruskal–Wallis test is significant at the $p \leq 0.05$. TPC—total phenolic content (mg 100 g^{-1}); HCA—hydroxycinnamic acids (mg 100 g^{-1}); FL—flavonols (mg 100 g^{-1}); CT—condensed tannins (mg 100 g^{-1}); Antioxidant activity—DPPH (µmol 100 g^{-1}) and FRAP (µmol 100 g^{-1}); SSC—soluble solids content (°Brix).

3.2. The Changes in BACs, Antioxidant Activity, SSC, and pH in Strawberry Juices after Hurdle Technology Processing and Storage

Before processing, the operating parameters need to be optimized to meet the high-quality requirements of the processed food. The results for the influence of hurdle parameters and storage on BAC, antioxidant activity, SSC, and pH of strawberry juices are presented in Table 3. Strawberry juices were found to be a good source of BACs, namely a TPC value of 117.93 ± 0.70 mg 100 g^{-1}. CT was the predominant bioactive compound detected (107.66 ± 0.23 mg 100 g^{-1}), followed by HCA (28.03 ± 0.28 mg 100 g^{-1}) and FL (13.38 ± 0.19 mg 100 g^{-1}). During storage, a TPC value of 117.93 ± 0.70 mg 100 g^{-1} was observed, and this value remained constant during the experimental period. The SSC value was also constant during storage, with an average value of 10.12 ± 0.01 °Brix. The results obtained are similar to those of Sulaiman et al. [40], where the SSC value remained constant in strawberry puree samples treated with ultrasound (24 kHz, 1.3 W g^{-1}, 33 °C) and stored at 3 °C for 30 days.

Table 3. Effect of hurdle technology on bioactive compounds content, antioxidant activity, SSC, and pH of strawberry juices during storage.

	n	TPC	HCA	FL	CT	DPPH	FRAP	SSC	pH
Storage		$p = 0.39$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.02$ †	$p = 0.26$ ‡	$p \leq 0.01$ †
0 days	18	118.54 ± 0.99 ^{a}	32.58 ± 0.40 ^{a}	14.26 ± 0.27 ^{a}	104.15 ± 0.33 ^{b}	299.30 ± 0.13 ^{a}	866.54 ± 7.66 ^{a}	10.11 ± 0.01 ^{a}	3.28 ± 0.01 ^{a}
7 days	18	117.31 ± 0.99 ^{a}	23.49 ± 0.40 ^{b}	12.50 ± 0.27 ^{b}	111.18 ± 0.33 ^{a}	288.57 ± 0.13 ^{b}	838.29 ± 7.66 ^{b}	10.13 ± 0.01 ^{a}	3.22 ± 0.01 ^{b}
PEF		$p = 0.48$ ‡	$p = 0.02$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.02$ †
1.5 min	12	118.19 ± 1.22 ^{a}	26.79 ± 0.49 ^{b}	14.15 ± 0.33 ^{a}	110.96 ± 0.40 ^{a}	292.77 ± 0.15 ^{b}	830.50 ± 9.38 ^{b}	9.85 ± 0.02 ^{c}	3.23 ± 0.01 ^{b}
3 min	12	118.85 ± 1.22 ^{a}	28.86 ± 0.49 ^{a}	13.57 ± 0.33 ^{a}	109.94 ± 0.40 ^{a}	294.69 ± 0.15 ^{a}	879.81 ± 9.38 ^{a}	10.01 ± 0.02 ^{b}	3.26 ± 0.01 ^{a}
4.5 min	12	116.75 ± 1.22 ^{a}	28.46 ± 0.49 ^{a}	12.44 ± 0.33 ^{b}	102.09 ± 0.40 ^{b}	294.34 ± 0.15 ^{a}	846.92 ± 9.38 ^{b}	10.49 ± 0.02 ^{a}	3.26 ± 0.01 ^{a}
HPU		$p = 0.02$ †	$p = 0.63$ ‡	$p = 0.02$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.26$ ‡
2.5 min	12	118.51 ± 1.22 ^{a,b}	28.42 ± 0.49 ^{a}	13.31 ± 0.33 ^{a,b}	107.96 ± 0.40 ^{b}	293.54 ± 0.15 ^{b}	854.81 ± 9.38 ^{a}	10.11 ± 0.02 ^{b}	3.25 ± 0.01 ^{a}
5 min	12	120.24 ± 1.22 ^{a}	27.84 ± 0.49 ^{a}	14.14 ± 0.33 ^{a}	111.46 ± 0.40 ^{a}	293.62 ± 0.15 ^{b}	877.46 ± 9.38 ^{a}	10.07 ± 0.02 ^{b}	3.25 ± 0.01 ^{a}
7.5 min	12	115.04 ± 1.22 ^{b}	27.84 ± 0.49 ^{a}	12.70 ± 0.33 ^{b}	103.57 ± 0.40 ^{c}	294.64 ± 0.15 ^{a}	824.96 ± 9.38 ^{b}	10.18 ± 0.02 ^{a}	3.26 ± 0.01 ^{a}
PEF + HPU (hurdle)		$p = 0.07$ ‡	$p = 0.59$ ‡	$p = 0.03$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	p =0.78 ‡	$p = 0.03$ †	$p = 0.76$ ‡
1.5 min + 2.5 min	12	122.79 ± 1.90 ^{a}	26.14 ± 0.84 ^{a}	12.80 ± 0.76 ^{b}	113.62 ± 0.52 ^{a}	290.94 ± 0.22 ^{c}	840.84 ± 17.48 ^{a}	9.93 ± 0.04 ^{a}	3.23 ± 0.01 ^{a}
1.5 min + 5 min	12	115.91 ± 1.90 ^{a}	26.81 ± 0.84 ^{a}	16.36 ± 0.76 ^{a}	110.47 ± 0.52 ^{b}	293.09 ± 0.22 ^{b}	825.53 ± 17.48 ^{a}	9.88 ± 0.04 ^{a}	3.23 ± 0.01 ^{a}
1.5 min + 7.5 min	12	115.86 ± 1.90 ^{a}	27.41 ± 0.84 ^{a}	13.29 ± 0.76 ^{b}	108.78 ± 0.52 ^{b}	294.30 ± 0.22 ^{a}	825.14 ± 17.48 ^{a}	9.75 ± 0.04 ^{b}	3.23 ± 0.01 ^{a}
PEF + HPU (hurdle)		$p = 0.39$ ‡	$p = 0.25$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.09$ ‡	$p \leq 0.01$ †	$p = 0.76$ ‡
3 min + 2.5 min	12	118.18 ± 2.15 ^{a}	29.23 ± 0.82 ^{a}	15.19 ± 0.41 ^{a}	110.25 ± 0.90 ^{a}	294.94 ± 0.25 ^{a}	836.0 5± 19.66 ^{a}	9.75 ± 0.03 ^{b}	3.25 ± 0.01 ^{a}
3 min + 5 min	12	116.99 ± 2.15 ^{a}	27.63 ± 0.82 ^{a}	11.95 ± 0.41 ^{c}	113.32 ± 0.90 ^{a}	293.64 ± 0.25 ^{b}	895.61 ± 19.66 ^{a}	9.80 ± 0.03 ^{b}	3.25 ± 0.01 ^{a}
3 min + 7.5 min	12	121.37 ± 2.15 ^{a}	29.72 ± 0.82 ^{a}	13.55 ± 0.41 ^{b}	106.25 ± 0.90 ^{b}	295.48 ± 0.25 ^{a}	907.78 ± 19.66 ^{a}	10.48 ± 0.03 ^{a}	3.28 ± 0.01 ^{a}
PEF + HPU (hurdle)		$p = 0.02$ †	$p = 0.08$ ‡	$p = 0.02$ †	$p \leq 0.01$ †	$p = 0.36$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.76$ ‡
4.5 min + 2.5 min	12	114.56 ± 2.26 ^{b}	29.89 ± 0.90 ^{a}	11.93 ± 0.51 ^{b}	99.99 ± 0.62 ^{b}	294.75 ± 0.32 ^{a}	887.54 ± 9.99 ^{a}	10.65 ± 0.03 ^{a}	3.26 ± 0.01 ^{a}
4.5 min + 5 min	12	127.80 ± 2.26 ^{a}	29.07 ± 0.90 ^{a}	14.11 ± 0.51 ^{a}	110.6 ± 0.62 ^{a}	294.13 ± 0.32 ^{a}	911.25 ± 9.99 ^{a}	10.53 ± 0.03 ^{b}	3.27 ± 0.01 ^{a}
4.5 min + 7.5 min	12	107.90 ± 2.26 ^{b}	26.40 ± 0.90 ^{a}	11.27 ± 0.51 ^{b}	95.69 ± 0.62 ^{c}	294.15 ± 0.32 ^{a}	741.97 ± 9.99 ^{b}	10.30 ± 0.03 ^{c}	3.26 ± 0.01 ^{a}
Dataset average	36	117.93 ± 0.70	28.03 ± 0.28	13.38 ± 0.19	107.66 ± 0.23	293.93 ± 0.09	852.41 ± 5.42	10.12 ± 0.01	3.25 ± 0.01

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; ‡ not significant factor in multifactor analysis. TPC—total phenolic content (mg 100 g^{-1}); HCA—hydroxycinnamic acids (mg 100 g^{-1}); FL—flavonols (mg 100 g^{-1}); CT—condensed tannins (mg 100 g^{-1}); Antioxidant activity—DPPH (µmol 100 g^{-1}) and FRAP-(µmol 100 g^{-1}); SSC—soluble solids content (°Brix). PEF—pulsed electric field (30 kV cm^{-1}, 100 Hz); HPU—high-power ultrasound (amplitude 25%, pulse 50%).

However, HCA and FL content decreased by 28% and 12%, respectively, during storage, as did DPPH and FRAP, which decreased by 4% and 3%, respectively, during the same period. The same trend was observed for pH but in a much lower range. Interestingly, the value of CT increased by 7%, perhaps implying that the losses of HCA and FL could be related to condensation to larger tannin molecules. This is also consistent with the observation that the amount of TPC remained constant since there was no real overall loss of polyphenols, as they probably only changed their form due to the condensation process. In addition, the results obtained are partly in accordance with our earlier work, in which HIPEF-treated strawberry juice samples exhibited a decrease in FL levels and an increase in CT levels during 7 days of cold storage [28].

When observing only PEF processing in the hurdle treatment, it can be seen that the total amount of TPC remained stable, regardless of how long the samples were exposed to PEF. These results are consistent with previously published work in which strawberry juices treated with HIPEF (40 and 50 kV cm^{-1}, 100 and 200 Hz) had no statistical effect on the changes in TPC amounts as a function of treatment duration of 3 and 6 min [28]. Of all the polyphenols studied, prolonged PEF treatment increased only HCA by 7% and DPPH by 6%. Similarly, for onion extracts, a positive correlation between antioxidant activity and electric field strength and duration of PEF treatment was found [41].

SSC and pH followed the same direction as the previously mentioned compounds but with a lower percentage (1%) at the lowest and the most intense exposure. Other polyphenols generally decreased with increasing duration of PEF treatment. Between 1.5

and 4.5 min of PEF, the percentage of FL and CT decreased by approximately 10% and 8%, respectively. Similarly, increasing PEF treatment duration from 3 to 6 minutes resulted in a statistically significant decrease in FL and CT [28]. Interestingly, FRAP followed a parabolic trend in which the highest value was observed at intermediate intensity and then fell to the initial level at the longest PEF treatment.

Independently, HPU did not affect HCA and pH, but the most intense exposure to this treatment decreased the levels of TPC, FL, CT, and FRAP. HPU treatments beyond 5 min decreased the content of TPC by 4%, FL by 10%, CT by 7%, and FRAP decreased by 6%. In a previous study with apple juice, HPU decreased the content of TPC by 32.94% FL by 21.66%, while the antioxidant activity decreased by 23.76% (DPPH) and 27.49% (FRAP) [42], suggesting that HPU in combination with other hurdles is less invasive to sensitive BACs in fruit juices. In HPU-processed strawberry juice, the processing time had no significant influence on the content of HCA, whereas the content of FL and CT was reduced by increasing the treatment duration from 5 to 10 min [27]. The observed trend is consistent with the obtained results, as the content of FL and CT increased when the treatment duration was increased from 5 to 7.5 min. In a study by Adekunte et al. [43], tomato juice was treated with HPU (20 kHz, 24.4–61.0 µm, 2–10 min), and the different operating times did not affect the changes in pH and SSC of the juice.

In our case, increasing the operating time from 2.5 to 5 min did not change the SSC value. However, when extended further to 7.5 min, an increase was observed. This could have a positive effect on stability during shelf-life, as a higher SSC value could inhibit the decrease in ascorbic acid and thus have a positive effect against oxidation [44].

When observing the impact of HPU on the content of bioactive antioxidants, a processing time of 5 min resulted in the highest content of TPC, FL, CT, and antioxidant activity (FRAP). Antioxidant bioactive compounds in the vacuole may be in soluble form or covalently bound to the cell wall matrix. Therefore, it is possible that the use of HPU favored the destruction of cell wall material and made it easier to release their constituents by cavitation collapsing around the colloidal particles [45]. A further extension to 5 min resulted in a reduction in these compounds. It is possible that this is a disintegration of the bioactive antioxidant due to long-term HPU treatment, which may cause excessive cavitation and cell breakup of the product [45]. DPPH was the only parameter that increased by almost half a percent after 5 min of HPU treatment, which may be due to the fact that it is related to the chemical changes and enhanced recovery of BACs.

To examine the effects of the hurdle technique, i.e., first combining the least exposure to PEF with all exposures to HPU, the following observations were made. Here, 1.5 min of PEF combined with 2.5, 5.0, and 7.5 min of HPU had no effect on TPC, HCA, FRAP, and pH. SSC and the levels of the other bioactive compounds decreased either linearly or parabolically with increasing HPU exposure, whereas DPPH was the only parameter that actually increased linearly with HPU exposure.

When the experiment was repeated with the same HPU settings but with an extension of PEF exposure to 3 min, the following was observed. Still, no changes were observed in the levels of TPC, HCA, FRAP, and PH. However, new patterns appeared in the data. Thus, FL and DPPH showed U-shaped curves; CT decreased linearly at 5%, while SSC increased linearly at 7% after 5 min exposure to HPU. To date, no studies have been performed on the influence of the treatment duration of the combination of PEF and HPU technology on the BACs and antioxidant capacities and physicochemical properties studied in this work.

The last part of the experiment included the highest PEF load (4.5 min) in combination with all previous HPU settings (2.5–7.5 min). Here, as before, no changes were observed in HCA, FRAP, and pH variables, but DPPH was added to this group of variables, while TPC showed significant changes in this hurdle setting. TPC, FL, and CT showed parabolic trends, meaning that the longest PEF exposure best matched with the mean exposure (5 min) to HPU. A linear trend was observed only for SSC, where the longest PEF/HPU exposure decreased the level of SSC by 3%. These data strongly suggest that the longest exposure to hurdle treatment causes the cellular material in the samples to degrade and

release polyphenols in the matrix until they are depleted, whereupon the mechanical forces of the hurdle technology (possibly HPU) begin to degrade them back to their original levels (or further). These data strongly suggest that optimizations are needed to identify "inflexion points" and ensure the highest possible nutritional value of the samples (in terms of polyphenolic compounds).

3.3. Optimization of Operating Parameters of Hurdle Technology for Strawberry Juice Treatments

Table 4 shows the mutual correlations between hurdle technology parameters and BAC content, antioxidant activity, SSC, and pH. Overall, prolonged storage decreased HCA, DPPH, and pH. El Darra et al. [46] recorded a decrease in pH when red grape extracts were stored during a 30-day of alcoholic fermentation. Moreover, prolonged exposure to PEF favored the increase in SSC and pH and the decrease in CT, while exposure to HPU showed no general direction. These results are in agreement with previous studies by Bebek Markovinović et al. [28], in which prolonged exposure of strawberry juice to HIPEF treatment from 3 to 6 min caused a statistically significant decrease in CT. TPC was positively associated with CT and FRAP, as this group of compounds was the most abundant in the samples and probably responsible for the antioxidant activity. With increasing HCA content, the content of FL increased, while the content of CT was reduced. This could be due to the fact that HCA condenses to tannins. In addition, HCA was strongly positively associated with DPPH, indicating that they contribute most to the antioxidant activity of the samples, and strongly negatively associated with decreasing pH, which makes sense since they contribute to the acidity of the samples. As the content of CT increased, DPPH and pH decreased, while FRAP increased.

Table 4. Mutual correlations of hurdle technology parameters on polyphenolic content, antioxidant capacities, SSC, and pH.

	Storage	**PEF Exposure**	**HPU Exposure**	**TPC** [1]	**HCA** [2]	**FL** [3]	**CT** [4]	**DPPH** [5]	**FRAP** [6]	**SSC** [7]	**pH**
Storage	1	0	0	−0.07	−0.85 *	−0.3	0.37 *	−0.96 *	−0.19	0.03	−0.73 *
PEF exposure		1	0	−0.07	0.13	−0.24	−0.38 *	0.11	0.09	0.75 *	0.30 *
HPU exposure			1	−0.17	−0.05	−0.08	−0.19	0.08	−0.16	0.08	0.09
TPC				1	0.08	0.14	0.69 *	0	0.74 *	0.09	0.19
HCA					1	0.38 *	−0.40 *	0.90 *	0.18	0.08	0.68 *
FL						1	−0.05	0.35 *	−0.05	−0.19	0.16
CT							1	−0.46 *	0.54 *	−0.33	−0.43 *
DPPH								1	0.17	0.04	0.74 *
FRAP									1	0.25	0.26
SSC										1	0.28
pH											1

* Correlation is significant at the $p \leq 0.05$. [1] TPC—total phenolic content (mg 100 g^{-1}); [2] HCA—hydroxycinnamic acids (mg 100 g^{-1}); [3] FL—flavonols (mg 100 g^{-1}); [4] CT—condensed tannins (mg 100 g^{-1}); [5] DPPH assay (µmol 100 g^{-1}); [6] FRAP assay (µmol 100 g^{-1}); [7] SSC—soluble solids content (°Brix). PEF—pulsed electric field (30 kV cm^{-1}, 100 Hz); HPU—high-power ultrasound (amplitude 25%, pulse 50%).

In order to obtain a product with preserved nutritional value, i.e., with a maximum content of native BACs, the operating parameters of the hurdle technology must be optimized. An optimal TPC of 125.81 mg 100 g^{-1} was obtained with a hurdle combination of 1.5 min PEF treatment and 2.5 min HPU treatment. Similarly, the highest CT content of 116.72 mg 100 g^{-1} was obtained with 1.5 min PEF and 3.2 min HPU treatment (Table 5, Figure 4). The best recovery of TPC and CT in strawberry juices was favored by shorter treatment time (4 min and 4.7 min of total treatment, respectively). Moreover, in this case, CT is one of the most abundant compounds observed in strawberry juices,

and due to its complex macromolecular structure, it is possible that a longer treatment time after the initial desirable extraction by the cavitation effect subsequently causes its destabilization and disintegration. Therefore, it is possible that CT and TPC correspond to a shorter treatment time, during which the destabilization of the cellular structure of the fruit tissue was achieved. This could facilitate the extraction of these compounds during the electroporation and cavitation process, but further exposure to these technologies would lead to their destabilization and destruction [47–49].

Table 5. Optimal hurdle parameters for maximum content of polyphenols and antioxidant activity in the samples.

	TPC	HCA	FL	CT	DPPH	FRAP
Storage (Days)	0.0	0.0	0.0	0.0	0.0	7.0
PEF treatment (min)	1.5	1.5	4.5	1.5	2.7	3.0
HPU treatment (min)	2.5	7.5	5.8	3.2	7.5	7.5
Optimal quantity (mg 100 g^{-1})	125.81	35.56	17.36	116.72	301.22	958.82

TPC—total phenolic content (mg 100 g^{-1}); HCA—hydroxycinnamic acids (mg 100 g^{-1}); FL—flavonols (mg 100 g^{-1}); CT—condensed tannins (mg 100 g^{-1}); Antioxidant activity—DPPH (μmol 100 g^{-1}) and FRAP (μmol 100 g^{-1}).

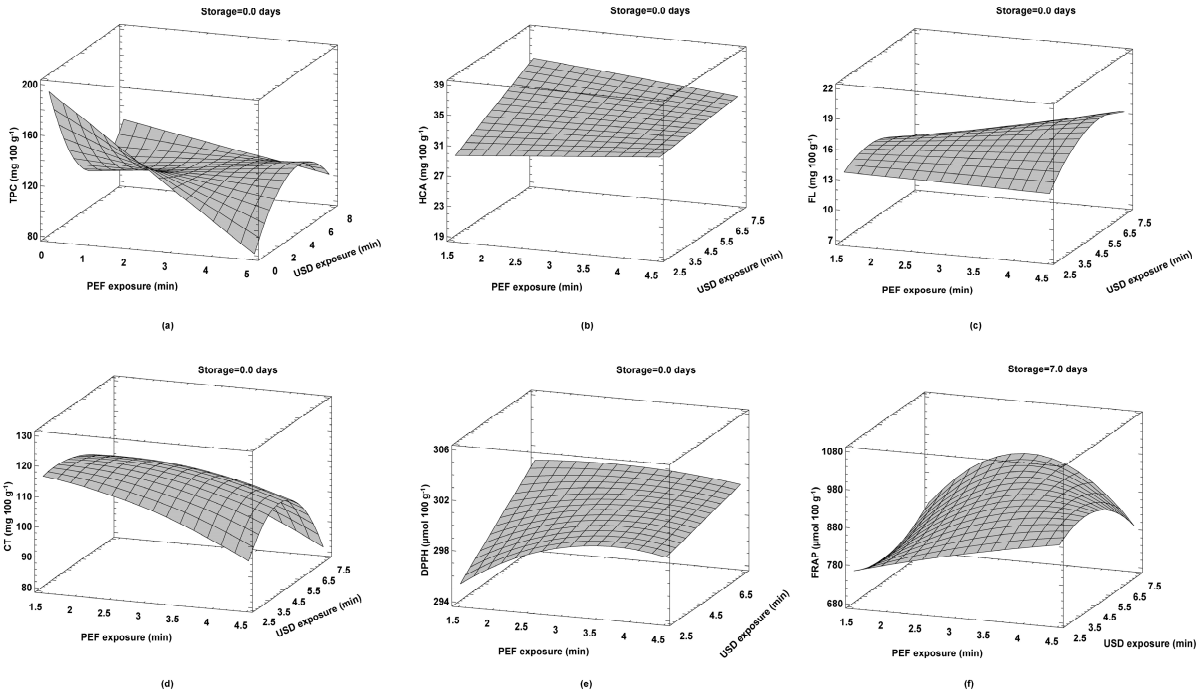


Figure 4. Optimal hurdle (PEF/HPU) treatment for maximum content of BACs and antioxidant activity: (**a**) total phenolic content (mg 100 g^{-1}); (**b**) hydroxycinnamic acids (mg 100 g^{-1}); (**c**) flavonols (mg 100 g^{-1}); (**d**) condensed tannins (mg 100 g^{-1}); (**e**) DPPH assay (μmol 100 g^{-1}); (**f**) FRAP assay (μmol 100 g^{-1}).

Similarly, the same PEF, but with a longer HPU exposure of 7.5 min, resulted in a maximum HCA content of 35.56 mg 100 g^{-1}. Longer exposure to both PEF (4.5 min) and

HPU (5.8 min) promoted an increased FL content, which was 17.36 mg 100 g^{-1}. In contrast to the recovery times of TPC and CT, the best yield of HCA and FL was obtained in longer hurdle treatments (9 min and 10.3 min, respectively). Koraqi et al. [50] showed that a longer time of ultrasound-assisted extraction increased the yield of phenolic and flavonoid compounds in relation to antioxidant activity values. At similar treatment times, 10.2 min and 10.5 min, the highest values of antioxidant capacities, DPPH and FRAP, were obtained. It seems that the best antioxidant activity in the samples was obtained at shorter exposure to PEF (2.7 min for DPPH; 3.0 min for FRAP) and longer exposure to HPU (both assays reached the maximum at 7.5 min). One of the possible explanations could be the fact that both components, HCA and FL, correlated positively with DPPH values (Table 3), and this could explain why it took a long time for complete extraction. As expected, all polyphenols show the highest values at the beginning of storage (0 days). These results are in contrast with previously published work, in which optimization of the parameters of the HIPEF technology (40 and 50 kV cm^{-1}, frequency 100 and 200 Hz, and treatment duration 3 and 6 min) showed that storage for 7 days at 4 °C favored the highest yield of TPC, HCA, and CT in strawberry juices [28]. This inconsistency could indicate a subsequent positive effect of the HIPEF technology, which caused easier straining of the extracted studied compounds during storage, which did not happen in our case because milder treatment conditions, i.e., hurdle technology, were applied. An interesting difference between the antioxidant assays relates to the duration of storage, where DPPH was highest at the beginning of storage, while FRAP peaked at the end of storage. This may be related to the subsequent extraction of BACs, which were not studied in this work and may have contributed in some way to the increased antioxidant activity during storage specifically demonstrated one antioxidant assay over the other.

4. Conclusions

Recently, there has been an increased search for sustainable processing for efficient preservation. In this regard, the hurdle concept is being explored, which combines the use of multiple hurdles (e.g., advanced thermal/non-thermal technology) with the goal of preserving the quality of functional foods. The results of this research confirm that PEF and HPU, in combination with chemometric optimization of the results, can be a good alternative to conventional preservation in strawberry fruit juice processing. Among the investigated BACs in strawberry juices, total phenols and hydroxycinnamic acids showed the highest stability when treated with hurdle technology, while total phenols were also the most stable during storage compared to the other individual groups of BACs. Considering physicochemical properties, BAC content, and antioxidant activity, the strawberry juice samples treated with a combination of PEF of 1.5 min and HPU of 2.5 min were most similar to the untreated samples stored for 0 days. The samples that were most similar to the controls after 7 days of storage were those treated with a combination of 1.5 min PEF and 2.5–7.5 min HPU. These results suggest that the combination of both technologies has a positive effect on maintaining the quality of strawberry juices, both after treatment and during cold chain storage.

By optimizing the hurdle technology parameters, the highest yield of BACs and antioxidant activity was obtained at 0 days of storage, except for the FRAP value, which was highest at 7 days of storage. The highest yield of TPC was obtained when treated with PEF (1.5 min) and HPU (2.5 min) with 125.81 mg 100 g^{-1}. This was followed by condensed tannins with 116.72 mg 100 g^{-1} when treated with PEF (1.5 min) and HPU (3.2 min), hydroxycinnamic acids when treated with PEF (1.5 min) and HPU (7.5 min), and finally flavonols when treated with PEF (4.5 min) and HPU (5.8 min). The highest DPPH values were measured in treatment with PEF (2.7 min) and HPU (7.5 min) and FRAP in treatment with PEF (3.0 min) and HPU (7.5 min).

It can be concluded that PEF and HPU in a hurdle combination can be considered for broader applications as two innovative technologies that complement each other thanks to their different mechanisms of action (e.g., electroporation, cavitation) and have a mutually

beneficial synergistic effect on the stability of BACs. Optimization performed with chemometric tools has shown that shorter treatment times are more favorable for maintaining the biological value of juices. However, this technology should be further tested for the stability of other quality parameters, which would confirm this concept as promising for the juice processing industry.

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References

1. Azam, M.; Ejaz, S.; Naveed Ur Rehman, R.; Khan, M.; Qadri, R. Postharvest Quality Management of Strawberries. In *Strawberry—Pre- and Post-Harvest Management Techniques for Higher Fruit Quality*; Asao, T., Asaduzzaman, M., Eds.; IntechOpen: London, UK, 2019.
2. Pap, N.; Fidelis, M.; Azevedo, L.; do Carmo, M.A.V.; Wang, D.; Mocan, A.; Pereira, E.P.R.; Xavier-Santos, D.; Sant'Ana, A.S.; Yang, B.; et al. Berry polyphenols and human health: Evidence of antioxidant, anti-inflammatory, microbiota modulation, and cell-protecting effects. *Curr. Opin. Food Sci.* **2021**, *42*, 167–186. [CrossRef]
3. Giampieri, F.; Forbes-Hernandez, T.Y.; Gasparrini, M.; Alvarez-Suarez, J.M.; Afrin, S.; Bompadre, S.; Quiles, J.L.; Mezzetti, B.; Battino, M. Strawberry as a health promoter: An evidence based review. *Food Funct.* **2015**, *6*, 1386–1398. [CrossRef] [PubMed]
4. Aaby, K.; Mazur, S.; Nes, A.; Skrede, G. Phenolic compounds in strawberry (*Fragaria* × *ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chem.* **2012**, *132*, 86–97. [CrossRef] [PubMed]
5. Alvarez-Suarez, J.M.; Mazzoni, L.; Forbes-Hernandez, T.Y.; Gasparrini, M.; Sabbadini, S.; Giampieri, F. The effects of pre-harvest and post-harvest factors on the nutritional quality of strawberry fruits: A review. *J. Berry Res.* **2014**, *4*, 1–10. [CrossRef]
6. Skupien, K.; Oszmianski, J. Comparison of six cultivars of strawberries (*Fragaria* × *ananassa* Duch.) grown in northwest Poland. *Eur. Food Res. Technol.* **2004**, *219*, 66–70. [CrossRef]
7. Bebek Markovinović, A.; Putnik, P.; Duralija, B.; Krivohlavek, A.; Ivešić, M.; Mandić Andačić, I.; Palac Bešlić, I.; Pavlić, B.; Lorenzo, J.M.; Bursać Kovačević, D. Chemometric Valorization of Strawberry (*Fragaria* × *ananassa* Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. *Foods* **2022**, *11*, 640. [CrossRef]
8. Cassani, L.; Tomadoni, B.; Moreira, M.R.; Agüero, M.V. Improving Quality Parameters of Functional Strawberry Juices: Optimization of Prebiotic Fiber Enrichment and Geraniol Treatment. *Food Bioprocess Technol.* **2018**, *11*, 2110–2124. [CrossRef]
9. Sui, X.; Bary, S.; Zhou, W. Changes in the color, chemical stability and antioxidant capacity of thermally treated anthocyanin aqueous solution over storage. *Food Chem.* **2016**, *192*, 516–524. [CrossRef]
10. Patras, A.; Brunton, N.P.; O'Donnell, C.; Tiwari, B.K. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci. Technol.* **2010**, *21*, 3–11. [CrossRef]
11. Kim, D.O.; Padilla-Zakour, O.I. Jam processing effect on phenolics and antioxidant capacity in anthocyanin-rich fruits: Cherry, plum, and raspberry. *J. Food Sci.* **2006**, *69*, S395–S400. [CrossRef]
12. Zia, S.; Khan, M.R.; Shabbir, M.A.; Aslam Maan, A.; Khan, M.K.I.; Nadeem, M.; Khalil, A.A.; Din, A.; Aadil, R.M. An Inclusive Overview of Advanced Thermal and Nonthermal Extraction Techniques for Bioactive Compounds in Food and Food-related Matrices. *Food Rev. Int.* **2020**, *38*, 1166–1196. [CrossRef]
13. Bursać Kovačević, D.; Maras, M.; Barba, F.J.; Granato, D.; Roohinejad, S.; Mallikarjunan, K.; Montesano, D.; Lorenzo, J.M.; Putnik, P. Innovative technologies for the recovery of phytochemicals from *Stevia rebaudiana* Bertoni leaves: A review. *Food Chem.* **2018**, *268*, 513–521. [CrossRef] [PubMed]

14. Koubaa, M.; Barba, F.J.; Bursać Kovačević, D.; Putnik, P.; Santos, M.D.; Queirós, R.P.; Moreira, S.A.; Inácio, R.S.; Fidalgo, L.G.; Saraiva, J.A. Pulsed electric field processing of fruit juices. In *Fruit Juices*; Academic Press: Cambridge, MA, USA, 2018; pp. 437–449.
15. Gabrić, D.; Barba, F.; Roohinejad, S.; Gharibzahedi, S.M.T.; Radojčin, M.; Putnik, P.; Bursać Kovačević, D. Pulsed electric fields as an alternative to thermal processing for preservation of nutritive and physicochemical properties of beverages: A review. *J. Food Process Eng.* **2018**, *41*, e12638. [CrossRef]
16. Tiwari, B.K. Ultrasound: A clean, green extraction technology. *TrAC Trends Anal. Chem.* **2015**, *71*, 100–109. [CrossRef]
17. Aaliya, B.; Valiyapeediyekkal Sunooj, K.; Navaf, M.; Parambil Akhila, P.; Sudheesh, C.; Ahmad Mir, S.; Sabu, S.; Sasidharan, A.; Theingi Hlaing, M.; George, J. Recent trends in bacterial decontamination of food products by hurdle technology: A synergistic approach using thermal and non-thermal processing techniques. *Food Res. Int.* **2021**, *147*, 110514. [CrossRef]
18. Arya, S.S.; Nachiappan, N.; Waghmare, R.; Bhat, M.S. Recent progress and future perspectives on non-thermal apple juice processing techniques. *Food Prod. Process. Nutr.* **2023**, *5*, 36. [CrossRef]
19. Krishnan Kesavan, R.; Begum, S.; Das, P.; Nayak, P.K. Hurdle effect of thermosonication and non-thermal processing on the quality characteristics of fruit juices: An overview. *J. Food Process Eng.* **2023**, *46*, e14310. [CrossRef]
20. Jin, T.Z.; Aboelhaggag, R.M. Combined Pulsed Electric Field with Antimicrobial Caps for Extending Shelf Life of Orange Juice. *Beverages* **2022**, *8*, 72. [CrossRef]
21. Shaik, L.; Chakraborty, S. Nonthermal pasteurization of pineapple juice: A review on the potential of achieving microbial safety and enzymatic stability. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 4716–4737. [CrossRef]
22. Mehta, D.; Yadav, S.K. Impact of atmospheric non-thermal plasma and hydrothermal treatment on bioactive compounds and microbial inactivation of strawberry juice: A hurdle technology approach. *Food Sci. Technol. Int.* **2019**, *26*, 3–10. [CrossRef]
23. Yildiz, S.; Pokhrel, P.R.; Unluturk, S.; Barbosa-Cánovas, G.V. Shelf life extension of strawberry juice by equivalent ultrasound, high pressure, and pulsed electric fields processes. *Food Res. Int.* **2021**, *140*, 110040. [CrossRef] [PubMed]
24. Yildiz, G.; Aadil, R.M. Comparison of high temperature-short time and sonication on selected parameters of strawberry juice during room temperature storage. *J. Food Sci. Technol.* **2019**, *57*, 1462–1468. [CrossRef] [PubMed]
25. Yildiz, S.; Pokhrel, P.R.; Unluturk, S.; Barbosa-Cánovas, G.V. Changes in Quality Characteristics of Strawberry Juice After Equivalent High Pressure, Ultrasound, and Pulsed Electric Fields Processes. *Food Eng. Rev.* **2020**, *13*, 601–612. [CrossRef]
26. Cassani, L.; Tomadoni, B.; Moreira, M.R.; Ponce, A.; Agüero, M.V. Optimization of inulin:Oligofructose proportion and non-thermal processing to enhance microbiological and sensory properties of fiber-enriched strawberry juice. *LWT* **2017**, *80*, 446–455. [CrossRef]
27. Bebek Markovinović, A.; Putnik, P.; Bičanić, P.; Brdar, D.; Duralija, B.; Pavlić, B.; Milošević, S.; Rocchetti, G.; Lucini, L.; Bursać Kovačević, D. A chemometric investigation on the functional potential in high power ultrasound (HPU) processed strawberry juice made from fruits harvested at two stages of ripeness. *Molecules* **2022**, *28*, 138. [CrossRef]
28. Bebek Markovinović, A.; Putnik, P.; Stulić, V.; Batur, L.; Duralija, B.; Pavlić, B.; Vukušić Pavičić, T.; Herceg, Z.; Bursać Kovačević, D. The application and optimization of HIPEF technology in the processing of juice from strawberries harvested at two stages of ripeness. *Foods* **2022**, *11*, 1997. [CrossRef]
29. Yuan, B.; Danao, M.-G.C.; Stratton, J.E.; Weier, S.A.; Weller, C.L.; Lu, M. High pressure processing (HPP) of aronia berry purée: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. *Innov. Food Sci. Emerg. Technol.* **2018**, *47*, 249–255. [CrossRef]
30. Howard, L.R.; Clark, J.R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238–1247. [CrossRef]
31. Sun, B.; Ricardo-da-Silva, J.M.; Spranger, I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. [CrossRef]
32. Shortle, E.; O'Grady, M.N.; Gilroy, D.; Furey, A.; Quinn, N.; Kerry, J.P. Influence of extraction technique on the anti-oxidative potential of hawthorn (*Crataegus monogyna*) extracts in bovine muscle homogenates. *Meat Sci.* **2014**, *98*, 828–834. [CrossRef]
33. Benzie, I.F.F. An automated, specific, spectrophotometric method for measuring ascorbic acid in plasma (EFTSA). *Clin. Biochem.* **1996**, *29*, 111–116. [CrossRef]
34. Maza, M.A.; Pereira, C.; Martínez, J.M.; Camargo, A.; Álvarez, I.; Raso, J. PEF treatments of high specific energy permit the reduction of maceration time during vinification of Caladoc and Grenache grapes. *Innov. Food Sci. Emerg. Technol.* **2020**, *63*, 102375. [CrossRef]
35. Wang, J.; Wang, J.; Ye, J.; Vanga, S.K.; Raghavan, V. Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control* **2019**, *96*, 128–136. [CrossRef]
36. Putnik, P.; Pavlić, B.; Šojić, B.; Zavadlav, S.; Žuntar, I.; Kao, L.; Kitonić, D.; Kovačević, D.B. Innovative hurdle technologies for the preservation of functional fruit juices. *Foods* **2020**, *9*, 699. [CrossRef]
37. Manzoor, M.F.; Ahmed, Z.; Ahmad, N.; Aadil, R.M.; Rahaman, A.; Roobab, U.; Rehman, A.; Siddique, R.; Zeng, X.A.; Siddeeg, A. Novel processing techniques and spinach juice: Quality and safety improvements. *J. Food Sci.* **2020**, *85*, 1018–1026. [CrossRef]
38. Makroo, H.A.; Saxena, J.; Rastogi, N.K.; Srivastava, B. Ohmic heating assisted polyphenol oxidase inactivation of watermelon juice: Effects of the treatment on pH, lycopene, total phenolic content, and color of the juice. *J. Food Process. Preserv.* **2017**, *41*, e13271. [CrossRef]

39. Assiry, A. Degradation kinetics of ascorbic acid during ohmic heating with stainless steel electrodes. *J. Appl. Electrochem.* **2003**, *33*, 187–196. [CrossRef]
40. Sulaiman, A.; Farid, M.; Silva, F.V.M. Strawberry puree processed by thermal, high pressure, or power ultrasound: Process energy requirements and quality modeling during storage. *Food Sci. Technol. Int.* **2016**, *23*, 293–309. [CrossRef] [PubMed]
41. Liu, Z.-W.; Zeng, X.-A.; Ngadi, M. Enhanced extraction of phenolic compounds from onion by pulsed electric field (PEF). *J. Food Process. Preserv.* **2018**, *42*, e13755. [CrossRef]
42. Bursać Kovačević, D.; Bilobrk, J.; Buntić, B.; Bosiljkov, T.; Karlović, S.; Rocchetti, G.; Lucini, L.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. High-power ultrasound altered the polyphenolic content and antioxidant capacity in cloudy apple juice during storage. *J. Food Process. Preserv.* **2019**, *43*, e14023. [CrossRef]
43. Adekunte, A.O.; Tiwari, B.K.; Cullen, P.J.; Scannell, A.G.M.; O'Donnell, C.P. Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chem.* **2010**, *122*, 500–507. [CrossRef]
44. Sobral, M.M.C.; Nunes, C.; Maia, A.; Ferreira, P.; Coimbra, M.A. Conditions for producing long shelf life fruit salads processed using mild pasteurization. *LWT-Food Sci. Technol.* **2017**, *85*, 316–323. [CrossRef]
45. Nguyen, C.; Nguyen, H. Ultrasonic Effects on the Quality of Mulberry Juice. *Beverages* **2018**, *4*, 56. [CrossRef]
46. El Darra, N.; Grimi, N.; Maroun, R.G.; Louka, N.; Vorobiev, E. Pulsed electric field, ultrasound, and thermal pretreatments for better phenolic extraction during red fermentation. *Eur. Food Res. Technol.* **2012**, *236*, 47–56. [CrossRef]
47. Roobab, U.; Aadil, R.M.; Madni, G.M.; Bekhit, A.E.-D. The impact of nonthermal technologies on the microbiological quality of juices: A review. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 437–457. [CrossRef]
48. Chemat, F.; Zill-e-Huma; Khan, M.K. Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. Sonochemistry* **2011**, *18*, 813–835. [CrossRef]
49. Barba, F.J.; Parniakov, O.; Pereira, S.A.; Wiktor, A.; Grimi, N.; Boussetta, N.; Saraiva, J.A.; Raso, J.; Martin-Belloso, O.; Witrowa-Rajchert, D.; et al. Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Res. Int.* **2015**, *77*, 773–798. [CrossRef]
50. Koraqi, H.; Petkoska, A.T.; Khalid, W.; Sehrish, A.; Ambreen, S.; Lorenzo, J.M. Optimization of the extraction conditions of antioxidant phenolic compounds from strawberry fruits (*Fragaria* × *ananassa* Duch.) using response surface methodology. *Food Anal. Methods* **2023**, *16*, 1030–1042. [CrossRef]

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Article

High-Power Ultrasound (HPU) and Pulsed Electric Field (PEF) in the Hurdle Concept for the Preservation of Antioxidant Bioactive Compounds in Strawberry Juice—A Chemometric Evaluation—Part II

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Abstract: In this work, the influence of high-power ultrasound (HPU) followed by pulsed electric field (PEF) in the hurdle concept (HPU + PEF) on the content of biologically active compounds (BACs) and antioxidant activity in strawberry juices stored at 4 °C/7 days was investigated. The HPU was performed with an amplitude of 25% and pulse of 50% during 2.5, 5.0 and 7.5 min, while the PEF was performed with an electric field strength of 30 kV cm^{-1} and frequency of 100 Hz during 1.5, 3 and 4.5 min. The results obtained indicate that the synergy of the mechanisms of action for technologies in the hurdle concept plays a critical role in the stability of BACs and antioxidant activity. Juices treated with HPU + PEF hurdle technology and kept at 4 °C for 7 days showed a statistically significant decrease in all BACs, antioxidant capacity and pH. Shorter HPU + PEF treatment times favored the preservation of BACs in juices. Regarding total phenolic compounds, flavonols, condensed tannins and antioxidant capacity, optimization of hurdle parameters showed that a shorter HPU treatment time of 2.5 min provided the best yield of these compounds. In summary, by optimizing and adjusting the parameters of the HPU/PEF technology, it is possible to produce functional strawberry juice.

Keywords: hurdle concept; non-thermal technology; functional juice; bioactive compounds; storage

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1. Introduction

A diet rich in fruits and vegetables has a helpful effect on human health and general well-being due to the effects of various ingredients, such as polyphenols, carotenoids, tocopherols, vitamins, minerals, fibers and others [1]. In recent years, fresh fruits and vegetables have been increasingly consumed in the form of pressed juices, smoothies and fermented beverages [2]. The quality of fruit juices depends on physical, organoleptic and microbiological aspects, as well as enzymatic activity. The shelf life of the product can be shortened by the action of enzymes, but also by the growth of microorganisms and/or oxidation reactions. In order to inactivate enzymes, but also to preserve health properties, fruit juices are traditionally preserved by heat treatment [3]. Nevertheless, increased temperatures have a negative impact, not only on the bioactive composition, but also on the color parameters and sensory properties, which is why there has been a growing interest in the application of non-thermal preservation methods in recent years [4].

Though non-thermal treatments do not appear to be as invasive as thermal treatments, the global effect depends on the matrix of the food to be processed [5]. Therefore, it is necessary to choose the most suitable non-thermal process with validated processing conditions

to preserve all the nutrients and the original organoleptic properties [6]. Nowadays, consumers are increasingly attracted to minimally processed food without additives, proven safety and longer shelf life while maintaining biological and functional properties [7]. Due to these facts, the food industry combines different technologies in food processing, i.e., it uses the so-called concept of "hurdle technology", in which a well-defined sequence of process hurdles is applied that the microorganisms present cannot "overcome" [8]. The aim of using "hurdle technology" is to simultaneously improve nutritional and sensory quality and increase food safety [9]. The main benefit of the use of hurdle processing, which consists of the combination of different technologies, is the synergistic effect of different mechanisms that achieve an improved preservation effect [9]. By carefully combining hurdles, each individual process can be carried out under much milder conditions than when applied separately. The resulting product can be considered microbiologically stable and at the same time exhibit improved functionality [8].

Recently, pulsed electric field (PEF) and high-power ultrasound (HPU) technologies have shown promising results in the processing of fruit juices to obtain stable and biologically valuable functional products [10–12]. PEF technology is characterized by the inactivation of microorganisms and enzymes, which can be a substitute to thermal pasteurization [13]. Depending on applied process parameters, such as the strength of the electric field or the number of pulses, the effect of PEF can produce different effects and can therefore be used for different purposes [14,15].

Although the mechanism of action at the cellular level is still not reliably defined, the most common explanation of the mechanism is supported by the phenomenon of electroporation and electropermeability of the cell membrane, whereby membrane damage occurs with the formation of (i)reversible ruptures as a result of exposure to an external electric field [16,17]. PEF processing can also provide products with improved antioxidant properties [18]. For example, strawberry juice treated with PEF shows a retention of antioxidant capacity of 75% to 100% in comparison to untreated juice [19]. The HPU is based on cavitation effect, which can lead to physical and chemical changes in the material. The effect of HPU creates a longitudinal mechanical wave in the liquid medium and an alternating pressure change occurs, i.e., phases of compression and expansion [17], which leads to the occurrence of cavitation. During cavitation, gas bubbles form in the medium, the volume of which increases from cycle to cycle until a critical size is reached. At the moment the critical size is reached, vapor condensation and implosion of the bubbles occur, and the molecules collide at high speed, creating the so-called shock waves. Shock waves cause very high temperatures (up to 5500 K) and pressures (up to 100 MPa). The formation of cavitation is in relation to processing parameters (e.g., frequency, intensity), the product characteristics (e.g., viscosity, density) and environmental conditions (e.g., temperature, pressure) [20].

The preservation of food and beverages using the hurdle concept is increasingly being researched to replace thermal preservation processes with sustainable technologies as thermal processing can have an undesirable effect on the food quality. In the last 5 years, only a few studies have studied the impact of hurdle technology on the quality of strawberry juice. The influence of the combination of ultrasound (40 kHz; 180 W; 0, 15 and 30 min) and natural antimicrobial additives (geraniol and pomegranate extract) on the microbial, sensory and nutritional quality of strawberry juice was studied by Tomadoni et al. [21]. Emamifar and Mohamadizadeh [22] studied the effect of ultrasound and antimicrobial ZnO nanostructure packaging on the quality of fresh strawberry juice (*Fragaria* × *anannasa* Duch., cultivar 'Parous') during cold storage for 35 days. However, the combination of HPU and PEF technologies has not yet been tested on strawberry juice, with the exception of a previously published paper, in which the technology was tested on strawberry juice in the PEF + HPU sequence [12]. Precisely because of the different mechanisms, it is of great importance to select the optimal sequence of hurdle technologies under optimized processing conditions. The aim of this work was therefore to investigate the effects of hurdle technology, i.e., the combination of HPU (amplitude 25%; pulse 50%; 2.5, 5.0 and 7.5 min)

followed by PEF (30 kV cm^{-1}; 100 Hz; 1.5, 3.0 and 4.5 min) on the stability of polyphenolic compounds and antioxidant capacity in strawberry juices during 7-day storage at 4 °C. In the final consideration, the results of this research will be compared with the results of previous research [12] to better understand the mechanism and influence of hurdle technology in the processing of functional strawberry juices.

2. Materials and Methods

2.1. Juice Samples

Strawberries (*Fragaria* × *ananassa* Duch, cultivar 'Albion') were grown and harvested in 2022 at Jagodar HB in Donja Lomnica, Croatia. After harvesting, the fruits were transported to the laboratory, de-stemmed, washed, dried and stored at −18 °C. The juice was processed in a Kuvings B6000 slow juicer (240 W, speed 60 rpm; VerVita d.o.o., Zagreb, Croatia). The experiment was carried out according to the experimental research design (Table 1).

Table 1. Experimental research design.

Juice Sample	Storage (Days)	Hurdle Processing	HPU Treatment (min)	PEF Treatment (min)
1	0	Control	0	0
2	0	HPU + PEF	2.5	1.5
3	0	HPU + PEF	2.5	3.0
4	0	HPU + PEF	2.5	4.5
5	0	HPU + PEF	5.0	1.5
6	0	HPU + PEF	5.0	3.0
7	0	HPU + PEF	5.0	4.5
8	0	HPU + PEF	7.5	1.5
9	0	HPU + PEF	7.5	3.0
10	0	HPU + PEF	7.5	4.5
11	7	Control	0	0
12	7	HPU + PEF	2.5	1.5
13	7	HPU + PEF	2.5	3.0
14	7	HPU + PEF	2.5	4.5
15	7	HPU + PEF	5.0	1.5
16	7	HPU + PEF	5.0	3.0
17	7	HPU + PEF	5.0	4.5
18	7	HPU + PEF	7.5	1.5
19	7	HPU + PEF	7.5	3.0
20	7	HPU + PEF	7.5	4.5

Control—untreated strawberry juice; and HPU + PEF—strawberry juice samples processed with HPU and PEF technology in hurdle concept.

2.2. High-Power Ultrasound (HPU) and Pulsed Electric Field (PEF) in Hurdle Concept for Processing Strawberry Juice

A total of 200 mL of strawberry juice was processed with HPU and then with PEF. For the HPU treatment, the Hielscher UP400St (400 W, 24 kHz) device (Hielscher Ultrasonics GmbH, Teltow, Germany) with a titanium horn Ø 22 mm was used, so that the maximum energy density was up to 300 W/cm^2. The HPU process parameters were amplitude of 25% (11.5 µm), pulse of 50% and treatment times of 2.5, 5.0 and 7.5 min. The duty cycle between pause and ultrasound treatment was set to 50%, which means that an ultrasound treatment lasted 0.5 s with a pause of 0.5 s.

A batch PEF system with the HVG60/1 HIPEF device (Impel d.o.o., Zagreb, Croatia), consisting of a Magna control unit, a high-voltage power source and a high-voltage pulse generator, was used for the PEF treatment. The treatment chamber consisted of 2 circular plates assembled in the form of a cylinder with a capacity of 200 mL. The treatments were performed with an electric field strength of 30 kV cm^{-1} at a frequency of 100 Hz. The total treatment times were 1.5, 3.0 and 4.5 min with the pulses of 1 µs. The ground electrode and the high-voltage electrode were 2.5 cm apart. A total of 18 juices were treated with the

hurdle concept, and two samples were control samples, which were untreated and served to compare the effect of the hurdle concept.

Throughout processing with the HPU, followed by PEF technology, the temperature was monitored before and after treatment using a PCE-777 infrared thermometer (PCE Instruments, Southampton, UK). The average initial temperature of the juices before processing was 15.67 °C, after the HPU treatment 19.55 °C and after the PEF treatment 17.97 °C; therefore, the effect of temperature can be ignored. At the end of each treatment, all the samples were kept in sterilized, hermetically sealed glass bottles. One batch of juices was analyzed immediately after treatment, and another batch was kept at 4 °C/7 days. All juice samples were tested for physico-chemical properties (pH and SSC), the content of BACs and antioxidant activity.

2.3. pH and Soluble Solids Content (SSC)

A Mettler Toledo FiveEasy pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland) and digital refractometer (ATAGO Pal-3 digital refractometer, ATAGO Co., Tokyo, Japan) were used for determination of pH and SSC, respectively.

2.4. Extraction Procedure

An extraction protocol was adopted from the literature [23]. The strawberry juice sample (5 g) was mixed with 1% formic acid in 80% methanol (v/v) (20 mL). Ultrasound-assisted extraction was performed in an ultrasonic bath (DT 514 H Sonorex Digitec 13.5 L, 860 W, 40 kHz, Bandelin electronic, Berlin, Germany) at 50 °C for 15 min. After filtration, the supernatant was transferred to a volumetric flask (25 mL) and made up with the extraction solvent. The extracts were kept at −18 °C in an inert gas atmosphere until analysis.

2.5. The Content of Bioactive Compounds

For all spectrophotometric determinations, a spectrophotometer (LLG-uniSPEC 2 Spectrophotometer, Buch and Holm, Meckenheim, Germany) was used. Total phenolic content (TPC) was determined by the modified method described in the literature by measuring absorbance at 725 nm [24]. Hydroxycinnamic acids (HCAs) and flavonols (FLs) were determined by the spectrophotometric method using 1 g L^{-1} HCl solution in 96% ethanol and 2 g L^{-1} HCl solution in distilled water by measuring the absorbance at 320 nm and 360 nm, respectively [25]. In addition, a modified spectrophotometric method was used to determine condensed tannins (CTs) using a 25% solution of sulfuric acid in methanol and a 1% solution of vanillin in methanol by measuring the absorbance at 500 nm [26]. The results of TPC were expressed as mg gallic acid equivalent (GAE) per 100 g sample, and HCA content and FLs were expressed as mg chlorogenic acid equivalent (CAE) per 100 g sample and mg quercetin equivalent (QE) per 100 g sample, respectively. The results of CTs were expressed as mg catechin equivalent (CA) per 100 g of sample. The determination procedure is described in detail in the first part of the published research paper [12].

2.6. In Vitro Antioxidant Activity Assay

In this study, two assays DPPH and FRAP, based with specific mechanisms of action, were used [27,28]. For the DPPH assay, a 0.5 mM DPPH solution was used, and the colorimetric response was recorded at 517 nm. For the FRAP assay, the FRAP reagent was used, the reaction was thermostatted at 37 °C for 10 min and the absorbance was recorded at 593 nm. The results obtained with both assays were expressed as µmol Trolox equivalent (TE) per 100 g of sample. The detailed protocol can be found in the first part of the published research paper [12].

2.7. Statistical Analysis

Full factorial randomized experimental designs were used for the experiments (n = 40) (Table 1). All BAC contents and antioxidant activities were the dependent variables. HPU treatment (2.5, 5.0 and 7.5 min), PEF treatment (1.5, 3.0 and 4.5 min) and storing time (0 and

7 days) were all independent factors. For the experimental dataset, descriptive statistics were used to evaluate the basic data. Multiple analysis of variance (MANOVA) was used to examine differences between treatments (continuous variables). To evaluate correlations between pairs of continuous variables, the Pearson coefficient was used. Exploratory hierarchical Ward cluster analysis was used to measure standardized similarities in samples, and the Kruskal–Wallis test was used for nonparametric analysis. Significance levels for rejection of a null hypothesis were $\alpha \leq 0.05$ for all tests. IBM SPSS Statistics (v.24) was used for analyses, and Statgraphics Centurion® (StatPoint Technologies, Inc., Warrenton, VA, USA) was used for experimental design.

3. Results and Discussion

3.1. Chemometrics Evaluation of Hurdle-Treated Samples against Untreated Samples

An exploratory hierarchical Ward's cluster analysis was performed to determine the influence of hurdle processing (HPU + PEF) on the quality of strawberry juice. When all samples were analyzed for standardized similarities (TPC, HCA, FL, CT, DPPH, FRAP, SSC, pH), the samples treated with HPU for 2.5 min and 1.5 min with PEF followed by HPU for 5.0 min and 4.5 min with PEF were most similar to the control samples at 0 days of storage. These findings were similar to our previous results where we studied the reverse order of hurdle technologies. On day 0 of storage, samples treated with PEF for 1.5 min and 2.5 min with HPU were most similar to controls [12]. On day 7 of storage, samples treated with HPU for 5.0 min and 3.0 min with PEF were most similar to controls (Figure 1).

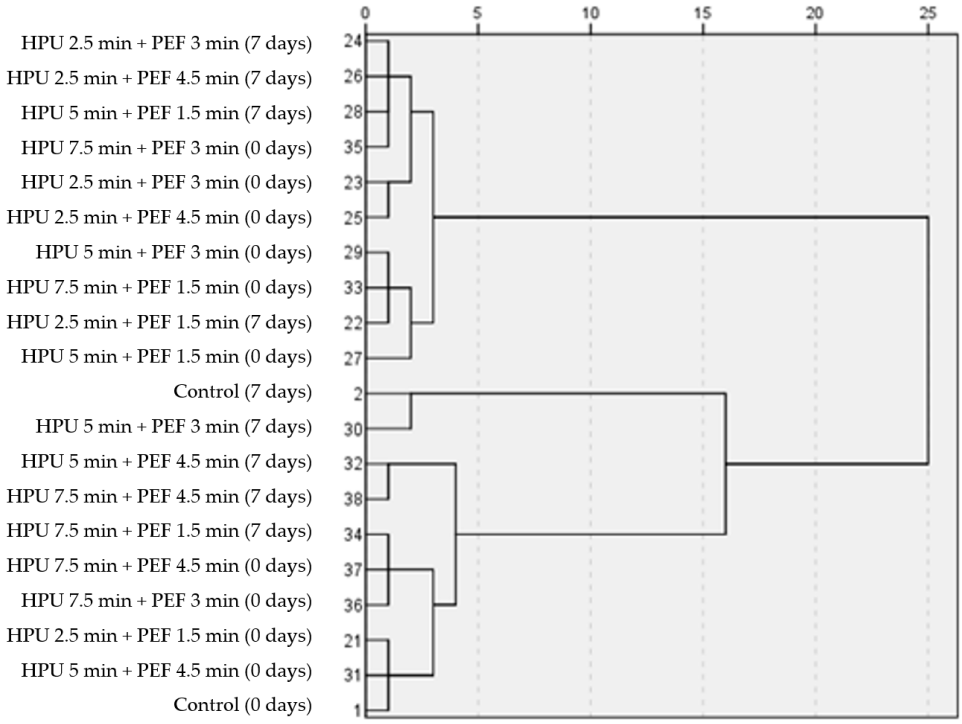

Figure 1. Results of the hierarchical clustering for averaged and standardized juice samples.

However, compared to the PEF + HPU combination treatment, the samples most similar to the untreated sample after one week of storage differ in the duration of the PEF treatment (PEF + HPU—1.5 min and HPU + PEF—3 min).

The untreated samples did not differ from the samples treated with the hurdle technology with respect to the BACs analyzed and antioxidant activity. There were only differences in FRAP and pH, where the experimental samples had higher median values than the control samples, and in SSC, when this was reversed (i.e., the control samples were more acidic than the hurdle samples, had lower FRAP antioxidant activity and higher SSC; Figure 2). The differences in the antioxidant capacity of DPPH and FRAP in the treated

juice samples may be attributed to the different sensitivity of these methods to the same fruit BACs [29]. In addition, the treated strawberry juice samples exhibited decreased SSC levels, which is consistent with the results of Koners et al. [30], who found a reduction in SSC levels in the excess sludge during the PEF treatment parameters (energy input of 100 kJ kg^{-1} at 15 kV cm^{-1} and sludge retention time of 14 days).

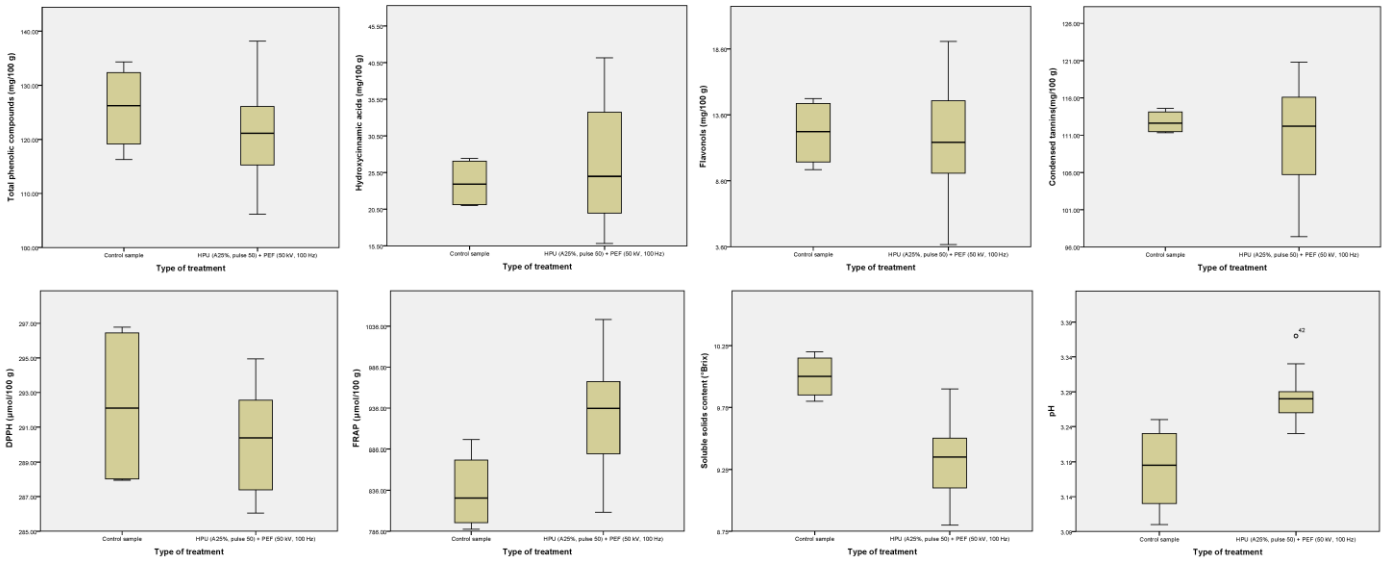

Figure 2. Median values of BAC content, antioxidant activity, SSC and pH in control vs. hurdle-treated samples.

Compared to the results of the reverse treatment sequence (PEF + HPU) [12], there are still common results of an increase in pH for the treated samples, which could be attributed to the possibility of electrolysis or electrochemical interactions during the treatment [31]. Table 2 shows the numerical values of the Kruskal–Wallis test of the hurdle-treated samples compared to the untreated samples, which correlates with the graphical representation in Figure 3.

Table 2. Results for Kruskal–Wallis test (hurdle treatment vs. control samples).

	TPC	HCA	FL	CT	DPPH	FRAP	SSC	pH
Chi-Square	1.37	0.10	0.03	0.03	1.17	6.84	8.9	9.11
df	1	1	1	1	1	1	1	1
Sig.	0.24	0.75	0.86	0.86	0.28	≤0.01 *	≤0.01 *	≤0.01 *

* Kruskal–Wallis test is significant at $p \leq 0.05$. TPC—total phenolic content (mg 100 g^{-1}); HCA—hydroxycinnamic acid (mg 100 g^{-1}); FL—flavonol (mg 100 g^{-1}); CT—condensed tannin (mg 100 g^{-1}); antioxidant activity—DPPH (µmol 100 g^{-1}) and FRAP (µmol 100 g^{-1}); and SSC—soluble solids content (°Brix).

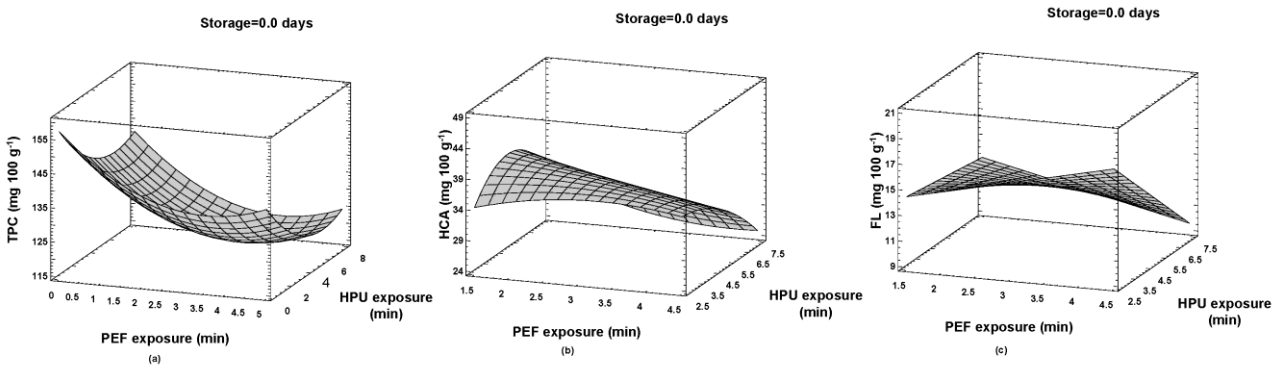

Figure 3. *Cont.*

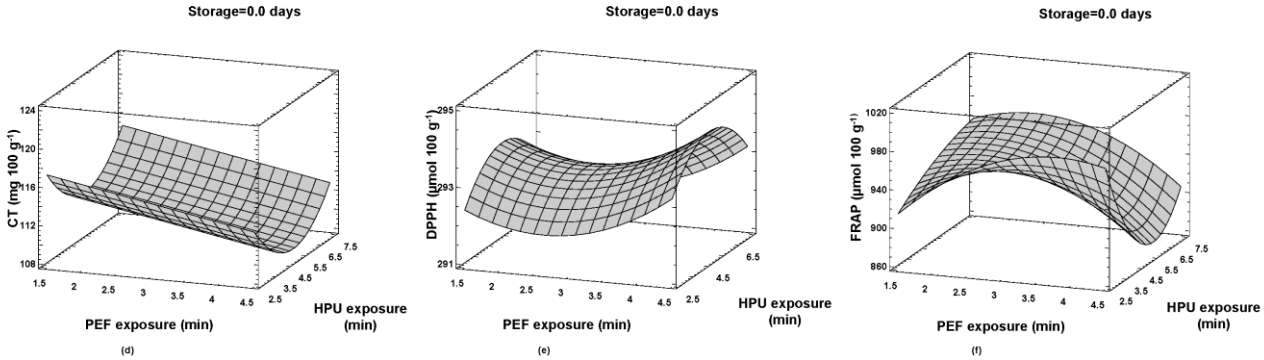

Figure 3. Optimal processing parameters (HPU + PEF) for highest level of BACs and antioxidant activity: (**a**) total phenolic content (TPC mg 100 g^{-1}); (**b**) hydroxycinnamic acids (HCAs mg 100 g^{-1}); (**c**) flavonols (FLs mg 100 g^{-1}); (**d**) condensed tannins (CTs mg 100 g^{-1}); (**e**) DPPH assay (DPPH µmol 100 g^{-1}); and (**f**) FRAP assay (FRAP µmol 100 g^{-1}).

3.2. The Changes in Strawberry Juices after Hurdle Processing and Storage with Respect to BACs, Antioxidant Activity, SSC and pH

Table 3 shows the effects of the processing parameters of the hurdle technology (HPU + PEF) and storage on the content of BACs, antioxidant activity, SSC and pH of the treated strawberry juices. In the previous study, the effects of the hurdle concept, but in the order of PEF + HPU, on the stability of BACs, SSC and pH during storage were investigated [12]. This study therefore aimed to investigate the effect of different sequences of these technologies on the quality of functional strawberry juices and to explore the synergy of these technologies in maintaining quality.

The average values of BACs in the treated juice samples were as follows: 120.56 ± 0.44 mg 100 g^{-1} TPC, 27.01 ± 0.25 mg 100 g^{-1} HCA, 11.55 ± 0.16 mg 100 g^{-1} FL and 109.99 ± 0.31 mg 100 g^{-1} CT. Yildiz et al. [32] found a slightly higher TPC (137.59 ± 1.93 mg GEA 100 mL^{-1}) in strawberry juice after the HPU treatment, as well as after the PEF treatment (144.97 ± 1.52 mg GEA 100 mL^{-1}), but the authors treated the clear strawberry juice under different operating conditions for the PEF and HPU technologies. Comparing the results obtained with the previous [12], it can be seen that the average values of TPC, HCA and CT were higher in the samples treated with HPU + PEF than in the samples treated with PEF + HPU. The average value of antioxidant activity measured by the DPPH method for the juice samples treated first with PEF and then with HPU technology was 293.93 ± 0.09 µmol 100 g^{-1}, while it was 290.24 ± 0.11 µmol 100 g^{-1} for the juices treated in the reverse order. The opposite trend was observed for the FRAP method, where an average value of 852.41 ± 5.42 µmol 100 g^{-1} was obtained for juice samples treated first with the PEF and then with the HPU technology, and an average value of 926.18 ± 5.51 µmol 100 g^{-1} was obtained for juices treated in reverse order of technology. Regarding the antioxidant activity, the results of the same samples measured by different methods for the determination of antioxidant activity sometimes differ due to the different sensitivity of these methods for the same BAC structures [29]. Also, the antioxidant activity measured by DPPH and FRAP showed lower values when treated with HPU + PEF compared to PEF + HPU. These trends in the results could be based on the assumption that electroporation is less damaging to cell membranes than cavitation during HPU treatment, which is more damaging to membranes [33]. These results suggest that there is a synergistic relationship between the mechanisms of action of these two technologies in the processing of strawberry juices and that the order of these technologies in the hurdle concept influences the stability of BACs and antioxidant activity.

Table 3. The influence of hurdle technology (HPU + PEF) on BACs, antioxidant activity, SSC and pH of strawberry juices throughout storage.

32	n	TPC	HCA	FL	CT	DPPH	FRAP	SSC	pH
Storage		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.03$ †	$p \leq 0.01$ †	$p = 0.08$ ‡
0 days	18	124.35 ± 0.63 ^{a}	33.01 ± 0.67 ^{a}	13.86 ± 0.23 ^{a}	113.58 ± 0.45 ^{a}	292.86 ± 0.15 ^{a}	940.18 ± 7.79 ^{a}	9.36 ± 0.01 ^{a}	3.29 ± 0.01 ^{a}
7 days	18	116.78 ± 0.63 ^{b}	21.02 ± 0.67 ^{b}	9.25 ± 0.23 ^{b}	108.40 ± 0.45 ^{b}	287.62 ± 0.15 ^{b}	913.44 ± 7.79 ^{b}	9.30 ± 0.01 ^{b}	3.27 ± 0.01 ^{a}
HPU		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.20$ ‡
2.5 min	12	126.02 ± 0.77 ^{a}	28.35 ± 0.82 ^{a}	13.83 ± 0.28 ^{a}	116.30 ± 0.55 ^{a}	289.81 ± 0.19 ^{b}	961.62 ± 9.54 ^{a}	9.27 ± 0.01 ^{b}	3.27 ± 0.01 ^{a}
5.0 min	12	118.52 ± 0.77 ^{b}	29.93 ± 0.82 ^{b}	11.71 ± 0.28 ^{b}	108.06 ± 0.55 ^{b}	290.96 ± 0.19 ^{a}	905.76 ± 9.54 ^{b}	9.63 ± 0.01 ^{a}	3.29 ± 0.01 ^{a}
7.5 min	12	117.14 ± 0.77 ^{b}	22.76 ± 0.82 ^{c}	9.14 ± 0.28 ^{c}	108.61 ± 0.55 ^{b}	289.95 ± 0.19 ^{b}	913.06 ± 9.54 ^{b}	9.08 ± 0.01 ^{c}	3.28 ± 0.01 ^{a}
PEF		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.28$ ‡	$p \leq 0.01$ †	$p = 0.16$ ‡
1.5 min	12	124.48 ± 0.77 ^{a}	28.66 ± 0.82 ^{a}	12.64 ± 0.28 ^{a}	113.12 ± 0.55 ^{a}	290.12 ± 0.19 ^{b}	931.60 ± 9.54 ^{a}	9.41 ± 0.01 ^{a}	3.29 ± 0.01 ^{a}
3.0 min	12	118.07 ± 0.77 ^{b}	27.31 ± 0.82 ^{b}	10.96 ± 0.28 ^{b}	110.70 ± 0.55 ^{b}	289.70 ± 0.19 ^{b}	934.81 ± 9.54 ^{a}	9.38 ± 0.01 ^{a}	3.27 ± 0.01 ^{a}
4.5 min	12	119.14 ± 0.77 ^{b}	25.06 ± 0.82 ^{c}	11.07 ± 0.28 ^{b}	109.14 ± 0.55 ^{b}	290.91 ± 0.19 ^{a}	914.03 ± 9.54 ^{a}	9.20 ± 0.01 ^{b}	3.27 ± 0.01 ^{a}
HPU + PEF (hurdle)		$p = 0.10$ ‡	$p = 0.10$ ‡	$p = 0.20$ ‡	$p = 0.07$ ‡	$p = 0.82$ ‡	$p = 0.05$ †	$p \leq 0.01$ †	$p = 0.06$ ‡
2.5 min + 1.5 min	12	129.54 ± 2.04 ^{a}	28.43 ± 1.42 ^{a}	13.47 ± 1.55 ^{a}	115.87 ± 2.09 ^{a}	289.55 ± 0.32 ^{a}	918.05 ± 16.33 ^{b}	9.43 ± 0.02 ^{a}	3.30 ± 0.01 ^{a}
2.5 min + 3.0 min	12	123.06 ± 2.04 ^{a}	29.77 ± 1.42 ^{a}	14.70 ± 1.55 ^{a}	118.87 ± 2.09 ^{a}	289.79 ± 0.32 ^{a}	981.50 ± 16.33 ^{a}	9.25 ± 0.02 ^{b}	3.25 ± 0.01 ^{a}
2.5 min + 4.5 min	12	125.46 ± 2.04 ^{a}	26.85 ± 1.42 ^{a}	13.30 ± 1.55 ^{a}	114.15 ± 2.09 ^{a}	290.08 ± 0.32 ^{a}	985.32 ± 16.33 ^{a}	9.13 ± 0.02 ^{c}	3.26 ± 0.01 ^{a}
HPU + PEF (hurdle)		$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.08$ ‡	$p \leq 0.01$ †	$p = 0.82$ ‡	$p = 0.02$ †	$p \leq 0.01$ †	$p = 0.06$ ‡
5.0 min + 1.5 min	12	123.21 ± 0.65 ^{a}	32.73 ± 0.83 ^{a}	12.94 ± 1.55 ^{a}	113.01 ± 0.91 ^{a}	290.87 ± 0.32 ^{a}	951.30 ± 13.70 ^{a}	9.33 ± 0.02 ^{a}	3.28 ± 0.01 ^{a}
5.0 min + 3.0 min	12	113.64 ± 0.65 ^{b}	30.59 ± 0.83 ^{a}	10.92 ± 1.55 ^{a}	103.26 ± 0.91 ^{b}	289.88 ± 0.32 ^{a}	883.21 ± 13.70 ^{b}	9.90 ± 0.02 ^{b}	3.29 ± 0.01 ^{a}
5.0 min + 4.5 min	12	118.70 ± 0.65 ^{c}	26.46 ± 0.83 ^{b}	11.27 ± 1.55 ^{a}	107.90 ± 0.91 ^{c}	292.14 ± 0.32 ^{a}	882.76 ± 13.70 ^{b}	9.68 ± 0.02 ^{c}	3.30 ± 0.01 ^{a}
HPU + PEF (hurdle)		$p = 0.02$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.82$ ‡	$p = 0.10$ ‡	$p \leq 0.01$ †	$p = 0.06$ ‡
7.5 min + 1.5 min	12	120.68 ± 1.33 ^{a}	24.83 ± 0.57 ^{a}	11.52 ± 0.37 ^{a}	110.49 ± 0.70 ^{a}	289.93 ± 0.32 ^{a}	925.44 ± 19.10 ^{a}	9.48 ± 0.02 ^{a}	3.29 ± 0.01 ^{a}
7.5 min + 3.0 min	12	117.50 ± 1.33 ^{a,b}	21.57 ± 0.57 ^{b}	7.27 ± 0.37 ^{c}	109.97 ± 0.70 ^{a}	289.42 ± 0.32 ^{a}	939.74 ± 19.10 ^{a}	8.98 ± 0.02 ^{b}	3.28 ± 0.01 ^{a}
7.5 min + 4.5 min	12	113.25 ± 1.33 ^{b}	21.86 ± 0.57 ^{b}	8.63 ± 0.37 ^{b}	105.38 ± 0.70 ^{b}	290.50 ± 0.32 ^{a}	874.00 ± 19.10 ^{a}	8.80 ± 0.02 ^{c}	3.26 ± 0.01 ^{a}
Dataset average	36	120.56 ± 0.44	27.01 ± 0.25	11.55 ± 0.16	109.99 ± 0.31	290.24 ± 0.11	926.18 ± 5.51	9.33 ± 0.01	3.28 ± 0.01

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; and ‡ not significant factor in multifactor analysis. TPC—total phenolic content (mg 100 g^{-1}); HCA—hydroxycinnamic acid (mg 100 g^{-1}); FL—flavonol (mg 100 g^{-1}); CT—condensed tannin (mg 100 g^{-1}); antioxidant activity—DPPH (μmol 100 g^{-1}) and FRAP (μmol 100 g^{-1}); and SSC—soluble solids content (°Brix). HPU—high-power ultrasound (amplitude 25%, pulse 50%); and PEF—pulsed electric field (30 kV cm^{-1}, 100 Hz).

All juice samples showed higher TPC, HCA, FL, CT, DPPH, FRAP and SSC on the first day of storage compared to day 7. However, the pH remained constant for all samples throughout the storage period. After 7 days of storage, there was a significant decrease in TPC (6.2%) and CT (4.56%) with the greatest decrease observed in HCA (36.32%) and FL (33.26%). SSC also decreased during the storage period. However, when the hurdle concept included PEF + HPU, higher proportions of all BACs were determined on the first day of storage, with the exception of CT, where higher concentrations were observed after 7 days of storage [12]. PEF treatment, applied in several studies, also promoted the extraction of tannins, and PEF showed significant effects on the condensation of tannins [34,35]. Thus, these results suggest that the sequence of technologies applied may have an impact on the stability of CTs during storage.

The antioxidant activity of juices treated with hurdles (DPPH and FRAP) decreased during storage in juice samples treated with both hurdle concepts (HPU + PEF, PEF + HPU) [12]. The observed decrease could be related to an enhanced tendency for polymerization of the polyphenols, which reduces the availability of hydroxyl groups for antioxidant potential. A higher degree of polymerization leads to improved molecular complexity and stericity, which may result in a lower availability of hydroxyl groups to scavenge DPPH radicals, leading to a corresponding decrease in antioxidant capacity [36]. In the study by Odriozola-Serrano et al. [37], the antioxidant capacity measured with the DPPH and ABTS methods after storage (7 days/4 °C) was higher in treated juices than in fresh strawberry juice. From this, the authors conclude that processing plays an imperative role in obtaining safe and stable juices, but also supports to preserve their antioxidant potential during storage [37].

In order to better understand the synergetic relationships between the two technologies, the influence of both technologies on the quality of the juices tested was investigated. The process parameters were previously optimized for both HPU and PEF technologies [11], so the influence of treatment duration was investigated in this study.

A longer duration of HPU treatment resulted in a statistically significant decrease in the values of TPC, FL, CT and FRAP. As already mentioned for storage, the decrease was greatest for FL at 33%. HCA, DPPH and SSC increased with a 5 min treatment and decreased significantly thereafter. One possible reason for the decrease in the content of BACs is their degradation upon prolonged exposure to HPU, which could be due to the oxidative degradation of phenolic compounds. In addition, the free radicals generated by acoustic cavitation can potentially oxidize polyphenolic compounds or induce the hydrolysis of the phenolic glycoside forms and promote the formation of aglycone structures that are less stable than their glycosidic moieties [38]. The pH remained constant without influence of HPU duration. However, this trend was not observed in the PEF + HPU hurdle treatment. Here, it was shown that the HPU treatment of 5 min gave the best results for almost all quality parameters investigated [12].

Prolonged PEF treatment led to a significant decrease in TPC, HCA, FL and CT. Interestingly, the antioxidant activity measured with DPPH was highest at the longest PEF duration (5 min) while no significant effect of PEF duration was observed for the antioxidant activity measured with the FRAP method. The results obtained contradict the research results of Odriozola-Serrano et al. [37], in which the authors observed a significant decrease in the antioxidant capacity of strawberry juice with an increasing duration of PEF treatment (100–2000 µs) and electric field strength (20–35 kV cm^{-1}). One possible explanation is that as a result of PEF treatment, various reactions such as hydroxylation, methylation, isoprenylation, dimerization and/or glycosylation, which induce modifications between the different phenolic compounds, may occur to different extents during processing, so that such new structures may affect the antioxidant capacity [39]. Moreover, the duration of PEF treatment had no significant effect on the application of pH, but SSC decreased significantly with a longer treatment duration. This trend in the stability of BACs was not observed with the reverse order of processing technologies (PEF + HPU) [12], suggesting that the same technologies have a different synergistic effect on the stability of BACs depending on whether they are applied as the first or second technology in the hurdle sequence.

The HPU treatment of 2.5 min in combination with PEF (1.5–4.5 min) had no statistically significant effect on the values of BACs, DPPH and pH. The antioxidant activity measured with the FRAP assay was higher when the hurdle treatment lasted longer (PEF at 3 min and 4.5 min). The pH of the treated juices was constant, while the SSC was highest in the shortest treatment (HPU 2.5 min + PEF 1.5 min). In a study where PEF with the shortest treatment time (1.5 min) was combined with HPU treatments (2.5–7.5 min), there were still significant changes in the concentrations of FL and CT, and DPPH, again confirming that the sequence of PEF and HPU technologies plays an important role in the stability of BACs in strawberry juices [12].

The impact of ultrasound and PEF on the quality of spinach juice was investigated by Faisal Manzoor et al. [40]. First, an ultrasonic treatment (40 kHz, 200 W, 30 °C, 21 min) was performed in an ultrasonic bath, followed by a PEF treatment (1 kHz, 60 mL min^{-1}, 30 °C, 335 µs, 9 kV cm^{-1}). As in previous studies, higher levels of TPC (12%), flavonoids (10%), FL (23%), antioxidant capacity, anthocyanins (15%), carotenoids (18%), chlorophyll (17%) and vitamins were found compared to untreated samples and samples treated individually with PEF and HPU. The authors also note that the membrane permeabilization induced by PEF treatment enhances the extraction of intracellular contents, leading to an increase in extraction effectiveness and yield of intracellular metabolites. In addition, the authors note that the increase in TPC during sonication is likely related to the release of bound phenols due to cavitation-induced damage to the cell membrane.

However, the extension of HPU treatment to 5 min in combination with PEF (1.5–4.5 min) did not change the contents of FL, DPPH and pH, but led to a decrease in the contents of TPC, HCA, CT and FRAP. Interestingly, the SSC increased after 3 min of PEF exposure and decreased with further exposure, but not below the initial level. Comparing the previous results, where the HPU treatment lasted 2.5 min, with these, where the HPU treatment time is twice as long (5 min), it can be seen that the prolongation of the HPU treatment still has a significant impact on the structures of certain BACs and thus strongly influences their stability. This indicates that both technologies need to be carefully optimized to produce high-quality functional strawberry juices.

A further extension of the HPU application time to 7.5 min followed by treatment with PEF lowers the content of all BACs, while the antioxidant activity remains unchanged. Here, all bioactive compounds showed the highest levels at the PEF treatment (1.5 min), but this trend changed at a PEF treatment of 3 min. PEF treatment at 3 min together with HPU at 7.5 min had a negative effect on the contents of TPC and HCA. The content of CT decreased with PEF treatment at 4.5 min. Interestingly, the most invasive treatment for FL stability was HPU 7.5 min + PEF 4.5 min.

Furthermore, FRAP, DPPH and pH were not affected by this combination of hurdle technologies (HPU 7.5 min + PEF 1.5–4.5 min), while SSC decreased with increasing duration. Here in the HPU + PEF combination, without influence of treatment duration, no statistical changes in DPPH values were observed, although variations in BACs were detected. This could be due to the different contributions to antioxidant capacity by different chemical bioactive structures [41], as not all BACs in strawberry juice were determined in this study. However, in the reverse sequence treatment, PEF + HPU, longer treatments favored higher DPPH values, while in the longest treatments (PEF 4.5 min + HPU 2.5/5/7.5 min), there was no significant difference depending on the selected time combinations. In the PEF + HPU sequence, FRAP values were also unchanged in the PEF 1.5 min + HPU 2.5/5/7.5 min and PEF 3.0 min + HPU 2.5/5/7.5 min treatments, and the difference only occurred in the longest PEF treatment of 4.5 min [12]. These results confirm once again that the sequence of combinations as well as the optimization of PEF and HPU technologies significantly influence each tested parameter, supporting the thesis that their synergistic effect is of crucial importance for the final quality of processed strawberry juices.

To date, no data on ultrasound (US) hurdle treatment and PEF technology in the processing of strawberry juices have been found in the literature with which to compare the results obtained.

In a recent study, the hurdle concept was applied to the processing of strawberry juice by combining atmospheric cold plasma (dielectric barrier discharge at 60 kV, 50 Hz, 10 and 15 min) and hydrothermal treatment (121 °C, 10 min at 10 lbf in^{-2}) to preserve the antioxidant bioactives. The authors concluded that a 10 min cold plasma treatment combined with hydrothermal processing preserves the biological quality of the treated strawberry juices [42]. Manzoor et al. [43] investigated the effects of the combination of US (40 kHz, 200 W, 35 °C, 20 min), PEF (flow rate 40 mL min^{-1}, 18 kV cm^{-1}, 500 µs, 1 kHz) and their combination PEF + US on the BAC content of almond extract. According to their results, the TPC increased in samples treated separately with PEF and US and in the samples treated with a combination of PEF + US compared to untreated samples. This study shows that the combination of PEF + US leads to higher yields of all analyzed BACs, such as TPC, TF, CT and total anthocyanins, than when these two technologies were used independently. The authors conclude that the reason for the higher content in the PEF + US treatment could be due to the different chemical effects of PEF and US on the plant matrix.

3.3. Optimization of Hurdle Technology Operating Parameters for the Treatment of Strawberry Juice

As shown in Table 4, all polyphenolic groups and DPPH decreased significantly during prolonged storage of strawberry juice after treatment with HPU and PEF, while no correlation was found for pH and SSC. These results differ from those obtained by PEF + HPU treatment [12], where storage has no significant effect on TPC and FL and a positive effect on the increase in CT. However, these results are in accordance with those of Nadeem et al. [44], who discovered that mixed carrot and grape juices treated with ultrasound (20 kHz; amplitude 70%; 2, 4 and 6 min) showed a gradual decrease in phenolics and flavonoids content with prolonged storage, but less than untreated or chemically preserved juice. Initial exposure to HPU strongly decreased TPC, FL, CT and FRAP values while the other variables did not correlate. Also in this case, the results differ from previously reported work [12], where HPU treatment had no effect on BAC content.

Table 4. Mutual correlations of hurdle technology parameters on polyphenolic content, antioxidant activities, SSC and pH.

	Storage	HPU Exposure	PEF Exposure	TPC [1]	HCA [2]	FL [3]	CT [4]	DPPH [5]	FRAP [6]	SSC [7]	pH
Storage	1	0	0	−0.53 *	−0.81 *	−0.55 *	−0.40 *	−0.93 *	−0.24	−0.09	−0.29
HPU exposure		1	0	−0.50 *	−0.31	−0.46 *	−0.49 *	0.02	−0.36 *	−0.23	0.11
PEF exposure			1	−0.30	−0.20	−0.15	−0.25	0.11	−0.13	−0.26	−0.27
TPC				1	0.51 *	0.69 *	0.75 *	0.43 *	0.55 *	0.02	0.05
HCA					1	0.68 *	0.39 *	0.77 *	0.29	0.36 *	0.30
FL						1	0.60 *	0.51 *	0.45 *	0.21	0.08
CT							1	0.37 *	0.74 *	−0.14	−0.06
DPPH								1	0.17	0.15	0.30
FRAP									1	−0.22	−0.22
SSC										1	0.50 *
pH											1

* Correlation is significant at the $p \leq 0.05$; [1] TPC—total phenolic content (mg 100 g^{-1}); [2] HCA—hydroxycinnamic acid (mg 100 g^{-1}); [3] FL—flavonol (mg 100 g^{-1}); [4] CT—condensed tannin (mg 100 g^{-1}); [5] DPPH assay (µmol 100 g^{-1}); [6] FRAP assay (µmol 100 g^{-1}); and [7] SSC—soluble solids content (°Brix). HPU—high-power ultrasound (amplitude 25%, pulse 50%); and PEF—pulsed electric field (30 kV cm^{-1}, 100 Hz).

As expected, TPC was strongly positively correlated with all polyphenolic groups and with the two corresponding antioxidant activities (DPPH and FRAP), but not with SSC and pH. In other words, with the increase in all polyphenolic groups, the TPC and their antioxidant activity increased, which is quite logical and perhaps indicates a good quality of analytical measurement of polyphenols. These results are partly different from the previously mentioned results [12], where TPC correlated positively only with CT and FRAP values.

HCA was strongly positively correlated with FL, CT, DPPH and SSC, indicating the expected correlation between the measured variables (e.g., relationship of HCA to condensation to tannins). FL also showed a significant positive correlation with CT content and both antioxidant activities. These results are in partial accordance with those obtained

by PEF + HPU treatment [12], where HCAs were significantly correlated with all BAC, DPPH and pH values, while FLs were significantly positively correlated only with DPPH. CT was correlated significantly positively with the values of both antioxidant capacities (Table 4). These results are in partial agreement with earlier studies with PEF + HPU treatment [12], in which CT is significantly negatively correlated with DPPH and positively correlated with FRAP and pH values. As for antioxidant capacity, DPPH was strongly positively correlated with HCA, FL and CT, implying that they are mainly responsible for the antioxidant activity in the samples. FRAP correlated mainly with FL and CT (and the previously mentioned TPC), suggesting that they are the main contributors to the antioxidant activity measured by this assay. The SSC value correlates significantly positively with pH, which was not the case in the previous studies with PEF + HPU treatment, where no correlation was found [12].

The optimization of the HPU + PEF process parameters was carried out to find the operating parameters with which the highest content of BACs and antioxidant activity can be achieved (Table 5). For all BACs and antioxidant activity, the highest levels were obtained with a shorter storage time (0 days). The same results for storage time were obtained for the optimal treatment of PEF + HPU in a previously published work [12]. The highest content of TPC/CT of 129.35 and 117.19 mg 100 g^{-1} was obtained for a 2.5 min HPU treatment and a 1.5 min PEF treatment, respectively. The highest content of HCA required a longer HPU treatment of 4.7 min, but a similar PEF treatment (as for TPC/CT) of 1.5 min for the highest content of 39.15 mg 100 g^{-1}. The highest content of FL (18.04 mg 100 g^{-1}) in the samples was observed with an HPU treatment of 2.5 min and a PEF treatment of 4.5 min. Overall, the best DPPH activity (294.25 µmol 100 g^{-1}) was observed at 5 min HPU and 4.5 min PEF. Finally, the strongest antioxidant activity, assessed by FRAP (987.75 µmol 100 g^{-1}), was observed at 2.5 min HPU exposure and 3.8 min PEF. In summary, higher levels of polyphenols and antioxidant activity favored shorter exposure times, both for HPU (up to 2.5 min) and PEF (up to 1.5 min). The shorter exposure time for certain technologies could be explained by the effective and rapid destabilization of the cell membrane under the influence of electroporation and cavitation phenomena, consequently, by the easier extraction of said compounds into the extracellular space [45–47].

Table 5. Optimal hurdle parameters for maximum content of polyphenols and antioxidant activity in the samples.

	TPC	HCA	FL	CT	DPPH	FRAP
Storage (days)	0.0	0.0	0.0	0.0	0.0	0.0
HPU treatment (min)	2.5	4.7	2.5	2.5	5.0	2.5
PEF treatment (min)	1.5	1.5	4.5	1.5	4.5	3.8
Optimal quantity (mg 100 g^{-1})	129.35	39.15	18.04	117.19	294.25	987.75

TPC—total phenolic content (mg 100 g^{-1}); HCA—hydroxycinnamic acid (mg 100 g^{-1}); FL—flavonol (mg 100 g^{-1}); CT—condensed tannin (mg 100 g^{-1}); antioxidant activity—DPPH (µmol 100 g^{-1}) and FRAP (µmol 100 g^{-1}); storage (days); HPU—high-power ultrasound (amplitude 25%, pulse 50%, measured in min); and PEF—pulsed electric field (30 kV cm^{-1}, 100 Hz, measured in min).

On the one hand, when comparing PEF + HPU and HPU + PEF in terms of optimal BAC quantity and antioxidant activity [12], it can be seen that the HPU + PEF hurdle concept was more favorable as it can produce strawberry juices with improved functional properties (Figure 3). On the other hand, comparing the results of PEF + HPU and HPU + PEF [12] in terms of required operation time, it can be seen that when PEF is the first technology in the hurdle concept, followed by HPU, the longer HPU treatment is required to obtain the highest content of BACs studied, with the exception of TPC, and thus a higher energy input is required to obtain higher yields of the targeted BACs. Even without influencing which technology is the first hurdle in processing, the same PEF duration is required for

PEF + HPU and HPU + PEF to obtain the maximum content of BACs. However, the strawberry juices treated with PEF + HPU achieved maximum antioxidant activity with a shorter PEF duration compared with the HPU + PEF combination, which again speaks in favor of the economically more favorable PEF + HPU processing.

4. Conclusions

Strawberry juice obtained from the 'Albion' cultivar can be considered as a functional food because of its high level of BACs and noteworthy antioxidant activity. Hurdle technology could be a good choice to prevent the quality of treated juices. The selection of HPU and PEF technologies in the hurdle concept proved to be a good resolution in the processing of functional strawberry juices. The results obtained show that it is not only important to optimize the process parameters of each technology, but also that the order of technology application must be studied, as it significantly affects the bioactive potential and antioxidant capacity of the treated strawberry juices. This supports the thesis that the synergistic effect of the selected technologies is crucial when defining the hurdle concept and should not be neglected.

The results obtained show that the sequence of HPU + PEF technologies was more efficient in the preservation of BACs in strawberry juices than PEF + HPU treatments since greater stability of the studied compounds was achieved with the same treatment durations. Considering the order of the applied technologies, it can be seen that the stability of TPC, CTs and pH is better influenced by the order of HPU + PEF treatment than by PEF + HPU. The results related to antioxidant activity obtained by the FRAP method show the same trend.

When the samples were initially treated by HPU, a longer duration of HPU treatment decreased the content of all BACs. The same was observed with PEF treatment. As with the combination of PEF + HPU treatment, shorter HPU + PEF treatments had a more favorable effect on the retention of BACs in strawberry juice. After the HPU + PEF treatment, reduced contents of all BACs during storage were observed. The results of the analysis of the effects of storage on the antioxidant capacity of the samples indicate a decrease in the antioxidant capacity, regardless of the order of treatment applied.

In terms of TPC, FL and CT as well as FRAP antioxidant capacity, the shorter HPU treatment time (2.5 min) is most favorable when the hurdle technology is applied in the HPU + PEF combination; while, for HCA and DPPH, the antioxidant capacity requires a slightly longer treatment. As with the combination of PEF + HPU, higher levels of BACs are obtained with a shorter PEF treatment (less than 5 min).

In summary, hurdle technology as an innovative processing concept with selected PEF and HPU technologies can be considered as a sustainable technology that has a broad industrial application perspective in the processing of functional strawberry juices.

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References

1. Wallace, T.C.; Bailey, R.L.; Blumberg, J.B.; Burton-Freeman, B.; Chen, C.y.O.; Crowe-White, K.M.; Drewnowski, A.; Hooshmand, S.; Johnson, E.; Lewis, R.; et al. Fruits, vegetables, and health: A comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. *Crit. Rev. Food Sci. Nutr.* **2019**, *60*, 2174–2211. [CrossRef]
2. Ruxton, C.H.S.; Myers, M. Fruit Juices: Are They Helpful or Harmful? An Evidence Review. *Nutrients* **2021**, *13*, 1815. [CrossRef]
3. Iqbal, A.; Murtaza, A.; Hu, W.; Ahmad, I.; Ahmed, A.; Xu, X. Activation and inactivation mechanisms of polyphenol oxidase during thermal and non-thermal methods of food processing. *Food Bioprod. Process.* **2019**, *117*, 170–182. [CrossRef]
4. Cano-Lamadrid, M.; Artés-Hernández, F. Thermal and Non-Thermal Treatments to Preserve and Encourage Bioactive Compounds in Fruit- and Vegetable-Based Products. *Foods* **2022**, *11*, 3400. [CrossRef]
5. Jadhav, H.B.; Annapure, U.S.; Deshmukh, R.R. Non-thermal Technologies for Food Processing. *Front. Nutr.* **2021**, *8*, 657090. [CrossRef]
6. Chacha, J.S.; Zhang, L.; Ofoedu, C.E.; Suleiman, R.A.; Dotto, J.M.; Roobab, U.; Agunbiade, A.O.; Duguma, H.T.; Mkojera, B.T.; Hossaini, S.M.; et al. Revisiting Non-Thermal Food Processing and Preservation Methods—Action Mechanisms, Pros and Cons: A Technological Update (2016–2021). *Foods* **2021**, *10*, 1430. [CrossRef]
7. Granato, D.; Barba, F.J.; Bursać Kovačević, D.; Lorenzo, J.M.; Cruz, A.G.; Putnik, P. Functional Foods: Product development, technological trends, efficacy testing, and safety. *Annu. Rev. Food Sci. Technol.* **2020**, *11*, 93–118. [CrossRef]
8. Putnik, P.; Pavlić, B.; Šojić, B.; Zavadlav, S.; Žuntar, I.; Kao, L.; Kitonić, D.; Kovačević, D.B. Innovative hurdle technologies for the preservation of functional fruit juices. *Foods* **2020**, *9*, 699. [CrossRef]
9. Rahman, M.S. Hurdle technology in food preservation. In *Minimally Processed Foods*; Food Engineering Series; Springer: Berlin/Heidelberg, Germany, 2015; pp. 17–33.
10. Bebek Markovinović, A.; Putnik, P.; Bičanić, P.; Brdar, D.; Duralija, B.; Pavlić, B.; Milošević, S.; Rocchetti, G.; Lucini, L.; Bursać Kovačević, D. A chemometric investigation on the functional potential in high power ultrasound (HPU) processed strawberry juice made from fruits harvested at two stages of ripeness. *Molecules* **2022**, *28*, 138. [CrossRef]
11. Bebek Markovinović, A.; Putnik, P.; Stulić, V.; Batur, L.; Duralija, B.; Pavlić, B.; Vukušić Pavičić, T.; Herceg, Z.; Bursać Kovačević, D. The application and optimization of HIPEF technology in the processing of juice from strawberries harvested at two stages of ripeness. *Foods* **2022**, *11*, 1997. [CrossRef]
12. Bebek Markovinović, A.; Stulić, V.; Putnik, P.; Birkić, A.; Jambrović, M.; Šaško, D.; Ljubičić, J.; Pavlić, B.; Herceg, Z.; Bursać Kovačević, D. Pulsed electric field (PEF) and high-power ultrasound (HPU) in the hurdle concept for the preservation of antioxidant bioactive compounds of strawberry juice—A chemometric evaluation—Part I. *Foods* **2023**, *12*, 3172. [CrossRef]
13. Nowosad, K.; Sujka, M.; Pankiewicz, U.; Kowalski, R. The application of PEF technology in food processing and human nutrition. *J. Food Sci. Technol.* **2020**, *58*, 397–411. [CrossRef]
14. Zhang, C.; Lyu, X.; Arshad, R.N.; Aadil, R.M.; Tong, Y.; Zhao, W.; Yang, R. Pulsed electric field as a promising technology for solid foods processing: A review. *Food Chem.* **2023**, *403*, 134367. [CrossRef]
15. Razola-Díaz, M.d.C.; Tylewicz, U.; Rocculi, P.; Verardo, V. Application of pulsed electric field processing in the food industry. In *Non-Thermal Food Processing Operations*; Jafari, S.M., Therdthai, N., Eds.; Woodhead Publishing: Sawston, UK, 2023; pp. 257–298.
16. Salehi, F. Physico-chemical properties of fruit and vegetable juices as affected by pulsed electric field: A review. *Int. J. Food Prop.* **2020**, *23*, 1036–1050. [CrossRef]
17. Arshad, R.N.; Abdul-Malek, Z.; Munir, A.; Buntat, Z.; Ahmad, M.H.; Jusoh, Y.M.M.; Bekhit, A.E.-D.; Roobab, U.; Manzoor, M.F.; Aadil, R.M. Electrical systems for pulsed electric field applications in the food industry: An engineering perspective. *Trends Food Sci. Technol.* **2020**, *104*, 1–13. [CrossRef]
18. Narendar, A.; Hanani, N.; Syafrial, S. Factors that influence technical efficiency of organic rice in Sumberngepoh Village, Lawang District, Malang Regency. *Agric. Soc. Econ. J.* **2018**, *18*, 79–85. [CrossRef]
19. Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT—Food Sci. Technol.* **2009**, *42*, 93–100. [CrossRef]
20. Tiwari, B.K. Ultrasound: A clean, green extraction technology. *Trends Anal. Chem.* **2015**, *71*, 100–109. [CrossRef]
21. Tomadoni, B.; Cassani, L.; Moreira, M.D.R.; Ponce, A.; Agüero, M.V. Natural antimicrobials combined with ultrasound treatments to enhance quality parameters and safety of unpasteurized strawberry juice. *Int. J. Fruit Sci.* **2019**, *20*, S178–S197. [CrossRef]
22. Emamifar, A.; Mohamadizadeh, M. Influence of sonication and antimicrobial packaging-based nano-ZnO on the quality of fresh strawberry juice during cold storage. *J. Food Meas. Charact.* **2020**, *14*, 3280–3290. [CrossRef]
23. Bursać Kovačević, D.; Putnik, P.; Dragović-Uzelac, V.; Pedisić, S.; Režek Jambrak, A.; Herceg, Z. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chem.* **2016**, *190*, 317–323. [CrossRef]

24. Yuan, B.; Danao, M.-G.C.; Stratton, J.E.; Weier, S.A.; Weller, C.L.; Lu, M. High pressure processing (HPP) of aronia berry purée: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. *Innov. Food Sci. Emerg. Technol.* **2018**, *47*, 249–255. [CrossRef]
25. Howard, L.R.; Clark, J.R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238–1247. [CrossRef]
26. Sun, B.; Ricardo-da-Silva, J.M.; Spranger, I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. [CrossRef]
27. Shortle, E.; O'Grady, M.N.; Gilroy, D.; Furey, A.; Quinn, N.; Kerry, J.P. Influence of extraction technique on the anti-oxidative potential of hawthorn (*Crataegus monogyna*) extracts in bovine muscle homogenates. *Meat Sci.* **2014**, *98*, 828–834. [CrossRef]
28. Benzie, I.F.F. An automated, specific, spectrophotometric method for measuring ascorbic acid in plasma (EFTSA). *Clin. Biochem.* **1996**, *29*, 111–116. [CrossRef] [PubMed]
29. Hartmann, A.; Patz, C.-D.; Andlauer, W.; Dietrich, H.; Ludwig, M. Influence of processing on quality parameters of strawberries. *J. Agric. Food Chem.* **2008**, *56*, 9484–9489. [CrossRef] [PubMed]
30. Koners, U.; Toepfl, S.; Heinz, V.; Camacho, P.; Ginestet, P.; Knorr, D. Application of pulsed electric field treatment for sludge reduction on waste water treatment plants. In Proceedings of the 2nd European Pulsed Power Symposium, Hamburg, Germany, 20–23 September 2004.
31. Assiry, A. Degradation kinetics of ascorbic acid during ohmic heating with stainless steel electrodes. *J. Appl. Electrochem.* **2003**, *33*, 187–196. [CrossRef]
32. Yildiz, S.; Pokhrel, P.R.; Unluturk, S.; Barbosa-Cánovas, G.V. Changes in Quality Characteristics of Strawberry Juice After Equivalent High Pressure, Ultrasound, and Pulsed Electric Fields Processes. *Food Eng. Rev.* **2020**, *13*, 601–612. [CrossRef]
33. Medina-Meza, I.G.; Boioli, P.; Barbosa-Cánovas, G.V. Assessment of the effects of ultrasonics and pulsed electric fields on nutritional and rheological properties of raspberry and blueberry purees. *Food Bioprocess Technol.* **2015**, *9*, 520–531. [CrossRef]
34. Maza, M.A.; Pereira, C.; Martínez, J.M.; Camargo, A.; Álvarez, I.; Raso, J. PEF treatments of high specific energy permit the reduction of maceration time during vinification of Caladoc and Grenache grapes. *Innov. Food Sci. Emerg. Technol.* **2020**, *63*, 102375. [CrossRef]
35. Zhao, D.; Zeng, X.-A.; Sun, D.-W.; Liu, D. Effects of pulsed electric field treatment on (+)-catechin–acetaldehyde condensation. *Innov. Food Sci. Emerg. Technol.* **2013**, *20*, 100–105. [CrossRef]
36. Pinelo, M.; Manzocco, L.; Nuñez, M.J.; Nicoli, M.C. Interaction among phenols in food fortification: Negative synergism on antioxidant capacity. *J. Agric. Food Chem.* **2004**, *52*, 1177–1180. [CrossRef] [PubMed]
37. Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Phenolic acids, flavonoids, vitamin C and antioxidant capacity of strawberry juices processed by high-intensity pulsed electric fields or heat treatments. *Eur. Food Res. Technol.* **2008**, *228*, 239–248. [CrossRef]
38. Bursać Kovačević, D.; Bilobrk, J.; Buntić, B.; Bosiljkov, T.; Karlović, S.; Rocchetti, G.; Lucini, L.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. High-power ultrasound altered the polyphenolic content and antioxidant capacity in cloudy apple juice during storage. *J. Food Process. Preserv.* **2019**, *43*, e14023. [CrossRef]
39. Morales-de la Peña, M.; Salvia-Trujillo, L.; Rojas-Graü, M.A.; Martín-Belloso, O. Changes on phenolic and carotenoid composition of high intensity pulsed electric field and thermally treated fruit juice–soymilk beverages during refrigerated storage. *Food Chem.* **2011**, *129*, 982–990. [CrossRef]
40. Faisal Manzoor, M.; Ahmed, Z.; Ahmad, N.; Karrar, E.; Rehman, A.; Muhammad Aadil, R.; Al-Farga, A.; Waheed Iqbal, M.; Rahaman, A.; Zeng, X.A. Probing the combined impact of pulsed electric field and ultra-sonication on the quality of spinach juice. *J. Food Process. Preserv.* **2021**, *45*, e15475. [CrossRef]
41. Giampieri, F.; Alvarez-Suarez, J.M.; Battino, M. Strawberry and Human Health: Effects beyond Antioxidant Activity. *J. Agric. Food Chem.* **2014**, *62*, 3867–3876. [CrossRef]
42. Mehta, D.; Yadav, S.K. Impact of atmospheric non-thermal plasma and hydrothermal treatment on bioactive compounds and microbial inactivation of strawberry juice: A hurdle technology approach. *Food Sci. Technol. Int.* **2019**, *26*, 3–10. [CrossRef]
43. Manzoor, M.F.; Zeng, X.-A.; Rahaman, A.; Siddeeg, A.; Aadil, R.M.; Ahmed, Z.; Li, J.; Niu, D. Combined impact of pulsed electric field and ultrasound on bioactive compounds and FT-IR analysis of almond extract. *J. Food Sci. Technol.* **2019**, *56*, 2355–2364. [CrossRef]
44. Nadeem, M.; Ubaid, N.; Qureshi, T.M.; Munir, M.; Mehmood, A. Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrason. Sonochem.* **2018**, *45*, 1–6. [CrossRef] [PubMed]
45. Roobab, U.; Aadil, R.M.; Madni, G.M.; Bekhit, A.E.-D. The impact of nonthermal technologies on the microbiological quality of juices: A review. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 437–457. [CrossRef] [PubMed]

46. Chemat, F.; Zill-e-Huma; Khan, K.M. Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. Sonochem.* **2011**, *18*, 813–835. [CrossRef] [PubMed]
47. Barba, F.J.; Parniakov, O.; Pereira, S.A.; Wiktor, A.; Grimi, N.; Boussetta, N.; Saraiva, J.A.; Raso, J.; Martin-Belloso, O.; Witrowa-Rajchert, D.; et al. Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Res. Int.* **2015**, *77*, 773–798. [CrossRef]

Publication No.8: 3D Printing of Functional Strawberry Snacks: Food Design, Texture, Antioxidant Bioactive Compounds, and Microbial Stability

Publication No.8

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antioxidants

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3D Printing of Functional Strawberry Snacks: Food Design, Texture, Antioxidant Bioactive Compounds, and Microbial Stability

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Abstract: 3D printing technology (3DP) as additive manufacturing is an innovative design technology that can meet the individual nutritional and sensory needs of consumers. Therefore, the aim of this work was to apply 3DP in the production of a strawberry-based functional product with the addition of two hydrocolloids (corn and wheat starch) in three proportions (10, 15 and 20%) and to investigate the influence of 3DP process parameters on physico-chemical and textural properties, as well as the bioactive and antioxidant potential and microbiological stability, with(out) the addition of natural antimicrobial agents. Starch type had a significant effect on all tested bioactive compounds, as well as on starch content, except for total phenolic and hydroxycinnamic acid contents. Considering the content of bioactive compounds and antioxidant capacity, program 2 proved to be more suitable than program 1. All samples exhibited good textural properties, a high degree of stability and minimal geometric deviations. Regarding microbiological safety, no pathogenic bacteria were found in the 3DP samples during storage. The 3DP sample with added citral at a concentration of 75 mg L^{-1} showed the best microbiological quality. Ultimately, 3DP can be successfully used for the production of new strawberry-based functional products.

Keywords: additive manufacturing; functional food; strawberry; starch; bioactive compounds; antioxidant capacity; rheology; microbiology

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1. Introduction

Consumers' awareness between dietary habits and wellbeing is on a constant increase around the world, and it is particularly evident in the aftermath of the pandemic triggered by the SARS-CoV-2 virus. Research in 16 countries found that 42% of people will buy more functional foods in 2021 than in the previous year [1]. The increasing demand and consumption of functional foods is in line with guidelines for increased daily intake of fruits and vegetables with the aim of improving and maintaining health [2].

Fruits and vegetables are a rich source of various compounds, especially polyphenols, which have various beneficial effects. Due to its sensory properties (e.g., bright red color and pleasant aroma) and nutritional composition, strawberry is an attractive raw material for food manufacturing. Due to its beneficial effects on human health, strawberry is considered a functional food. This is contributed to by the bioactive compounds in the composition of strawberries, where the most important are polyphenols and their antioxidant properties,

able to prevent the formation of free radicals. They are also involved in regulating the gene expressions responsible for metabolism, cell survival/proliferation, and DNA-repair mechanisms [3].

Despite all these benefits, current global consumption of fruits (e.g., strawberries) and vegetables remains insufficient. According to the recommendation of the WHO, at least 400 g of fruits and vegetables should be consumed daily to benefit from their intake [4]. Here, 3D food printing (3DP) represents a great potential for the development of interesting and nutritionally adapted food choices for consumers due to the versality of interesting shapes and designs (especially for children) [5].

The 3DP is a food manufacturing process that creates three-dimensional edible shapes (extrudates) by applying material(s) layer by layer. This approach offers a technological solution for the production of personalized foods with control over calorie content, final product composition, dimensions, and taste [6]. For example, Derossi et al. [7] designed a fruit-based meal for children 3–10 years old given that a snack must provide 5–10% of the total daily energy intake. Banana was the principal component in the meal because of its sensorial acceptance among children, plus it adds to the viscosity of the mixture. The rest of the ingredients fostered nutritional potential and consistency of the mixture for the printing. Severini et al. [8] also base their research on improving the nutritional content of the 3D printed form by preparing a balanced meal with fruits and vegetables. As mentioned earlier, fruits and vegetables are raw materials rich in beneficial compounds that can be used for 3DP functional manufacturing. Such products can be additionally enriched by adding other functional constituents, such as bioactive compounds (BAC), extracts from medicinal plants, algae, functional peptides, probiotics, etc.

However, one of the major challenges for 3DP food is microbial safety [9]. For this reason, additives are often inserted to 3D-printed mixtures to extend the shelf-life of the foods. The shelf-life of fruit-based 3D-printed products can be extended by adding natural food-grade antimicrobials such as vanillin [10–12], citral [11,13–15], and geraniol [12,16]. For instance, Cassani et al. [12] in their study observed a significant reduction of natural microflora (4–6 log cycles) in strawberry juice treated with vanillin and geraniol vs. control (untreated) samples. They also found a significant effect of vanillin on the increase of phenolic content of 3DP products. Another study showed that the combination of vanillin and citral with a mild heating can be an alternative to thermal processing of orange juice [15].

Another challenge with 3DP of fruit is that fruit is a raw material that cannot be easily printed due to its low soluble solids content; instead, it is necessary to add additives to the pulp that help in optimizing the viscosity and texture. These are primary substances that achieve a certain degree of thickening, such as hydrocolloid carriers. The most commonly used hydrocolloids in the food industry are pectin, xanthan gum, starch, carrageenan, guar gum, etc. Yang et al. [17] studied the rheological properties of 3D lemon juice gels prepared with different types of starches (potato, sweet potato, wheat, and corn) and concluded that the type of starch carrier modified the viscosity and mechanical properties in the final product. Azam et al. [18] studied the effects of different proportions of potato starch (15, 20, 25, and 30%) in orange concentrate on the rheological properties of the final product and concluded that the addition of 20% starch carrier gave a product with the best mastication properties. Liu et al. [19] conducted a similar study adding 0, 1, 2, and 4% potato starch to a mixture of mashed potato and 15% trehalose and concluded that the addition of 2% starch gave a product with the best extrudability and printability. Mu et al. [20] concluded that the addition of different proportions of berry powders (5, 10, and 15%) to potato starch paste significantly increased the content of total phenols and antioxidant activity in the final product. Thus, to produce 3DP products with good nutritional and biological value, as well as satisfactory rheological and textural properties, it is necessary to choose a suitable carrier and optimize its ratios.

From the abovementioned, the derived aim of this work was to apply 3D printing technology in the production of a functional strawberry products using wheat and corn

starches in three different designs/proportions of 10%, 15%, and 20% (*w*/*w*) and to study the physico-chemical and rheological properties of the final products. The bioactive compounds and antioxidant capacity were monitored before and after the 3DP to evaluate the bioactive compounds' functionality. Since 3DP products have a very short shelf-life, a 10-day storage at 4 °C was tested with natural antimicrobials (citral and vanillin) for extending microbiological stability.

2. Materials and Methods

2.1. Chemical and Standards

HPLC 99% pure methanol obtained from Honeywell (Paris, France) was used as an extraction solvent, while the Folin–Ciocalteu reagent obtained from Fisher Scientific UK (Loughborough, UK) was used for spectrophotometric determination of total phenols. Hydrochloric acid (37%, *w*/*w*), sulfuric acid (96%, p.a.), sodium carbonate, anhydrous (99.5–100.5%), and formic acid (98%, p.a.) were obtained from Lach-ner (Neratovice, Czech Republic). Ethanol (96% pure) was obtained from Gram-mol (Zagreb, Croatia). Quercetin (95%), potassium acetate (99%), and aluminum chloride (98.5%) were purchased from Acros Organics (Guangzhou, China). Gallic acid standard (97.5–102.5%), DPPH (2,2-diphenyl-1-picrylhydrazyl radical), and TPTZ (2,4,6-tris-2-pyridyl-s-triazine) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Vanillin (99%), potassium chloride (99.0–100.5%), sodium acetate anhydride (99%), and chlorogenic acid (min. 95%) were purchased from Thermo Fisher (Kandel, Germany). Iron (III)-chloride hexahydrate and sodium acetate trihydrate resistant to potassium permanganate were obtained from Kemika (Zagreb, Croatia). Glacial acetic acid (≥99.8%) was purchased from Honeywell Fluka ™ (Seelze, Germany) and trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) used for the DPPH and FRAP method was purchased from Biosynth (Bratislava, Slovakia).

2.2. Preparation of Fruit Material

The basic raw material for the 3D printing mixture(s) was strawberry juice blends (*Fragaria x ananassa* Duch., cv. 'Albion'). The strawberries were purchased from Jagodar-HB Ltd. in Donja Lomnica, Zagreb County (Croatia). After the strawberries were delivered to the laboratory, they were destemmed, washed, dried with cellulose, packed in plastic bags, and stored at −18 °C. The strawberries were crushed and homogenized with a household blender to obtain a strawberry juice blend. The pure juice blends were used for the control samples, while starch was added to the other samples according to the experimental design (Table 1). Corn starch (Gustin Dr. Oetker, Janossomorja, Hungary) and wheat starch (Denes Natura Kft., Pécs, Hungary) were used in proportions of 10%, 15%, and 20% (*w*/*w*). The mixtures of strawberry pulp and various starches were continuously mixed and heated on a magnetic stirrer LLG-uniSTIRRER 7 (Lab w Group GmbH, Meckenheim, Germany) to a constant temperature (about 70 °C) to increase the viscosity and homogeneity. After heating, the mixtures were cooled down to a room temperature and subjected to 3D printing.

Table 1. Design of the experiment.

Sample	Starch Type	Starch Content	3D Program
1	Control sample	-	-
2	Corn starch	10%	Program 1
3	Corn starch	10%	Program 2
4	Corn starch	15%	Program 1
5	Corn starch	15%	Program 2
6	Corn starch	20%	Program 1
7	Corn starch	20%	Program 2
8	Wheat starch	10%	Program 1
9	Wheat starch	10%	Program 2
10	Wheat starch	15%	Program 1
11	Wheat starch	15%	Program 2
12	Wheat starch	20%	Program 1
13	Wheat starch	20%	Program 2

Sample 1—Control sample without added starch.

2.3. 3D Printing of Snacks

Foodini 3D printer (Natural Machines, Barcelona, Spain) was used to 3D print the mixtures. Capsules with a volume of 100 mL and a nozzle diameter of 4 mm were used and filled with mixtures of strawberry pulp and starches at room temperature. The filled capsules were inserted into a holder inside the 3D printer. The selection of the desired shape and other printing parameters were set using the Foodini Creator Software on the computer. A heart shape with three layers was selected for 3D printing. The 3D printing was performed with two different programs that differed in process parameters. Program 1 was set up with a printing speed of 8000 mm min^{-1}, a printing line thickness of 3.5 mm, a mixture flow rate of 1.4, and a nozzle height of the first layer of 6 mm. Program 2 was set up: Printing speed 14,000 mm min^{-1}, print line thickness 3.4 mm, mixture flow rate 1.65, and nozzle height of the first layer 4.5 mm. 3D printing was performed at room temperature. A mixture of strawberry pulp with the addition of wheat or corn starch in different percentages was printed by both programs (Table 1). Subsequently, pH and water activity were measured while extracts were prepared from samples for further analysis for determination of bioactive compounds and antioxidant capacities.

2.4. Characterization of 3DP Products

2.4.1. Determination of pH and Water Activity (a_w)

The pH was determined with a previously calibrated pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland), by immersing the combined electrode into a homogenized sample previously diluted with distilled water in a 1:1 ratio and reading the pH. The read results were expressed as the mean of two parallel measurements ± standard deviation.

Water activity was determined using an a_w meter (AquaLab, P08584, Decagon Devices, Pullman, WA, USA). The instrument was calibrated before the first use. For measurement, the sample was applied to a standard measuring cup so that the entire surface of the cup was covered with the sample. The results were expressed as the average of two parallel measurements ± standard deviation.

2.4.2. Determination of Bioactive Compounds and Antioxidant Capacity

Extraction of bioactive compounds was performed on an ultrasonic processor (UP400St, Hielscher Ultrasound Technology, Teltow, Germany) coupled with titanium DN22 (546 mm^2) sonotrode according to a modified protocol from the literature [21]. An aqueous

solution of methanol (80%, v/v) with 1% formic acid (v/v) was used as the extraction solvent. Ten grams of the sample was placed in a beaker and 40 mL of the extraction solvent was added. Then, ultrasound-assisted extraction was performed under the following conditions: Amplitude 50%, pulse 100%, and extraction time 5 min. After extraction, the extract was filtered into a 50 mL volumetric flask and made up with the extraction solvent. The obtained extracts were stored at 4 °C until the analysis. The prepared extracts were used for all determinations of bioactive compounds and antioxidant capacity with a spectrophotometer (LLG-uniSpec 2, Spectrophotometer, Meckenheim, Germany) in parallel determinations.

- Determination of Total Phenolic Content (TPC)

A modified Follin–Ciocalteu method from the literature was used to determine the TPC content [22] and the determination procedure is identical to previous work by Bebek Markovinović et al. [23]. The TPC was calculated using a calibration curve prepared with different concentrations of gallic acid solutions (10–250 mg L^{-1}), and the results were expressed as mg gallic acid equivalent (GAE) per 100 g of sample.

- Determination of Total Monomeric Anthocyanins (ANT)

Anthocyanins were determined by the spectrophotometric differential pH method [24], the determination procedure being identical to previous work by Bebek Markovinović et al. [23]. The concentration of monomeric anthocyanins in the sample was expressed as pelargonidin-3-glucoside equivalent (Pg-3-G) (mg 100 mL^{-1}).

- Determination of Total Flavonoids (TF)

A modified spectrophotometric method described in the literature [25] was used for the determination of TF. Briefly, 0.5 mL of the extract, 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water were pipetted sequentially into a test tube. The blank sample was prepared in the same way, but instead of the extract, an extraction solvent was taken, and instead of 10% aluminum chloride, distilled water was added. The reaction mixture was briefly homogenized on a vortex device, and after 30 min, the absorbance was measured at a wavelength of 415 nm. Parallel measurements were performed for each sample. A quercetin standard solution (10–200 mg L^{-1}) was used to construct the calibration curve, and results were expressed as mg quercetin equivalent (QE) per 100 g of sample.

- Determination of Total Hydroxycinnamic Acids (HCA) and Total Flavonols (FL)

HCA and FL were determined using a modified spectrophotometric method [26] and the determination procedure is identical to previous work by Bebek Markovinović et al. [23]. The HCA content was calculated using a calibration curve generated with different concentrations of chlorogenic acid (10–600 mg L^{-1}), and the results were expressed as mg chlorogenic acid equivalent (CAE) per 100 g or 100 mL of the sample. The FL in the extracts was calculated from a calibration curve obtained with different concentrations of quercetin solution (10–600 mg L^{-1}) and the results were expressed as mg quercetin equivalent (QE) per 100 g of the sample.

- Determination of Condensed Tannins (CT)

CT was determined by a modified spectrophotometric method [27] and the procedure for determination is identical to previous work by Bebek Markovinović et al. [23]. The CT in the extracts were calculated from a calibration curve obtained with different concentrations of catechin solution (10–120 mg L^{-1}), and the results were expressed as mg catechin equivalent (CA) per 100 g of the sample.

- Determination of Antioxidant Capacity (AOC)

1. DPPH Method
   The antioxidant activity was determined using the spectrophotometric DPPH method described in the literature [28]. Briefly, 1.5 mL of the properly diluted

extract and 3 mL of a 0.5 mM DPPH solution were pipetted into a glass test tube. As a control, 1.5 mL of 100% methanol and 3 mL of 0.5 mM DPPH solution were pipetted. Pure methanol was used as a blank. After setting up the reaction, the test tubes were kept in the dark for 20 min and then the absorbance was measured at 517 nm. Parallel measurements were performed for each sample. Antioxidant activity was calculated using a calibration curve generated with different concentrations of Trolox solution (10–150 µM) and the results were expressed as µM rolox equivalent (TE) per 100 g of the sample.

2. FRAP (Ferric Reducing Antioxidant Power) Method
Another method used to determine antioxidant activity was the spectrophotometric FRAP method described in the literature [29]. Briefly, 600 µL of the previously appropriately diluted extract and 4.5 mL of the FRAP reagent (prepared from acetate buffer (0.3 M), 2.5 mL of TPTZ reagent (2,4,6-tris-2-pyridyl-s-triazine; 10 mM), and 2.5 mL of iron (III) chloride (20 mM) in a ratio of 10:1:1) were pipetted into the glass tubes. Briefly, the reaction mixture was homogenized on a vortex shaker (Grant Instruments Ltd., Cambs, England) and thermostatted in a water bath at 37 °C for 10 min. For the blank sample, the determination procedure was identical except that the extraction solvent was used instead of the extract. After 10 min, the absorbance was read at 593 nm. Ferric reducing antioxidant power was calculated from a calibration curve obtained with different concentrations of Trolox solution (10–150 µM), and the results were expressed as mM Trolox equivalent (TE) per 100 g of the sample.

2.5. Determination of Color Parameters

Color analysis was performed using a Lovibond colorimeter (LC 100, Lovibond—Tintometer Ltd., Amesbury, UK). Prior to analysis, the colorimeter was calibrated with an integrated white reference slider. Color was expressed as L^*, a^*, and b^* values where L^* indicates lightness, a^* redness, and b^* yellowness. All readings were taken at five random locations from the surface of each sample and the average was used to express the color of the individual sample.

Color difference (ΔE^*), chroma (C^*), and hue angle (h) were calculated using Equations (1)–(3) [30,31]:

$$\Delta E^* = \sqrt{\left(\overline{L^*} - \overline{L_0^*}\right)^2 + \left(\overline{a^*} - \overline{a_0^*}\right)^2 + \left(\overline{b^*} - \overline{b_0^*}\right)^2} \quad (1)$$

$$C^* = \sqrt{\left(\overline{a^*}\right)^2 + \left(\overline{\mathrm{b}^*}\right)^2} \quad (2)$$

$$h^\circ = \mathrm{tg}^{-1}\left(\frac{\overline{b^*}}{\overline{a^*}}\right) \quad (3)$$

where $\overline{L^*}$, $\overline{a^*}$, $\overline{b^*}$ are the mean values of the measured L^*, a^*, b^* parameters of the 3D-printed samples; and $\overline{L_0^*}$, $\overline{a_0^*}$, $\overline{b_0^*}$ are mean values of the measured L^*, a^*, b^* parameters of the control sample.

2.6. Texture Analysis

Texture properties were determined using a TA.HD.plus Texture Analyser (Stable Micro System, Godalming, UK). With different extensions, two tests were performed: a penetration test and an extrusion test.

2.6.1. Penetration Test

Tests were performed using a spherical probe of 4 mm. Texture analyzer settings were set to: pre-test speed, 1 mm s^{-1}; test speed 0.5 mm s^{-1}; post-test speed 10 mm s^{-1}; deformation distance 6 mm; and trigger force 2 g. The measurements were carried out at

room temperature. Mean values in triplicates were reported as the maximum force (F_p) (hardness) and work (W_p). The sample strength was presented as the breaking force (N) concerning deformation distance (mm).

2.6.2. Forward Extrusion Test

Tests were conducted with an extrusion cell (sample container and the piston disc). The sample container can settle the base disc with an outlet diameter of 3 mm. Selected diameters were chosen depending on the consistency of the sample. The parameters were as follows: pre-test speed 1 mm s^{-1}; test speed 1 mm s^{-1}; post-test speed 10 mm s^{-1}; deformation distance 20 mm; and trigger force 10 g (0.098 N). A compression force–time curve was obtained. The measurements were carried out at room temperature, results were calculated from triplicated observations and shown as the mean extrusion force (F) (firmness) and work (W).

2.7. Microscopic Analysis

The microstructures of the printed samples were evaluated using a digital microscope with an adjustable maximum magnification of 500× (Dino-Lite Edge Digital Microscope AM 7915 MZT, Dino-Lite Europe/IDCP B.V., Almere, The Netherlands). Images were captured and recorded using Dino Capture 2.0 software (Dino-Lite Europe/IDCP B.V., Almere, The Netherlands).

2.8. Particle Size Distribution and Dimension Measurement of 3DP Samples

The particle size distribution was measured using laser diffraction analysis (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK) with the liquid dispersion system Hydro 2000S. Approximately 10 g of each sample was dispersed in 30 mL of distilled water to obtain a homogeneous solution adequate for measuring at the minimum achieved degree of obscuration. The particle diameters were expressed over: d (0.1)—10% of the volume distribution is below the observed diameter; d (0.5)—median diameter, 50% of the volume distribution is below, and 50% is above the observed diameter; d (0.9)—90% of the volume distribution is below the observed diameter; D (3.2)—surface weighted mean diameter (Sauter mean diameter); D (4.3)—volume weighted mean diameter (De Brouckere mean diameter).

Dimensions of 3DP samples were measured over three geometric values (length × width × height). Changes in a variety of dimensions were detected using a digital caliper with an accuracy of 0.01 mm. The default dimensions of the 3DP product according to both programs were: 53 mm × 51 mm × 12 mm (length × width × height).

2.9. Shelf-Life Study

The microbiological quality of 3DP samples was monitored during storage at 4 °C for 10 days. A total of 5 samples were analyzed as follows: (i) control sample—without antimicrobial agents; (ii) 3DP sample with the addition of vanillin 1 g L^{-1}; (iii) 3DP sample with the addition of vanillin 2 g L^{-1}; (iv) 3DP sample with the addition of citral 75 mg L^{-1}; and (v) 3DP sample with the addition of citral 150 mg L^{-1}. The selected antimicrobial concentrations were adopted and modified according to previous findings [15,32].

Classical microbiological methods were employed, and microorganisms selected for the analysis were in accordance with the prescribed regulations for foodstuffs (EC, 2073/2005) [33]. Here, 1 g of a 3D-printed sample was homogenized in 9 mL of sterile deionized water and serial dilutions were made before plating. For the determination of total aerobic mesophilic bacteria (AMB), the pour plate method was used and for other bacteria, the spread plate method was employed on appropriate selective media. AMB count was determined after incubation on Nutrient agar (Merck, Darmstadt, Germany) at 37 °C for 24–48 h. Molds and yeasts were determined after incubation on Potato Dextrose agar (Biolife, Milan, Italy) at 25 °C for 96 h. Enumeration of *Enterobacteriaceae* was conducted on Violet Red Bile Glucose (VRBG) agar (Biolife, Milan, Italy) after incubation at 37 °C for

48 h. *Salmonella* sp. detection was conducted in a Rappaport–Vassiliadis (RV) Salmonella enrichment broth (Merck, Darmstadt, Germany), and by subculturing on Xylose Lysine Deoxycholate (XLD) agar (Biolife, Milan, Italy) at 37 °C for 24–48 h. All experiments were conducted in triplicate and the results were expressed as the colony forming units per gram of 3D-printed sample (CFU g^{-1}).

2.10. Statistical Analysis

Descriptive statistics were used to characterize pH, a_w, bioactive compounds, and antioxidant capacities of the 3DP samples. Multivariate analysis of variance (MANOVA) simultaneously tested associations among dependent and independent variables. The significance level for all tests was $\alpha \leq 0.05$, and the results were analyzed using SPSS software (v.22) (IBM, Chicago, IL, USA). Statistical analysis of texture analysis, particle size distribution and color parameters were performed using "Statistica 12" software (TIBCO, Palo Alto, CA, USA).

3. Results and Discussion

3.1. The Influence of 3DP Technology on a_w and pH

Considering that fruit is a raw material with a low pH but also with a high water-activity, in this study, the influence of the process parameters of the 3DP technology on the stability of these parameters was monitored (Table 2). The average water activity in the 3D-printed samples was 0.95%. In contrast to the program type, which had no statistically significant effect on the a_w value, the starch type and starch content did have a significant effect on this variable. Here, samples with wheat starch had a higher a_w value than samples with corn starch. The results of Dogan et al. [34] showed that among the starches tested, such as modified corn starch, unmodified corn starch, modified potato starch, tapioca starch, potato starch, and wheat starch, corn starch had the best swelling capacity. Our results could be explained by the fact that corn starch has a better swelling power and binds to water better than wheat starch (which reduced the a_w values in the samples). Increasing the starch content also resulted in an increase in the a_w value compared to the initial value at 10% starch content. This could be due to the fact that glucose and sucrose, which are naturally present in strawberries, compete for water binding [35,36] and are inversely related to the a_w value. Thus, as starch content increases, the concentration of these sugars in the samples decreases, which may be reflected in an increase in a_w values.

Table 2. Association of 3DP parameters with the contents of a_w and pH in the 3DP samples.

Variable	*n*	a_w	pH
Starch type		$p \leq 0.01$ †	$p \leq 0.01$ †
Corn	12	0.94 ± 0.0^{b}	3.38 ± 0.0^{b}
Wheat	12	0.95 ± 0.0^{a}	3.50 ± 0.0^{a}
Starch content		$p \leq 0.01$ †	$p \leq 0.01$ †
10%	8	0.94 ± 0.0^{b}	3.25 ± 0.0^{b}
15%	8	0.96 ± 0.0^{a}	3.53 ± 0.0^{a}
20%	8	0.95 ± 0.0^{a}	3.53 ± 0.0^{a}
3D Program		$p = 0.12$ ‡	$p \leq 0.01$ †
Program 1	12	0.95 ± 0.0^{a}	3.35 ± 0.0^{b}
Program 2	12	0.95 ± 0.0^{a}	3.53 ± 0.0^{a}
Dataset average	24	0.95 ± 0.0	3.53 ± 0.0

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; ‡ not a significant factor in multifactor analysis. a_w—activity of water (%).

The average pH of 3D-printed samples was 3.53. All three variables studied, starch type, starch content, and 3D program, had a statistically significant effect on the pH of the samples. Higher pH values were found for the 3D printed product with wheat starch

at higher proportions of added starch (15% and 20% vs. 10%) printed with program 2. These results agree with the findings of Rababah et al. [37], who found a significant effect of the addition of hydrocolloids on pH in gelatinized strawberry products. Moreover, Bravo-Núñez et al. [38] indicated that the interaction between starch and proteins could affect pH. Therefore, it is possible that studied starches bind differently to strawberry proteins and thus affect the pH of the 3DP product.

3.2. The Influence of 3DP Technology on the Stability of Bioactive Compounds and Antioxidant Capacity

The results for the effect of 3DP processing parameters on the bioactive compounds content of and antioxidant capacity in the 3DP samples are shown in Table 3.

Table 3. Association of 3DP processing parameters with the contents of bioactive compounds and antioxidant capacity in the 3DP samples.

Variable	*n*	TPC	HCA	FL	TF	ANT	CT	DPPH	FRAP
Starch type		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.04$ †	$p = 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
Corn	12	74.47 ± 1.08 ^{b}	19.44 ± 0.25 ^{a}	6.90 ± 0.16 ^{a}	5.30 ± 0.05 ^{a}	7.50 ± 0.03 ^{b}	43.90 ± 0.27 ^{b}	487.83 ± 0.41 ^{a}	1.19 ± 0.02 ^{b}
Wheat	12	82.66 ± 1.08 ^{a}	13.64 ± 0.25 ^{b}	3.70 ± 0.16 ^{b}	4.50 ± 0.05 ^{b}	7.61 ± 0.03 ^{a}	45.01 ± 0.27 ^{a}	486.08 ± 0.41 ^{b}	1.29 ± 0.02 ^{a}
Starch content		$p = 0.54$ ‡	$p = 0.09$ ‡	$p \leq 0.01$ †	$p = 0.04$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
10%	8	77.4 ± 1.32 ^{a}	16.00 ± 0.30 ^{a}	4.47 ± 0.20 ^{c}	4.86 ± 0.06 ^{a,b}	7.82 ± 0.04 ^{a}	45.02 ± 0.33 ^{b}	515.76 ± 0.50 ^{a}	1.24 ± 0.02 ^{b}
15%	8	79.48 ± 1.32 ^{a}	17.04 ± 0.30 ^{a}	6.06 ± 0.20 ^{a}	5.04 ± 0.06 ^{a}	7.94 ± 0.04 ^{a}	47.14 ± 0.33 ^{a}	489.15 ± 0.50 ^{b}	1.32 ± 0.02 ^{a}
20%	8	78.83 ± 1.32 ^{a}	16.58 ± 0.30 ^{a}	5.37 ± 0.20 ^{b}	4.80 ± 0.06 ^{b}	6.91 ± 0.04 ^{b}	41.20 ± 0.33 ^{c}	455.97 ± 0.50 ^{c}	1.16 ± 0.02 ^{c}
3D Program		$p = 0.03$ †	$p = 0.08$ ‡	$p = 0.09$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.02$ †	$p \leq 0.01$ †	$p = 0.08$ ‡
Program 1	12	76.62 ± 1.08 ^{b}	16.21 ± 0.25 ^{a}	5.32 ± 0.16 ^{a}	4.79 ± 0.05 ^{b}	7.43 ± 0.03 ^{b}	43.96 ± 0.27 ^{b}	485.26 ± 0.41 ^{b}	1.22 ± 0.02 ^{a}
Program 2	12	80.51 ± 1.08 ^{a}	16.87 ± 0.25 ^{a}	5.28 ± 0.16 ^{a}	5.01 ± 0.05 ^{a}	7.69 ± 0.03 ^{a}	44.95 ± 0.27 ^{a}	488.66 ± 0.41 ^{a}	1.27 ± 0.02 ^{a}
Dataset average	24	78.57 ± 1.08	16.54 ± 0.18	5.30 ± 0.12	4.90 ± 0.04	7.56 ± 0.02	44.46 ± 0.19	486.96 ± 0.29	1.24 ± 0.01

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; ‡ not a significant factor in multifactor analysis. TPC—Total phenolic compounds (mg 100 g^{-1}); HCA—Hydroxycinnamic acids (mg 100 g^{-1}); FL—Flavanols (mg 100 g^{-1}); TF—Total flavonoids (mg 100 g^{-1}); ANT—Monomeric anthocyanins (mg 100 g^{-1}); CT—Condensed tannins (mg 100 g^{-1}); DPPH assay (μM); FRAP assay (mM).

The mean value of total phenols in the 3DP samples was 78.57 ± 1.08 mg 100 g^{-1} sample. Of all analyzed bioactive components, condensed tannins were the most abundant (44.46 ± 0.19 mg 100 g^{-1}), followed by hydroxycinnamic acids (16.54 ± 0.18 mg 100 g^{-1}), anthocyanins (7.56 ± 0.02 mg 100 g^{-1}), flavanols (5.30 ± 0.12 mg 100 g^{-1}), and finally, total flavonoids (4.90 ± 0.04 mg 100 g^{-1}).

The type of starch carrier had a statistically significant effect on all bioactive compounds tested. The content of total phenols, anthocyanins, and condensed tannins was higher in 3D-printed samples with wheat starch, while the content of hydroxycinnamic acids, flavanols, and total flavonoids was higher in samples with corn starch.

Starch carrier content affected ($p \leq 0.05$) contents of all bioactive compounds except those of total phenols and hydroxycinnamic acids. Increasing the starch content increased almost all dependent variables, up to 15% of the content. After the addition of 15% starch, the experimental mean values tended to either decrease or remain the same. Increasing the content of starch carriers (from 15% to 20%) in mixtures for 3D printing leads to a decrease in the mass fraction of all studied bioactive compounds. This result is expected considering that mixtures with a higher content of starch carriers have a lower content of the fruit component, which is the source of the bioactive compounds. Moreover, these results indicated that the addition of starch at the level of 15% is optimal for the preservation of bioactivity of the samples.

The type of 3D program had a statistically significant effect on the content of almost all bioactive compounds, except for hydroxycinnamic acids and flavonols, which were not affected. Program 2 compared to Program 1 increased the content of TPC (5%), TF (5%), ANT (4%), and CT (2%). Considering that the printing programs differed from each other

in terms of the speed of nozzle movement, the speed of extrusion of the mixture from the nozzle, the thickness of the printing line, and the distance of the nozzle from the surface when printing the first layer, it is possible that due to the change in the different printing parameters, there was a faster degradation of TPC, TF, ANT, and CT with program 1, and consequently, their content differed according to the 3D-printing program.

The antioxidant activity of the 3DP samples was determined using two in vitro assays. The average values for DPPH and FRAP were 486.96 ± 0.29 μM and 1.24 ± 0.01 mM, respectively (Table 3). Starch type affected antioxidant capacity, with higher DPPH values detected in 3DP samples with corn starch and higher FRAP values in 3DP samples with wheat starch. Considering the starch content, the FRAP values followed the trend of the content of other bioactive compounds, i.e., with increasing starch content, the FRAP value increased, but mostly only up to a proportion of 15%, after which it tended to decrease with further increasing starch content. An exception was DPPH, whose value decreased with increasing starch content (−12%). These results are consistent with a similarly designed study by Mu et al. [20]. In their study, potato starch pastes were fortified with 5, 10, and 15% strawberry or blackcurrant powders, and significantly higher DPPH and FRAP levels were found in such pastes compared to pure starch paste. In other words, DPPH and FRAP values correlated with the content of total phenols from berry powders [20]. In our case, there was an expected decrease in the value of DPPH antioxidant capacity, since with an increase in the proportion of starch carriers, the proportion of strawberry pulp rich in bioactive compounds decreased. Regarding the type of 3D program, FRAP was not changed when a different programming of the 3D printer was selected. On the other hand, compared to Program 1, Program 2 increased the DPPH value by 0.7% and the FRAP value by 4%. These results somehow suggest that program 2 is more suitable for 3D printing strawberry-starch products, not only because of the higher antioxidant capacities, but also because of the higher levels of other biologically active compounds compared to Program 1.

Overall, for the production of functional foods, it is of particular importance to choose raw materials with a favorable bioactive compositions [39]. However, during processing, the quality of the raw material may be compromised and the final product may fall short of the starting raw material in terms of bioactive potential and antioxidant capacity. In this regard, 3DP technology with the principle of cold extrusion promises production of functional foods in which the deterioration of the quality of the original raw material would be extremely low [40]. However, the addition of various additives may affect the quality, which remains to be investigated.

3.3. The Influence of 3DP Technology on Color Parameters, Microscopic Analysis, and 3DP Dimension Measurement

Color is a key attribute that determines consumers' acceptance of a food product and influences their purchasing behavior [30,31]. Table 4 shows the CIEL*a*b* color parameters in respect to variations in 3DP program, starch type and starch content. As can be seen, with no influence of 3DP operating conditions, ΔE* values greater than 12 represent a large color difference that can be easily perceived by human eyes [41].

Table 4. The CIEL*a*b* color parameters in control and 3DP samples.

3DP Program	Type of Starch	Starch Content (%)	L*	a*	b*	C*	h (°)	ΔE*
Control sample	/	/	24.84	30.42	20.88	36.90	34.46	/
Program 1	corn	10	34.16	22.64	7.50	23.85	18.33	18.07
	corn	15	35.90	24.12	10.82	26.44	24.16	16.22
	corn	20	44.10	27.32	12.22	29.93	24.09	21.34
	wheat	10	38.64	27.52	11.76	29.93	23.14	16.79
	wheat	15	34.44	22.64	9.50	24.55	22.76	16.78
	wheat	20	39.54	24.46	9.74	26.33	21.71	19.38
Program 2	corn	10	34.20	23.10	8.86	24.74	20.98	16.91
	corn	15	33.52	22.54	10.04	24.67	24.01	15.97
	corn	20	43.94	26.68	12.98	29.67	25.94	21.01
	wheat	10	34.90	27.52	11.76	29.93	23.14	13.88
	wheat	15	33.40	22.8	11.10	25.36	25.96	15.07
	wheat	20	39.24	23.22	10.14	25.34	23.59	19.35

Control sample—sample without added starch; L*—lightness; a*—redness; b*—yellowness; ΔE*—color difference; C*—chroma; h*—hue angle.

Significant influence of 3DP operating conditions on colorimetric parameters were given in Table 5. The changes in lightness (L*) were influenced by the proportion of starch added, while the type of starch and type of printing program did not vary (Table 5).

Table 5. Influence of 3DP processing parameters on color, texture, particle diameters, and product dimensions expressed by *p*-value *.

Parameter	3DP Program	Type of Starch	Starch Content
L*	0.34	0.47	≤0.01 *
a*	0.72	0.82	0.29
b*	0.60	0.80	0.59
C*	0.89	0.82	0.42
h	0.17	0.66	0.11
Fp	0.07	0.03 *	0.04 *
Wp	0.11	0.26	0.02 *
F	1.00	≤0.01 *	0.02 *
W	0.99	≤0.01 *	0.02 *
D (3.2)	0.95	0.05 *	≤0.01 *
D (4.3)	0.95	0.05 *	≤0.01 *
d (0.1)	0.82	≤0.01 *	≤0.01 *
d (0.5)	0.85	0.85	≤0.01 *
d (0.9)	0.25	0.20	≤0.01 *
Length	0.51	0.06	0.23
Width	0.52	0.54	0.40
Height	0.76	0.78	0.16

L*—lightness; a*—redness; b*—yellowness; C*—chroma; h*—hue angle; F—extrusion force (firmness) (N); W—work at extrusion force (Nmm); F_p –maximum force (hardness) (N); W_p—work at maximum force (Nmm); D (3.2)—surface weighted mean diameter (Sauter mean diameter); D (4.3)—volume weighted mean diameter (De Brouckere mean diameter); d (0.1)—10% of the volume distribution is below the observed diameter; d (0.5)—median diameter, 50% of the volume distribution is below, and 50% is above the observed diameter; d (0.9)—90% of the volume distribution is below the observed diameter. *—statistically significant ($p < 0.05$).

Increasing the proportion of the two added starches (corn and wheat) from 0 to 20% increased the lightness of samples (Table 4). Figure 1 shows photographs of printed strawberry samples with 10% (A), 15% (B), and 20% (C) starch content, confirming the information given previously. This was aligned with previous results about content of polyphenols, and antioxidant activity, as some of them are main carries of the color in the strawberry pulp (e.g., anthocyanins). So, it makes sense that samples were lighter with an addition of starch.

Figure 1. Microscopic pictures (500×) of 3DP samples: Influence of added starch, 10% (**A**); 15% (**B**); 20% (**C**), on the lightness (L*).

As shown in Table 5, the 3DP processing parameters did not significantly affect changes in the dimension of the 3D-printed product. Considering the results of mechanical properties, it was expected that the variation in starch type and starch content affected the variation in observed product dimensions. All samples show a high level of stability and minimum deviations in geometry (Table 6). This is very important for larger food manufacturing and the associations among food design in terms of a content and its geometric form (i.e., regardless of the added ingredient shape remains unchanged).

Table 6. Influence of 3DP processing parameters on the dimension of 3DP samples.

3DP Program	Type of Starch	Starch Content (%)	Length (mm)	Width (mm)	Height (mm)
Program 1	corn	10	53.36 ± 0.53	51.22 ± 0.49	12.24 ± 0.29
	corn	15	52.11 ± 0.56	51.45 ± 0.25	12.41 ± 0.23
	corn	20	52.12 ± 0.66	51.76 ± 0.31	12.49 ± 0.38
	wheat	10	52.25 ± 0.31	51.33 ± 0.51	11.72 ± 0.76
	wheat	15	52.26 ± 0.41	51.36 ± 0.14	12.25 ± 0.38
	wheat	20	51.76 ± 0.38	50.98 ± 0.91	12.36 ± 0.88
Program 2	corn	10	52.12 ± 0.89	51.11 ± 0.75	11.87 ± 0.48
	corn	15	53.28 ± 0.39	51.25 ± 0.57	11.81 ± 0.71
	corn	20	52.23 ± 0.62	51.34 ± 0.67	12.66 ± 0.43
	wheat	10	52.25 ± 0.54	51.25 ± 0.55	12.41 ± 0.27
	wheat	15	51.56 ± 0.37	51.46 ± 0.77	12.49 ± 0.57
	wheat	20	51.22 ± 0.67	52.91 ± 0.39	12.55 ± 0.64

Results are presented as an average value of triplicate measurements ± STDEV.

The samples containing 10% starch were visually much darker than the samples with 20% starch. A similar phenomenon was reported by Liu et al. [42], who investigated the effect of soluble starch-based additives on the color of 3D-printed beef, and found that the incorporation of starch-based additives gave the samples a lighter color. Zhang et al. [43] also found that the addition of starch increased the brightness of surimi beef gels. Furthermore, changing the printing programs, starch types (corn and wheat), and starch content did not result in significant changes in a*, b*, C*, and h ($p > 0.05$). All printed

samples contained approximately equal amounts of a*, b*, C*, and h despite the different printing programs, starch types, and starch contents, and the only difference between samples was brightness.

3.4. The Influence of 3DP Technology on Texture Properties and Particle Size Distribution

Changing the proportion of the two added starches resulted in a significant change in the work (W_p) and force (F_p) required to penetrate the samples, as shown in Table 7.

Table 7. Influence of 3DP parameters on textural properties of samples.

3DP Program	Type of Starch	Starch Content (%)	F (N)	W (Nmm)	F_p (N)	W_p (Nmm)
Program 1	corn	10	53.538	535.200	0.143	0.115
	corn	15	50.437	504.279	0.278	0.116
	corn	20	95.881	958.52	0.18	0.129
	wheat	10	98.543	985.129	0.073	0.074
	wheat	15	177.914	1778.729	0.147	0.149
	wheat	20	162.755	1627.201	0.139	0.145
Program 2	corn	10	39.367	393.599	0.084	0.077
	corn	15	37.723	377.149	0.14	0.092
	corn	20	92.904	917.987	0.173	0.106
	wheat	10	99.029	990.085	0.085	0.081
	wheat	15	182.785	1827.473	0.126	0.118
	wheat	20	187.259	1872.168	0.108	0.143

F—extrusion force (firmness) (N); W—work at extrusion force (Nmm); F_p—maximum force (hardness) (N); W_p—work at maximum force (Nmm).

Force (F_p) and Work (W_p) increased with increasing starch content, implying sturdier (firmer) texture in the samples. A similar trend was reported by Yang et al. [44], who studied the effect of potato starch (10, 12.5, 15, 17.5, and 20 g 100 g^{-1}) on the textural properties of lemon juice gels. In addition, Feng et al. [45] added potato starch (2, 4, 6, and 8%) to improve the printability of Nostoc sphaeroides gel, while Dong et al. [46] examined the addition of starch (2, 6, 8, and 10%) to surimi-based printing mixtures. In all cases, with the increase of starch content in the gels, their hardness also increased and they were more resistant to external damage. It is assumed that the increase in starch content leads to a greater number of starch molecules per unit volume, thus increasing the probability of intermolecular hydrogen bonding, which leads to greater compactness of the network structure and thus, to the strength of the samples [17,46]. In addition, the F_p values were also affected by added types of starch (Table 5). Here, samples containing corn starch exhibited significantly higher penetration force than those containing wheat starch.

We showed that the force and work required for the extrusion of mixtures significantly depend on starch types and its contents. Similar to the previously described results of the penetration test, the values for F and W increased with increasing starch content. Although increasing both starch types increased F and W values, the extent of the increase was greater for the wheat vs. the corn. This was in contrast to the results of the penetration test, where samples with corn starch had higher hardness than samples with wheat. Zheng et al. [47] found that wheat starch forms stronger starch gels than corn starch when performing texture analysis on 3D-printed samples of wheat and corn starch. This suggests that the reason for the difference in gel strength may be the higher amylose content of wheat starch compared to corn starch. It can be assumed that the extrusion test still describes the texture of the observed samples more reliably than a penetration test. Measurement errors in the case of a penetration test are possible due to the specific geometry of the 3D-printed samples and the flat-bottomed cylindrical probe chosen as an extension for the texture analyzer. Considering that the surface of the measured samples was not completely flat and smooth, the use of spherical probes instead of cylindrical probes could increase the precision of the mentioned method.

In addition, textural properties are also affected by printing parameters such as nozzle diameter, nozzle height, extrusion speed, printing speed, and line thickness [48,49], which was not the case here. Namely, the selected printing programs (programs 1 and 2) did not significantly affect the texture of the samples, although they differed from each other in the first layer nozzle height, print speed, line thickness, and extrusion speed. It is assumed that this is not due to insufficient deviations in the values of the specified parameters according to which the programs differed.

The variations in starch concentration resulted in significant changes in the values of d (0.1), d (0.5), d (0.9), D (3.2), and D (4.3). As shown in Table 8, an increase in starch content resulted in a decrease in the observed particle size distribution parameters. By decreasing the values of d (0.1), d (0.5), d (0.9), D (3.2), and D (4.3), the increase in smaller particles becomes more evident. In addition, the change of d (0.1), D (3.2), and D (4.3) was also significantly affected by the starch type ($p \leq 0.05$). As shown in Table 8, the samples containing corn and wheat starch at the same concentration had lower particle size distribution values than the samples with wheat starch added.

Table 8. Influence of 3DP processing parameters on particle diameters of 3DP samples.

3DP Program	Type of Starch	Starch Content (%)	D (3.2) (μm)	D (4.3) (μm)	d (0.1) (μm)	d (0.5) (μm)	d (0.9) (μm)
Program 1	corn	10	49.56	23.563	21.391	122.724	598.039
	corn	15	25.763	116.602	12.901	35.68	357.256
	corn	20	22.458	76.682	11.468	31.748	234.055
	wheat	10	44.827	176.767	22.855	74.155	489.584
	wheat	15	30.226	90.956	15.759	46.31	168.747
	wheat	20	27.99	94.021	14	43.511	287.481
Program 2	corn	10	44.133	221.151	19.313	95.741	591.564
	corn	15	25.945	119.374	12.852	36.627	364.902
	corn	20	21.959	84.936	10.757	31.725	262.309
	wheat	10	48.053	201.603	24.092	84.512	541.467
	wheat	15	33.328	126.287	16.924	51.738	362.298
	wheat	20	28.011	92.548	13.804	43.546	270.289

D (3.2)—surface weighted mean diameter (Sauter mean diameter); D (4.3)—volume weighted mean diameter (De Brouckere mean diameter); d (0.1)—10% of the volume distribution is below the observed diameter; d (0.5)—median diameter, 50% of the volume distribution is below and 50% is above the observed diameter; d (0.9)—90% of the volume distribution is below the observed diameter.

The volume size distribution (Figure 2) shows that the addition of 20% starch increased interval particles of size 2–85 μm compared to the reference sample without starch and a decrease in particle size between 85 and 1673 μm.

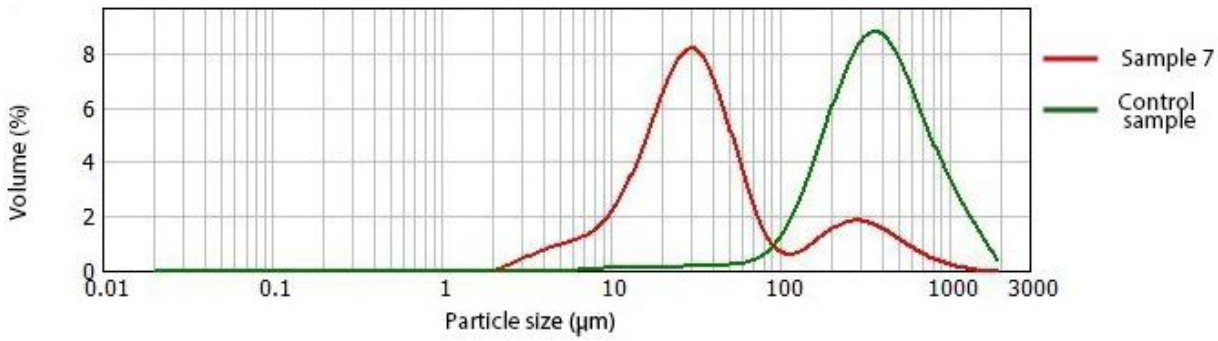

Figure 2. Volume size distribution of control sample (green), and sample 7 with 20% of corn starch (red).

By comparing the particle size distribution results with the extrusion test results, it was observed that samples with smaller particles were more difficult to extrude than samples with larger particle sizes. The correlation of the particle size distribution with the textural

properties of the printed samples was also observed in the results of the penetration test, where the strength of the printed samples increased as the proportion of smaller particles increased. Wilms et al. [50] demonstrated a correlation between individual material properties, e.g., particle size, and the extrusion process. According to the previously mentioned studies, the Consistency factor (K) is inversely proportional to the particle size. On the other hand, the consistency factor affects the apparent viscosity and thus, the pressure required for the extrusion process, which increases when the K value is increased. This explains the increase in the maximum force and work required to extrude strawberry samples when the particle size is decreased. In addition, the reduction in particle size can be used to improve processability [50].

3.5. Microbial Analyses of 3DP Samples

Microbial analysis of 3DP samples was conducted during the 10 days of storage at 4 °C. Results are presented in Table 9. Results indicated that none of the samples contained pathogenic bacteria or yeasts and molds, even after 10 days of storage. Total bacterial count was above the desired limit for the samples 2 and 4.

Table 9. Microbiological counts (CFU g^{-1}) of the 3DP samples during 10 days of storage at 4 °C.

Microorganism Type	Sample	Days of Storage				
		0	**2**	**4**	**7**	**10**
Aerobic mesophilic bacteria	control	1.5×10^2	9×10^2	1.8×10^3	n.d.	n.d.
	vanillin 1 g L^{-1}	n.d.	9×10^2	9×10^2	3.8×10^4 *	9×10^4 *
	vanillin 2 g L^{-1}	n.d.	n.d.	9×10^4 *	n.d.	9×10^2
	citral 75 mg L^{-1}	1.8×10^3	n.d.	n.d.	n.d.	n.d.
	citral 150 mg L^{-1}	1.5×10^2	9×10^2	$3{,}6 \times 10^3$	n.d.	n.d.
Enterobacteriaceae	control	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 1 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 2 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 75 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 150 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
Salmonella sp.	control	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 1 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 2 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 75 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 150 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
Escherichia coli	control	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 1 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 2 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 75 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 150 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
Yeasts and molds	control	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 1 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	vanillin 2 g L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 75 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.
	citral 150 mg L^{-1}	n.d.	n.d.	n.d.	n.d.	n.d.

Control—sample without antimicrobial agents; vanillin 1 g L^{-1}—3DP sample with addition of vanillin in a concentration of 1 g L^{-1}; vanillin 2 g L^{-1}—3DP sample with addition of vanillin in a concentration of 2 g L^{-1}; citral 75 mg L^{-1}—3DP sample with addition of citral in a concentration of 75 mg L^{-1}; citral 150 mg L^{-1}—3DP sample with addition of citral in a concentration of 150 mg L^{-1}; n.d.—not detected; above the safety limit of 10^4 CFU g^{-1}; *—not satisfactory criterion ($\leq 10^4$ CFU mL^{-1}).

The samples with added vanillin (1 g L^{-1}) were stable during the 4-day storage period, after which an increase in bacterial counts above the desired level was observed on days 7 and 10. Interestingly, in the samples with higher vanillin concentration (1 g L^{-1}), a high bacterial count was observed only on one day (4th day), after which the count was within the desired range. These results leave open the possibility of inadequate homogenization of the small amounts of the added microbial agent in the strawberry matrix, which would result in a localized presence of the inhibitory agent, while there could be areas of decreased antimicrobial activity. In addition, although the sampling of the products was done in triplicate and different parts of the product were analyzed, there could be areas of the product that are susceptible to faster spoilage because the conditions in the external and internal parts of the product are very different. In addition, a reduction in aerobic mesophilic bacteria was observed at the citral concentrations studied during the storage. The inhibitory effect of citral could play a role in inhibiting bacterial growth during storage. The presence of aerobic mesophilic bacteria observed before storage could be due to handling during the preparation of the product and is limited to the surface. In addition, the problem of homogenization of the antimicrobial agent in the sample could lead to different citral concentrations in the sample. Further studies should address the technical problem of delivery of antimicrobial agents in products of this type. From our results, it is clear that the product formation process is free from pathogenic contamination and that the addition of citral (75 mg L^{-1}) gave the best microbiological quality. However, the manufacturing methods need to be articulated to avoid localized antimicrobial activity in the final products.

4. Conclusions

The production of strawberry-based functional snacks with 3D printing technology had favorable effects on the retention and stability of the bioactive components and antioxidant capacity. The type of starch significantly affected the stability of all determined bioactive compounds and antioxidant capacity. The use of corn starch resulted in a higher level of hydroxycinnamic acids, flavanols, total flavonoids, and antioxidant capacity as determined by the DPPH method, while the product with wheat starch had higher levels of total phenols, anthocyanins, condensed tannins, and antioxidant capacity determined by the FRAP method. Starch content has a significant effect on almost all of the determined bioactive compounds and antioxidant capacity. To maintain the bioactive profile of the 3D-printed product and strong antioxidant capacity, a starch content of 15% is most favorable. The type of 3D printing program has a significant effect on the majority of the determined bioactive compounds and antioxidant capacity, where the use of program 2 resulted in greater stability of all analyzed components in the strawberry-based 3D printed product.

In terms of textural properties, the extrusion test better describes the mechanical properties of smaller particles affecting the strength and stability of 3DP products in relation to the applied 3DP processing parameters when both test mechanisms are used.

The microbiological safety of the product was confirmed as no pathogenic bacteria were detected in the samples during 10 days of storage at 4 °C. Further research should be directed towards the availability of antimicrobial agents in the strawberry matrices for 3D printing.

In conclusion, 3D printing can be considered a very promising technology with great potential for the development of innovative and customized functional products.

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References

1. Kerry Group. ProActive Health Division Global Survey. Available online: https://www.kerry.com/ (accessed on 29 November 2022).
2. Bursać Kovačević, D.; Brdar, D.; Fabečić, P.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. Strategies to achieve a healthy and balanced diet: Fruits and vegetables as a natural source of bioactive compounds. In *Agri-Food Industry Strategies for Healthy Diets and Sustainability*; Barba, F.J., Putnik, P., Bursać Kovačević, D., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 51–88.
3. Giampieri, F.; Forbes-Hernandez, T.Y.; Gasparrini, M.; Alvarez-Suarez, J.M.; Afrin, S.; Bompadre, S.; Quiles, J.L.; Mezzetti, B.; Battino, M. Strawberry as a health promoter: An evidence based review. *Food Funct.* **2015**, *6*, 1386–1398. [CrossRef]
4. WHO. Healthy Diet. Available online: https://www.who.int/news-room/fact-sheets/detail/healthy-diet (accessed on 29 November 2022).
5. Ricci, I.; Derossi, A.; Severini, C. 3D printed food from fruits and vegetables. In *Fundamentals of 3D Food Printing and Applications*; Academic Press: Cambridge, MA, USA, 2019; pp. 117–149.
6. Severini, C.; Derossi, A. Could the 3D printing technology be a useful strategy to obtain customized nutrition? *J. Clin. Gastroenterol.* **2016**, *50*, S175–S178. [CrossRef]
7. Derossi, A.; Caporizzi, R.; Azzollini, D.; Severini, C. Application of 3D printing for customized food. A case on the development of a fruit-based snack for children. *J. Food Eng.* **2018**, *220*, 65–75. [CrossRef]
8. Severini, C.; Derossi, A.; Ricci, I.; Caporizzi, R.; Fiore, A. Printing a blend of fruit and vegetables. New advances on critical variables and shelf life of 3D edible objects. *J. Food Eng.* **2018**, *220*, 89–100. [CrossRef]
9. Tran, J.L. Safety and labelling of 3D printed food. In *Fundamentals of 3D Food Printing and Applications*; Godoi, F.C., Bhandari, B.R., Prakash, S., Zhang, M., Eds.; Academic Press: Cambridge, MA, USA, 2019; pp. 355–371.
10. Cerrutti, P.; Alzamora, S.M.; Vidales, S.L. Vanillin as an antimicrobial for producing shelf-stable strawberry puree. *J. Food Sci.* **1997**, *62*, 608–610. [CrossRef]
11. Ferrario, M.; Fenoglio, D.; Chantada, A.; Guerrero, S. Hurdle processing of turbid fruit juices involving encapsulated citral and vanillin addition and UV-C treatment. *Int. J. Food Microbiol.* **2020**, *332*, 108811. [CrossRef]
12. Cassani, L.; Tomadoni, B.; Viacava, G.; Ponce, A.; Moreira, M.R. Enhancing quality attributes of fiber-enriched strawberry juice by application of vanillin or geraniol. *LWT—Food Sci. Technol.* **2016**, *72*, 90–98. [CrossRef]
13. Tabanelli, G.; Montanari, C.; Arioli, S.; Magnani, M.; Patrignani, F.; Lanciotti, R.; Mora, D.; Gardini, F. Physiological response of *Saccharomyces cerevisiae* to citral combined with thermal treatment. *LWT—Food Sci. Technol.* **2019**, *101*, 827–834. [CrossRef]
14. Patrignani, F.; Tabanelli, G.; Siroli, L.; Gardini, F.; Lanciotti, R. Combined effects of high pressure homogenization treatment and citral on microbiological quality of apricot juice. *Int. J. Food Microbiol.* **2013**, *160*, 273–281. [CrossRef]
15. Char, C.D.; Guerrero, S.N.; Alzamora, S.M. Mild thermal process combined with vanillin plus citral to help shorten the inactivation time for *Listeria innocua* in orange juice. *Food Bioprocess Technol.* **2008**, *3*, 752–761. [CrossRef]
16. Tomadoni, B.; Viacava, G.; Cassani, L.; Moreira, M.R.; Ponce, A. Novel biopreservatives to enhance the safety and quality of strawberry juice. *J. Food Sci. Technol.* **2015**, *53*, 281–292. [CrossRef]
17. Yang, F.; Guo, C.; Zhang, M.; Bhandari, B.; Liu, Y. Improving 3D printing process of lemon juice gel based on fluid flow numerical simulation. *LWT—Food Sci. Technol.* **2019**, *102*, 89–99. [CrossRef]
18. Azam, S.M.R.; Zhang, M.; Mujumdar, A.S.; Yang, C. Study on 3D printing of orange concentrate and material characteristics. *J. Food Process Eng.* **2018**, *41*, e12689. [CrossRef]
19. Liu, Z.; Zhang, M.; Bhandari, B.; Yang, C. Impact of rheological properties of mashed potatoes on 3D printing. *J. Food Eng.* **2018**, *220*, 76–82. [CrossRef]
20. Mu, J.; Wu, G.; Chen, Z.; Klosek, A.; Brennan, M.A.; Hui, X.; Brennan, C.S. The effects of blackcurrant and strawberry powder on the physicochemical and in vitro glycaemic response of starches derived from sweet potato (*Ipomoea batatas*) and potato (*Solanum tuberosum*). *Int. J. Food Sci. Technol.* **2021**, *56*, 6062–6072. [CrossRef]
21. Bursać Kovačević, D.; Putnik, P.; Dragović-Uzelac, V.; Pedisić, S.; Režek Jambrak, A.; Herceg, Z. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chem.* **2016**, *190*, 317–323. [CrossRef]

22. Yuan, B.; Danao, M.-G.C.; Stratton, J.E.; Weier, S.A.; Weller, C.L.; Lu, M. High pressure processing (HPP) of aronia berry purée: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. *Innov. Food Sci. Emerg. Technol.* **2018**, *47*, 249–255. [CrossRef]
23. Bebek Markovinović, A.; Putnik, P.; Stulić, V.; Batur, L.; Duralija, B.; Pavlić, B.; Vukušić Pavičić, T.; Herceg, Z.; Bursać Kovačević, D. The application and optimization of HIPEF technology in the processing of juice from strawberries harvested at two stages of ripeness. *Foods* **2022**, *11*, 1997. [CrossRef]
24. AOAC. *Official Methods of Analysis: Changes in Official Methods of Analysis Made at the Annual Meeting*; AOAC International: Rockville, MD, USA, 1990.
25. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* **2002**, *10*, 178–182.
26. Howard, L.R.; Clark, J.R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238–1247. [CrossRef]
27. Sun, B.; Ricardo-da-Silva, J.M.; Spranger, I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. [CrossRef]
28. Shortle, E.; O'Grady, M.N.; Gilroy, D.; Furey, A.; Quinn, N.; Kerry, J.P. Influence of extraction technique on the anti-oxidative potential of hawthorn (*Crataegus monogyna*) extracts in bovine muscle homogenates. *Meat Sci.* **2014**, *98*, 828–834. [CrossRef] [PubMed]
29. Benzie, I.F.F. An automated, specific, spectrophotometric method for measuring ascorbic acid in plasma (EFTSA). *Clin. Biochem.* **1996**, *29*, 111–116. [CrossRef] [PubMed]
30. Keerthana, K.; Anukiruthika, T.; Moses, J.A.; Anandharamakrishnan, C. Development of fiber-enriched 3D printed snacks from alternative foods: A study on button mushroom. *J. Food Eng.* **2020**, *287*, 110116. [CrossRef]
31. Putnik, P.; Bursać Kovačević, D.; Herceg, K.; Levaj, B. Influence of antibrowning solutions, air exposure, and ultrasound on color changes in fresh-cut apples during storage. *J. Food Process. Preserv.* **2017**, *41*, e13288. [CrossRef]
32. Orizano-Ponce, E.; Char, C.; Sepúlveda, F.; Ortiz-Viedma, J. Heat sensitization of Escherichia coli by the natural antimicrobials vanillin and emulsified citral in blended carrot-orange juice. *Food Microbiol.* **2022**, *107*, 104058. [CrossRef]
33. The European Parliament and the Council of the European Union. *Regulation (EC) No 2073/2005 of the European Parliament and of the Council of 29 April 2004 on Microbiological Criteria for Foodstuffs*; 338/1; OJEU: Brussels, Belgium, 2005.
34. Dogan, M.; Toker, O.S.; Goksel, M. Rheological behaviour of instant hot chocolate beverage: Part 1. Optimization of the effect of different starches and gums. *Food Biophys.* **2011**, *6*, 512–518. [CrossRef]
35. Evans, I.D.; Haisman, D.R. The effect of solutes on the gelatinization temperature range of potato starch. *Starch* **1982**, *34*, 224–231. [CrossRef]
36. Keysers, H. Unterschiede in der KristallisationsbestaÈndigkeit zwischen Invertzucker und High-Fructose-Glukosesirup sowie deren Weichhaltewirkung. *Zucker U. SuÈûwarenwirtschaft* **1982**, *35*, 147.
37. Rababah, T.M.; Al-u'datt, M.H.; Al-Mahasneh, M.A.; Feng, H.; Alothman, A.M.; Almajwal, A.; Yang, W.; Kilani, I.; Alhamad, M.N.; Ereifej, K.; et al. Effect of storage on the physicochemical properties, total phenolic, anthocyanin, and antioxidant capacity of strawberry jam. *J. Food Agric. Environ.* **2011**, *9*, 101–105.
38. Bravo-Núñez, Á.; Garzón, R.; Rosell, C.M.; Gómez, M. Evaluation of Starch–Protein Interactions as a Function of pH. *Foods* **2019**, *8*, 155. [CrossRef] [PubMed]
39. Granato, D.; Barba, F.J.; Bursać Kovačević, D.; Lorenzo, J.M.; Cruz, A.G.; Putnik, P. Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety. *Annu. Rev. Food Sci. Technol.* **2020**, *11*, 93–118. [CrossRef] [PubMed]
40. Tomašević, I.; Putnik, P.; Valjak, F.; Pavlić, B.; Šojić, B.; Bebek Markovinović, A.; Bursać Kovačević, D. 3D printing as novel tool for fruit-based functional food production. *Curr. Opin. Food Sci.* **2021**, *41*, 138–145. [CrossRef]
41. Chen, X.D. Food drying fundamentals. In *Drying Technologies in Food Processing*; Chen, X.D., Mujumdar, A.S., Eds.; Blackwell Publishing: Singapore, 2008; pp. 1–55.
42. Liu, P.; Dang, X.; Woo, M.W.; Chattha, S.A.; An, J.; Shan, Z. Feasibility study of starch-based biomass incorporated 3D printed beef. *Starch-Stärke* **2022**, *74*, 2200030. [CrossRef]
43. Zhang, F.; Fang, L.; Wang, C.; Shi, L.; Chang, T.; Yang, H.; Cui, M. Effects of starches on the textural, rheological, and color properties of surimi–beef gels with microbial tranglutaminase. *Meat Sci.* **2013**, *93*, 533–537. [CrossRef] [PubMed]
44. Yang, F.; Zhang, M.; Bhandari, B.; Liu, Y. Investigation on lemon juice gel as food material for 3D printing and optimization of printing parameters. *LWT—Food Sci. Technol.* **2018**, *87*, 67–76. [CrossRef]
45. Feng, C.; Zhang, M.; Bhandari, B. Controlling the three-dimensional printing mechanical properties of *Nostoc Sphaeroides* system. *Food Biophys.* **2019**, *15*, 240–248. [CrossRef]
46. Dong, X.; Huang, Y.; Pan, Y.; Wang, K.; Prakash, S.; Zhu, B. Investigation of sweet potato starch as a structural enhancer for three-dimensional printing of *Scomberomorus niphonius* surimi. *J. Texture Stud.* **2019**, *50*, 316–324. [CrossRef]
47. Zheng, L.; Yu, Y.; Tong, Z.; Zou, Q.; Han, S.; Jiang, H. The characteristics of starch gels molded by 3D printing. *J. Food Process. Preserv.* **2019**, *43*, e13993. [CrossRef]
48. Pereira, T.; Barroso, S.; Gil, M.M. Food texture design by 3D printing: A review. *Foods* **2021**, *10*, 320. [CrossRef]

49. Theagarajan, R.; Moses, J.A.; Anandharamakrishnan, C. 3D extrusion printability of rice starch and optimization of process variables. *Food Bioprocess Technol.* **2020**, *13*, 1048–1062. [CrossRef]
50. Wilms, P.; Daffner, K.; Kern, C.; Gras, S.L.; Schutyser, M.A.I.; Kohlus, R. Formulation engineering of food systems for 3D-printing applications—A review. *Food Res. Int.* **2021**, *148*, 110585. [CrossRef] [PubMed]

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Article

Characterization of Antioxidant Bioactive Compounds and Rheological, Color and Sensory Properties in 3D-Printed Fruit Snacks

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Abstract: The influence of wheat starch (6%, 8% and 10%, *w*/*w*) and a 3D printing program (program 1 vs. program 2) on the content of bioactive compounds, antioxidant capacity, color parameters and rheological and sensory properties was investigated in 3D strawberry and strawberry tree fruit snacks. Increasing the starch content led to a decrease in the content of almost all the bioactive compounds, while it had no effect on the antioxidant capacity. The printing program had no significant effect on the bioactive compounds (except hydroxycinnamic acids), antioxidant capacity and color parameters. A higher starch content improved the strength of the sample but had no effect on the mechanical properties. Smaller particles with a higher starch content improved the stability of the sample. In contrast to the programs, varying the starch content had a significant effect on all the color parameters except the a* values. Eight different sweeteners in two different concentrations were used for the sensory evaluation of the 3D-printed snacks. The variations in sweetener content only affected the sweet and harmonious taste. In summary, this study confirms the great potential of fruit bases for the production of 3D-printed snacks with excellent biological and rheological properties, which can be a step toward personalized food with the addition of sweeteners.

Keywords: strawberry; strawberry tree fruit; *Arbutus unedo* L.; 3D printing; quality

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1. Introduction

Due to growing consumer awareness and increased interest in nutritionally valuable foods, novel plant materials that could improve the nutritional, functional or sensory properties of foods are in high demand [1]. Of particular importance are plants that have not yet been sufficiently researched but show great potential for processing, such as the Mediterranean plant strawberry tree fruit (*Arbutus unedo* L.), which has been shown to have strong biological effects thanks to its antioxidant bioactive compounds [2]. It has already been shown that phytochemicals from *A. unedo* have the ability to slow down the oxidative process by inhibiting the harmful effects of free radicals, thus protecting the body against the development of numerous chronic diseases, such as cardiovascular and neurodegenerative diseases, diabetes and tumor diseases [3]. In addition, these fruits are characterized by an impressive amount of crude fiber, containing between 7.04 and 22.20 g of total dietary fiber per 100 g of fresh ripe strawberry tree fruit [4–7]. From a technological point of view, they could be highlighted as an interesting raw material for the production of functional food.

In response to solving global crises with a focus on sustainability in food production and processing, the fourth industrial revolution or Industry 4.0 was initiated by a combination of information and communication solutions that is now visible in numerous

sectors where, thanks to digitalization, it is significantly changing the way new products are designed or manufactured [8]. The guidelines for Industry 4.0 particularly emphasize the use of non-thermal processing technologies together with additive technologies, with three-dimensional printing (3DP) leading the way in food technology [9]. Three-dimensional printing is a relatively fast process of additive, layer-by-layer production in which computer models enable the production of 3D products in various shapes. This technology is already being explored for functional food design, so its application to various raw materials such as broccoli and carrots [10]; a blend of calcium caseinate powder, starch and medium-chain triglyceride powder [11]; a betaine-enriched oat-based blend [12]; orange by-products [13,14]; a gluten-free cereal blend [15,16]; and a pumpkin blend [17] has been investigated. However, fruit matrices for the 3DP are particularly challenging as it is difficult to produce a functional product that meets the nutritional, biological, textural and sensory requirements [18].

In a previous work, the influence of different amounts (10%, 15% and 20%) and types of starch (wheat vs. corn) and the 3DP programs on the stability of bioactive compounds, antioxidant capacity and textural properties of the strawberry-based 3D-printed product was investigated [19]. A similar study was also conducted with 3D-printed *A. unedo* products to optimize the 3DP technology for this fruit material [2]. Both studies showed a significant influence of the amount and type of starch as well as the 3DP processing parameters on the stability of the bioactive compounds, antioxidant capacity and textural properties. However, due to its chemical and textural properties, *A. unedo* proved to be an excellent raw material that can serve as an excellent basis for the development of various formulations of 3D functional products—in contrast to strawberry, which poses a major challenge due to its high water content.

Based on all the above, the aim of this work was to investigate the possibility of combining two fruit bases, *A. unedo* and strawberry, in the production of 3D-printed functional snacks. The idea is to improve the nutritional, biological, rheological and sensory properties of innovative functional 3D snack products by combining these two fruits. To this end, the influence of different amounts of wheat starch (6%, 8% and 10%) and the type of 3D printing program on the content of bioactive compounds (total phenolic content, total hydroxycinnamic acids, total flavonols and condensed tannins), pigments (monomeric anthocyanins, total carotenoids, chlorophyll A and chlorophyll B), antioxidant capacity (DPPH and FRAP), color and rheological properties of the 3D-printed snacks was investigated. The 3D-printed snack sample characterized by its best bioactive potential and rheological properties was selected for the continuation of the sensory acceptability study, where the addition of eight different sweeteners in two different concentrations was tested.

2. Materials and Methods

2.1. Fruit Material

The samples for the 3D snacks were produced from the fruits of strawberries (*Fragaria ananassa* × Duch., cv. 'Albion') and strawberry trees (*Arbutus unedo* L.). The strawberries were supplied by the company Jagodar-HB d.o.o. (Donja Lomnica, Zagreb County, Croatia). The fruits of *A. unedo* were collected in the southern part of the island of Lošinj (Primorsko-Goranska County, Croatia). After delivery to the laboratory, the fruits were washed, cleaned, dried and stored in plastic bags at −18 °C until the experiments. Wheat starch (Denes Natura Kft., Pécs, Hungary) was used as a hydrocolloid carrier to prepare the fruit mixture for 3D printing. All the sweeteners used for the sensory evaluation were purchased from the local market.

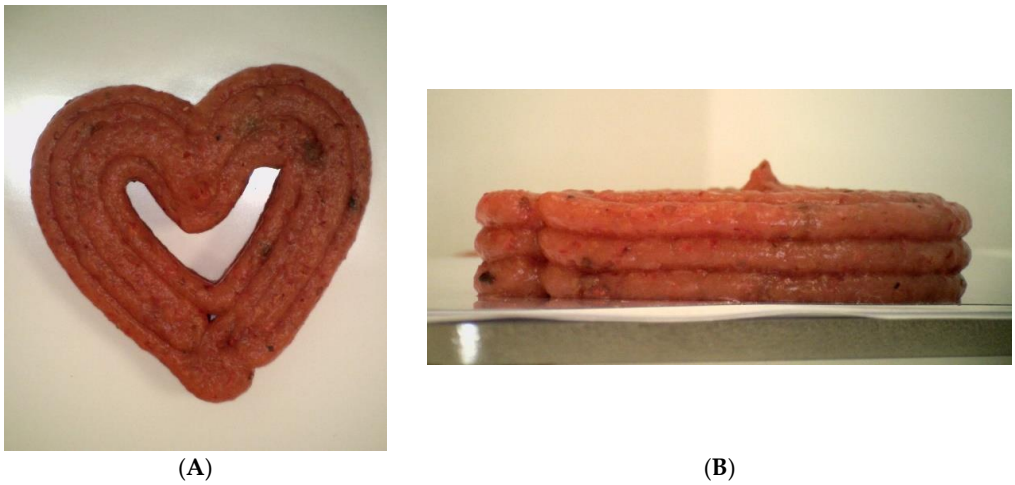
2.2. Preparation of Fruit Material for 3D Printing

Each of the fruits was thawed, homogenized with a Cordys SB-1 blender (MS Industrial Ltd., Hong Kong, China) and then mixed in a 1:1 ratio (*w*/*w*). The wheat starch (6%,

8% and 10% *w*/*w*) was added to the prepared fruit mixture to achieve an appropriate viscous texture. The mixture was then heated to 65 °C with constant stirring on an LLG-uniSTIRRER 7 magnetic stirrer (Lab Logistics Group GmbH, Meckenheim, Germany) to obtain a mixture with suitable viscosity for 3D printing.

2.3. Three-dimensional printing of Fruit Snacks

A Foodini 3D printer (Natural Machines, Barcelona, Spain) with a nozzle diameter of 4 mm was used for the 3DP of the functional snacks. A 3D heart shape (Figure 1A) with three layers (Figure 1B) was designed using the Foodini Creator computer program. The 3DP was performed with two different programs (P1 and P2), differing in the printing speed (8000 mm min^{-1} vs. 14000 mm min^{-1}), printing line thickness (3.5 mm vs. 3.4 mm), mixture flow rate (1.4 vs. 1.65 (dimensionless)) and nozzle height of the first layer (6 mm vs. 4.5 mm). The dimensions of the 3D-printed objects were 53 mm (length) × 51 mm (width) × 12 mm (height).



(**A**) (**B**)

Figure 1. Designed heart shape for 3D printing: top view (**A**) and side view (**B**).

The design of the 3DP experiments is presented in Table 1.

Table 1. Experimental plan for 3DP of fruit snacks.

Sample ID	Starch Content (%)	3D Program
1	0	P1
2	0	P2
3	6	P1
4	6	P2
5	8	P1
6	8	P2
7	10	P1
8	10	P2

2.4. Extraction of Antioxidant Bioactive Compounds

Ultrasound-Assisted Extraction (UAE) was performed to isolate the bioactive compounds from all the analyzed samples using a processor (UP400St Hielscher Ultrasound Technology, Teltow, Germany) equipped with a DN22 titanium sonotrode (546 mm^2) [20]. In brief, 1% formic acid in 80% methanol (*v*/*v*) was used as the extraction solvent. A total of 10 g of the sample was placed in an Erlenmeyer flask and 40 mL of the extraction solvent was poured over it. The UAE was performed at 50% amplitude and 100% pulse for 5 min. The extract was then filtered into a 50 mL flask and made up with the extraction solvent.

The extracts obtained were used in the procedures for the spectrophotometric determination of the total phenols, total flavonoids, hydroxycinnamic acids, flavonols, monomeric anthocyanins and condensed tannins as well as for the analysis of the antioxidant activities using the DPPH and FRAP methods. All the measurements were carried out in duplicate.

2.5. Determination of Polyphenolic Compounds

All the 3D-printed snacks were spectrophotometrically analyzed for the polyphenol content, pigments and antioxidant capacity using a UV–vis spectrophotometer (LLG-uniSPEC 2 spectrophotometer, Buch and Holm, Meckenheim, Germany).

2.5.1. Determination of Total Phenolic Content (TPC)

A total of 400 µL of the extract, 400 µL of the Folin Ciocalteu reagent and 4 mL of a 7.5% sodium carbonate solution were pipetted successively into the test tubes. The reaction mixture was allowed to stand at room temperature for 20 min. The absorbance was measured at 725 nm. The calibration curve was prepared with the solutions of different concentrations of gallic acid (10–250 mg L^{-1}). The equation of the calibration curve used to determine the total phenolic content was:

$$y = 0.0078x - 0.0032,$$

where

y—the absorbance of the sample at 725 nm;

x—the concentration of gallic acid (mg L^{-1}). The TPC results were expressed as mg gallic acid equivalents (GAEs) per 100 g sample [21].

2.5.2. Determination of Total Flavonoids (TF)

Briefly, 0.5 mL of the extract, 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water were added into a glass tube. A blank sample was prepared in the same way, but an extraction solvent was used instead of the extract and the same volume of distilled water (0.1 mL) was added instead of 10% aluminum chloride. The reaction mixture was then allowed to stand for 30 min and the absorbance was measured at 415 nm. Quercetin standard solutions (10–200 mg L^{-1}) were used to generate a calibration curve and the results were expressed as mg quercetin equivalents (QEs) per 100 g of sample[22].

2.5.3. Determination of Total Hydroxycinnamic Acids (HCAs) and Total Flavonols (FLs)

A total of 250 µL of the extract, 250 µL of 1 g L^{-1} HCl in 96% ethanol and 4.55 mL of 2 g L^{-1} HCl were pipetted into a glass tube. The absorbance was then measured at 320 nm for the HCA and at 360 nm for the FL. The HCA content was calculated from the calibration curve obtained from the solutions of different concentrations of chlorogenic acid (10–600 mg L^{-1}), and the results were expressed as the mg chlorogenic acid equivalent (CAE) per 100 g sample. The FL content was calculated from the calibration curve obtained from the solutions of different concentrations of quercetin (10–600 mg L^{-1}), and the results were expressed as the mg quercetin equivalent (QE) per 100 g of sample [23].

2.5.4. Determination of Condensed Tannins (CTs)

In total, 2.5 mL of 1% vanillin, 2.5 mL of 25% H_2SO_4 solution and 1 mL of the extract were pipetted into a glass tube. The mixture was mixed and allowed to stand at room temperature for 10 min. Then, the absorbance was measured at 500 nm. The calibration curve was prepared from the catechin solutions of different concentrations (10–120 mg L^{-1}) and the results were expressed as the mg catechin equivalent (CE) per 100 g of sample [24].

2.6. Determination of Pigments

2.6.1. Determination of Total Monomeric Anthocyanins (ANTs)

The anthocyanin content was determined using the pH differential method [25]. In total, 1 mL of the extract was mixed with 4 mL of potassium chloride buffer pH 1.0 (0.025 M) and also 1 mL of the extract was mixed with 4 mL of sodium acetate buffer pH 4.5 (0.4 M). After 20 min, the absorbance of the reaction mixtures was measured at 520 nm and 700 nm. The ANT content was expressed as the mg cyanidin-3-glucoside equivalent (Cy-3-Glc) per 100 g of sample.

2.6.2. Determination of Total Carotenoids (CARs), Chlorophyll A (CHL A) and Chlorophyll B (CHL B)

The determination of the CAR, CHL A and CHL B was performed using the previously established method [26]. In total, 5 g of the sample was placed in an Erlenmayer flask and 25 mL of the extraction solvent (80% acetone, *v*/*v*) was added. The prepared mixture was then extracted in an ultrasonic bath (DT 514 H SONOREX DIGITEC, 13.5L, 860W, 40 kHz, Bandelin electronic, Berlin, Germany) at 50 °C for 30 min. After the extraction, the samples were filtered into 25 mL volumetric flasks and filled up to the mark with 80% acetone. The absorbance was measured at 470 nm, 646.8 nm and 663.2 nm. The concentrations of the CAR, CHL A and CHL B were calculated according to the formula from the literature [26] and expressed in mg 100 g^{-1} of sample.

2.7. In Vitro Antioxidant Capacity (AOC)

2.7.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Activity Assay

The antiradical activities of the bioactive antioxidants were determined using the DPPH method [27]. In brief, 1.5 mL of the extract and 3 mL of 0.5 mM DPPH solution were pipetted into a test tube and kept at room temperature in the dark for 20 min. The absorbance was then measured at 517 nm. A calibration curve was constructed from different concentrations of Trolox solutions (10–150 µM) and the results were expressed as the µmol Trolox equivalent (TE) per 100 g of sample.

2.7.2. FRAP (Ferric-Reducing Antioxidant Power) Assay

The FRAP method was performed according to the literature protocol [28]. A total of 600 µL of extract and 4500 µL of FRAP reagent (prepared from acetate buffer (0.3 M), 2.5 mL of TPTZ reagent (2,4,6-tris-2-pyridyl-s-triazine; 10 mM) and 2.5 mL of iron (III) chloride (20 mM) in a 10:1:1 ratio) were pipetted into glass tubes, mixed and thermostatted at 37 °C for 10 min. The absorbance was then measured at 593 nm. A calibration curve was constructed from different concentrations of Trolox solutions (10–150 µM) and the results were expressed as the mmol Trolox equivalent (TE) per 100 g of sample.

2.8. Determination of Rheological Properties of 3DP Snacks

2.8.1. Texture Analysis

An evaluation of the rheological properties was conducted using the TA.HD plus Texture Analyser (Stable Micro System, Godalming, United Kingdom), applying two tests: the forward extrusion test and penetration test.

Forward Extrusion Test

The testing was conducted using an extrusion set (cylindrical sample container and a piston disc). The base disk is set at the bottom of the sample container with a central opening of 3 mm in diameter. The parameters are as follows: test speed, 1 mms^{-1}; outgoing speed, 10 mms^{-1}; and extrusion distance, 20 mm with trigger force 10 g.

The testing measures the compression force required for the piston to extrude the 3D-printed sample through the opening of the disk. Each sample batch was tested three times, and all the tests were performed at room temperature. The results are expressed as the mean extrusion force (F) (firmness) and work (W) required for extruding the samples.

Penetration Test

A spherical probe with a diameter of 4 mm was utilized during the test. The parameters were set to the following operating speeds: test speed, 0,5 mms^{-1}; outgoing speed, 10 mms^{-1}; and deformation distance, 6 mm with trigger force 2 g. Three parallel measurements were conducted at room temperature, and the results are presented as the maximum force (F_P) (hardness) and work (W_P).

2.8.2. Dimension Measurements

The dimensions of the samples are expressed in terms of the geometric parameters (length × width × height). The differences in the measured values were determined using a digital caliper with an accuracy of 0.01 mm. The minimal differences in the sample dimensions using different programs without starch content are program 1: 58 mm × 58 mm × 9.4 mm and program 2: 62 mm × 59 mm × 9 mm (length × width × height).

2.8.3. Particle Size Distribution

The particle size distribution of the samples in the Hydro 2000S system was determined using the laser diffraction method (Malvern Masterseizer 2000, Malvern Instruments Ltd., Worcestershire, UK). Within the cylinder, 10 g of dissolved sample was dispersed in 30 mL of distilled water, ensuring the homogeneity of the solution for the measurement and achieving a minimal degree of obscuration. The particle diameters were expressed over D (3.2); D (4.3); d (0.1); d (0.5); and d (0.9).

2.9. Determination of Instrumental Color

Color measurements were carried out for each trial using a Konica Minolta Spectrophotometer (CM-700d, Konica Minolta, Tokyo, Japan), which featured a D65 10° standard observer light source and a target mask CM-A183 with an 8 mm aperture, and a glass-covered cone. In each trial, the 3D-printed sample was compressed under the target mask of the spectrophotometer and the colorimetric parameters (L*, a* and b*) were measured. The color change (ΔE), chroma (C) and hue (H*) were calculated using the provided formulas:

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$H^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

where all ΔL^{*2}, Δa^{*2} and Δb^{*2} were calculated on the differences between the control and 3D-printed samples. All the measurements were conducted in triplicate.

2.10. Sensory Evaluation of 3D-Printed Snacks

All the 3D-printed snacks were sensory-evaluated using the Quantitative Descriptive Analysis (QDA) method [29]. Based on the results obtained in the determination of the

stability of the bioactive antioxidants, pigments and rheological properties by 3DP technology, a sample characterized by the best results was selected and a sensory evaluation was carried out with it. For this purpose, the addition of 8 different sweeteners was tested in 2 concentration levels (Table 2), which were determined in a preliminary sensory evaluation.

Table 2. Experimental design for the sensory evaluation of 3D-printed snacks.

Simpe ID	Sweeteners	Sweetener Content (%)
A	Control sample	Without sweetener
B1	Saccharose	6.1
B2	Saccharose	9.1
C1	Fructose	7.1
C2	Fructose	8.9
D1	Birch sugar (xylitol)	5.6
D2	Birch sugar (xylitol)	8.5
E1	Erythritol	3.2
E2	Erythritol	4.7
F1	Maple syrup	5.5
F2	Maple syrup	8.7
G1	Date syrup	5.2
G2	Date syrup	7.1
H1	Agave syrup	6.7
H2	Agave syrup	10.2
I1	Stevia and erythritol	2.5
I2	Stevia and erythritol	3.9

A team of 18 sensory panelists rated the sensory attributes using a line intensity scale, with the scores assigned on a scale of 0–7 to indicate the relative intensity of each attribute, with 0 indicating the complete absence of the sensory attribute and 7 indicating a very pronounced attribute. The samples were served in coded Petri dishes. A total of 12 sensory descriptors were evaluated, which included the following attributes: (i) color—intensity of orange color; (ii) odor—strawberry odor, off-odor; (iii) aroma—strawberry flavor, strawberry tree fruit flavor, off-flavor; (iv) taste—sweet taste, sour taste, harmony taste, off-taste; and (v) texture—homogeneity, glossy appearance.

2.11. Statistical Analysis

A multivariate analysis of variance (MANOVA) with Tukey's HSD was performed to simultaneously test the relationships between the dependent and categorical variables. The significance for all the tests was $p \leq 0.05$. All the results were analyzed using Statistica software (v. 14.1) [30]. The results of the rheological properties obtained were analyzed using Statistica 12 software. The statistical significance of the influence of the process parameters on the parameters of the descriptive statistics was determined by conducting a MANOVA. The results were considered statistically significant if $p \leq 0.05$ (95% significance level).

3. Results and Discussion

3.1. Characterization of Polyphenolic Compounds, Pigments and Antioxidant Capacity in 3D-Printed Snacks

Table 3 shows the results for the influence of four different proportions of wheat starch, namely, 0, 6, 8 and 10%, on the content of the polyphenolic compounds in the 3D samples. Considering all the phenolic compounds determined, condensed tannins were the most abundant (150.56 ± 3.31 mg 100 g^{-1}), followed by hydroxycinnamic acids (74.79 ± 2.03 mg 100 g^{-1}), flavonols (49.84 ± 1.31 mg 100 g^{-1}) and total flavonoids (9.86 ± 0.16 mg 100 g^{-1}). These results are consistent with the results of previous studies on the bioactive composition of strawberries [31].

When considering the influence of starch content, it can be seen that the content of total phenolic compounds, hydroxycinnamic acids and flavonols decreases with increasing starch content (0–10%). These results are consistent with previous reports [2], in which an increase in starch content from 4 to 8% led to a decrease in the content of total phenolic compounds. The results obtained indicate a negative effect of increasing starch content on the content of bioactive compounds, which is rather expected due to the higher content of non-phenolic compounds, i.e., starch. However, no such correlation was observed for the total flavonoids and condensed tannins. In the case of condensed tannins, it was found that there was no difference in their concentration when different proportions of starch were added. An unusual trend was observed for the content of total flavonoids, where the highest concentrations were recorded in samples without added starch (0%) and in samples with 8% starch. However, when the starch content was further increased to 10%, the TF content had the lowest value. The reason for this could be interactions within the matrix of the mixture, as well as interference in the spectrophotometric assay for the total flavonoids caused by non-flavonoid compounds. Namely, starch can react with other components of the mixture, such as some bioactive compounds, which can lead to an increase in their availability. In addition, the presence of starch can affect the solubility of bioactive compounds, which also affects their increase in concentration [32].

Table 3. Relationship between 3DP parameters and the content of bioactive compounds in the 3D-printed snacks.

Variable	n	TPC	HCAs	FLs	TFs	CTs
Starch level		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
0%	4	429.50 ± 5.87 ^{a}	84.99 ± 0.73 ^{a}	55.41 ± 0.54 ^{a}	10.66 ± 0.22 ^{a}	171.33 ± 1.66 ^{a}
6%	4	392.96 ± 5.87 ^{b}	78.16 ± 0.73 ^{b}	52.55 ± 0.54 ^{b}	9.65 ± 0.22 ^{b}	141.87 ± 1.66 ^{b}
8%	4	353.60 ± 5.87 ^{c}	71.11 ± 0.73 ^{c}	49.25 ± 0.54 ^{c}	9.90 ± 0.22 ^{a,b}	141.41 ± 1.66 ^{b}
10%	4	344.21 ± 5.87 ^{c}	64.89 ± 0.73 ^{d}	42.16 ± 0.54 ^{d}	9.22 ± 0.22 ^{b}	147.59 ± 1.66 ^{b}
3DP Program		$p = 0.43$ ‡	$p \leq 0.01$ †	$p = 0.05$ ‡	$p = 0.17$ ‡	$p = 0.34$ ‡
Program 1	8	382.48 ± 4.15 ^{a}	76.45 ± 0.52 ^{a}	50.47 ± 0.38 ^{a}	9.69 ± 0.15 ^{a}	149.71 ± 1.17 ^{a}
Program 2	8	377.65 ± 4.15 ^{a}	73.12 ± 0.52 ^{b}	49.22 ± 0.38 ^{a}	10.02 ± 0.15 ^{a}	151.39 ± 1.17 ^{a}
Dataset average	16	380.07 ± 9.09	74.79 ± 2.03	49.84 ± 1.31	9.86 ± 0.16	150.56 ± 3.31

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; and ‡ not significant factor in multifactor analysis. TPC—total phenolic content (mg GAE 100 g^{-1}); HCAs—hydroxycinnamic acids (mg CAE 100 g^{-1}); FLs—flavonols (mg QE 100 g^{-1}); TFs—total flavonoids (mg QE 100 g^{-1}); and CTs—condensed tannins (mg CE 100 g^{-1}).

The 3D printing of the samples was carried out with two different programs (P1 and P2). These programs differed in terms of the 3DP speed, the print line thickness, the flow rate of the mixture and the nozzle height of the first layer. The only significant influence of the 3DP program was observed for the HCA content as a lower amount of HCA was found in the samples printed with program 2 compared to program 1. These two programs may have a different influence on the bioactive compound content as the 3DP process parameters, such as the nozzle movement speed, pressure and flow rate, differ between these two programs [33]. Program 2 could lead to better preservation of the bioactive compounds, as it has a higher flow rate of the mixture and a higher printing speed compared to program 1. As a result, the processing time is shorter, which means that the bioactive compounds are exposed for less time to conditions that can degrade them. The influence of printing process parameters, i.e., printing programs, on the structural quality of 3D-printed products has already been investigated, but there is not yet enough data on their influence on the stability of bioactive compounds in a 3D-printed product.

The influence of the addition of starch and the 3D programs was also observed on the influence of the stability of the pigments and the antioxidant capacity in the 3D samples (Table 4). The highest concentrations of anthocyanins and chlorophylls were determined in the samples without added starch, while the starch content of 6% had the most favorable influence on the stability of the carotenoids. Furthermore, when the starch content was increased by 6–10%, no significant differences were found in the content of the anthocyanins, chlorophylls A and chlorophylls B in the 3DP samples. A different trend was observed for the carotenoids. Increasing the starch content had a negative effect on the stability of the carotenoids. Increasing the starch content from 0 to 10% had no significant effect on the DPPH values; while higher FRAP values were obtained for the 3D samples with 0–8% added starch, the samples with 10% had the lowest values.

Bebek Markovinović et al. [19] also observed a decrease in the content of carotenoids and antioxidant activity values determined by the FRAP method when the starch content in the 3DP strawberry-based products was increased to over 15%. The reason for the initial deviations from this trend in the carotenoid and FRAP values may be that this study used a strawberry and strawberry tree fruit-based blend, which has different rheological properties than a strawberry blend. Ultimately, the overall result of a decrease in the bioactive compound content was to be expected, as a printing mixture with higher starch content has a lower proportion of the fruit component, which is the source of the bioactive compounds. There is also the possibility of interactions between bioactive compounds and starch in 3DP products with higher starch content, which may result in lower levels of the bioactive compound. It is hypothesized that phenolic hydroxyl groups may interact with starch by forming non-covalent bonds, such as hydrogen bonds, and that electrostatic and ionic interactions may occur, leading to the formation of complex compounds. However, the addition of starch, which triggers such chemical reactions, can lead to an improvement in the nutritional and physico-chemical properties of the product and the digestibility of the starch [34].

Table 4. Relationship between 3DP parameters and the content of pigments and antioxidant capacity in the 3D-printed snacks.

Variable	n	ANT	CAR	CHLA	CHLB	DPPH	FRAP
Starch level		$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p = 0.07$ ‡	$p = 0.03$ †
0%	4	9.65 ± 0.16 ^{a}	0.58 ± 0.002 ^{b}	0.24 ± 0.01 ^{a}	0.40 ± 0.01 ^{a}	2.90 ± 0.07 ^{a}	290.38 ± 0.35 ^{a,b}
6%	4	7.75 ± 0.16 ^{b}	0.62 ± 0.002 ^{a}	0.11 ± 0.01 ^{b}	0.18 ± 0.01 ^{b}	3.00 ± 0.07 ^{a}	289.95 ± 0.35 ^{a,b}
8%	4	7.73 ± 0.16 ^{b}	0.52 ± 0.002 ^{c}	0.11 ± 0.01 ^{b}	0.17 ± 0.01 ^{b}	2.74 ± 0.07 ^{a}	290.82 ± 0.35 ^{a}
10%	4	8.09 ± 0.16 ^{b}	0.48 ± 0.002 ^{d}	0.12 ± 0.01 ^{b}	0.19 ± 0.01 ^{b}	2.73 ± 0.07 ^{a}	288.94 ± 0.35 ^{b}

3DP Program		$p = 0.64$ ‡	$p = 0.11$ ‡	$p = 0.12$ ‡	$p = 0.19$ ‡	$p = 0.15$ ‡	$p = 0.73$ ‡
Program 1	8	8.35 ± 0.11 [a]	0.55 ± 0.001 [a]	0.15 ± 0.004 [a]	0.25 ± 0.01 [a]	2.90 ± 0.05 [a]	289.96 ± 0.25 [a]
Program 2	8	8.27 ± 0.11 [a]	0.55 ± 0.001 [a]	0.14 ± 0.004 [a]	0.23 ± 0.01 [a]	2.78 ± 0.05 [a]	290.08 ± 0.25 [a]
Dataset average	16	8.31 ± 0.22	0.55 ± 0.01	0.14 ± 0.01	0.24 ± 0.03	2.84 ± 0.05	290.02 ± 0.26

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; and ‡ not significant factor in multifactor analysis. ANT—monomeric anthocyanin (mg Cy-3-Glc 100 g^{-1}); CAR—total carotenoid (mg 100 g^{-1}); CHL A—total chlorophyll A (mg 100 g^{-1}); CHL B—total chlorophyll B (mg 100 g^{-1}); DPPH assay (μmol TE 100 g^{-1}); and FRAP assay (mmol TE 100 g^{-1}).

The 3DP programs had no effect on the stability of the pigments and on the antioxidant capacity. The reason for this could be that the process parameters of these two programs are not sufficiently different to cause statistically significant changes in the above-mentioned parameters. It can also be assumed that some other bioactive compounds, which were not considered in this study, contribute to the antioxidant capacity, and the differences in the 3DP process parameters by the two programs had no effect on them.

Table 5 shows the correlations between the investigated bioactive compounds and the antioxidant capacity (DPPH and FRAP). The TPC correlates positively with the content of all the bioactive compounds, most strongly with the HCA and least with the TF. They also correlate with the DPPH. The other bioactive compounds examined also show a positive correlation with similar chemical structures. When looking at the antioxidant capacity, the DPPH correlates significantly with the TPC, HCA, FL and CAR, while there are no significant correlations with the other bioactive compounds. On the other hand, the FRAP does not correlate significantly with any of the bioactive compounds or DPPH. This suggests that the FRAP values are related to some other bioactive compounds that were not studied. Andrés et al. [35] showed that the FRAP capacity in a smoothie of orange juice, papaya juice, melon juice, carrot puree and skim milk correlated significantly with ascorbic acid and polyphenolic compounds but not with carotenoids.

Table 5. Mutual correlations of bioactive compounds and antioxidant capacity.

	TPC	**HCA**	**FL**	**TF**	**ANT**	**CT**	**CAR**	**CHL A**	**CHL B**	**DPPH**	**FRAP**
TPC		0.92 *	0.84 *	0.56 *	0.71 *	0.69 *	0.75 *	0.77 *	0.77 *	0.51 *	0.32
HCA	0.92 *		0.91 *	0.66 *	0.63 *	0.61 *	0.78 *	0.69 *	0.69 *	0.50 *	0.41
FL	0.84 *	0.91 *		0.63 *	0.44	0.42	0.86 *	0.54 *	0.55 *	0.55 *	0.44
TF	0.56 *	0.66 *	0.63 *		0.63 *	0.64 *	0.43	0.64 *	0.65 *	0.13	0.28
ANT	0.71 *	0.63 *	0.44	0.63 *		0.90 *	0.22	0.93 *	0.92 *	0.25	0.09
CT	0.69 *	0.61 *	0.42	0.64 *	0.90 *		0.19	0.81 *	0.80 *	0.07	0.05
CAR	0.75 *	0.78 *	0.86 *	0.43	0.22	0.19		0.33	0.35	0.68 *	0.25
CHL A	0.77 *	0.69 *	0.54 *	0.64 *	0.93 *	0.81 *	0.33		1.00 *	0.26	0.20
CHL B	0.77 *	0.69 *	0.55 *	0.65 *	0.92 *	0.80 *	0.35	1.00 *		0.27	0.20
DPPH	0.51 *	0.50 *	0.55 *	0.13	0.25	0.07	0.68 *	0.26	0.27		−0.03
FRAP	0.32	0.41	0.44	0.28	0.09	0.05	0.25	0.20	0.20	−0.03	

* Correlations are significant at $p < 0.05$. TPC—total phenolic content (mg GAE 100 g^{-1}); HCA—hydroxycinnamic acid (mg CAE 100 g^{-1}); FL—flavonol (mg QE 100 g^{-1}); TF—total flavonoid (mg QE 100 g^{-1}); ANT—monomeric anthocyanin (mg Cy-3-Glc 100 g^{-1}); CT—condensed tannin (mg CE 100 g^{-1}); CAR—total carotenoid (mg 100 g^{-1}); CHL A—total chlorophyll a (mg 100 g^{-1}); CHL B—total chlorophyll b (mg 100 g^{-1}); DPPH assay (μmol TE 100 g^{-1}); and FRAP assay (mmol TE 100 g^{-1}).

3.2. Characterization of Rheological Properties in 3D-Printed Snacks

3.2.1. Texture Analysis (Forward Extrusion and Penetration Test)

The results presented in Table 6 show that by applying program 2 and increasing the starch content in the printing mixture from 6% to 8%, the extrusion force does not change significantly. However, the total flow resistance through the cylinder increases considerably during the 3DP of the mixture with a starch content of 10%. The substantially higher flow resistance observed during the extrusion of the samples with the maximum starch content results from increased sample firmness. This is consistent with the findings of the studies conducted by Feng et al. [36], Dong et al. [37] and Yang et al. [38,39], who found that the starch content in the printing mixture is proportional to the sample firmness. By applying program 1, which has a lower printing speed, the extrusion force decreases as the starch content in the mixture increases. A higher starch content results in a more consistent sample structure, increasing the flow index.

Table 6. Influence of 3DP processing parameters on textural properties of 3D-printed snacks.

Sample	3DP Program	Starch Content (%)	F (N)	W (Nmm)	F_P (N)	W_P (Nmm)
1	1	0	16.89	168.76	0.01	0.01
2	2	0	7.58	75.79	0.01	0.01
3	1	6	454.73	4545.01	0.07	0.06
4	2	6	54.08	540.43	0.05	0.05
5	1	8	112.82	1127.64	0.05	0.06
6	2	8	66.77	667.37	0.05	0.06
7	1	10	75.69	756.59	0.07	0.07
8	2	10	1003.08	10,025.82	0.06	0.04

F—mean extrusion force (firmness) (N); W—work (Nmm); F_P—maximum force (hardness); and Wp—work (Nmm).

Consequently, the sample's fluidity increases and the total resistance during extrusion through the cylinder container decreases. One possible explanation for these results, whose statistical significance is shown in Table 7, is the significant influence of the apparent viscosity value indicating the pseudoplastic character of the extruded samples. Samples 3D printed with program 1 were expected to exhibit increased resistance with increasing starch content, but the predicted effect did not materialize due to changes in the rheological parameters. Accordingly, no significant deviations from the values of the extrusion force change were observed in the extrusion work values as the printing speed and starch content in the samples changed. The aforementioned are in accordance with the findings of the research by Bebek Markovinović et al. [2], which demonstrated that the force and work required for the extrusion of the mixture significantly depend on the starch content—specifically, increasing the starch content led to increased values of work and extrusion force. The extrusion work of samples, like the extrusion force, can be considered a relevant factor in determining the textural properties of samples, with the note that, in this case, the path (the distance travelled by the piston through the cylinder) is the main factor defining the total work or the area under the curve and, consequently, the maximum extrusion force, defining its initial and final firmness.

Table 7. Influence of 3DP process parameters on texture, particle diameters and dimension in 3D-printed snacks expressed by *p*-value *.

Parameter	3DP Program	Starch Content (%)
F	0.725588	0.695556
W	0.725577	0.695550
F_P	0.178047	0.158750
W_P	0.236516	0.876531
D [3.2]	0.949818	0.003742 *
D [4.3]	0.660607	0.024213 *
d [0.1]	0.730548	0.029678 *
d [0.5]	0.616439	0.004638 *
d [0.9]	0.603835	0.037802 *
Length	0.147469	0.754530
Width	0.659869	0.795063
Height	0.122649	0.334880

* Results are statistically significant at $p \leq 0.05$.

According to the results obtained from the forward extrusion test, the penetration test method did not affect significant deviations in the characterization of the mechanical properties. Because the test is performed with smaller probe contact areas where the sensitivity of force (F_P) (hardness) sensing is more pronounced and stresses are significantly lower, differences in the sample strength are even less pronounced, considering the starch content.

3.2.2. Dimension Measurements of 3D-Printed Snacks

An increase in the starch content and the application of different printing programs did not result in statistically significant deviations in the dimensions of the printed samples. Table 8 shows the influence of the 3DP program on the dimensions of the 3DP fruit snack. The samples exhibit stability in all three observed geometry directions.

Table 8. Influence of 3DP processing parameters on the dimension of 3D-printed snacks.

Sample	3DP Program	Starch Content (%)	Length (mm)	Width (mm)	Height (mm)
1	1	0	58.88 ± 0.11	58.69 ± 0.22	9.40± 0.09
2	2	0	62.01± 0.17	59.94 ± 0.19	9.39± 0.12
3	1	6	54.85 ± 0.21	53.33 ± 0.53	12.55 ± 0.14
4	2	6	55.70 ± 0.15	53.57 ± 0.31	12.12 ± 0.15
5	1	8	55.06 ± 0.07	51.57 ± 0.23	13.47 ± 0.52
6	2	8	55.60 ± 0.32	53.99 ± 0.44	12.83 ± 0.32
7	1	10	53.73 ± 0.56	54.18 ± 0.09	13.50 ± 0.17
8	2	10	55.98 ± 0.37	53.09 ± 0.21	11.98 ± 0.26

The results are presented as an average value of triplicate measurements ± standard deviation.

3.2.3. Particle Size Distribution

An increase in the starch content has shown an influence on all the parameters of the particle size distribution. The translation of the medians toward the interval of smaller

particle distribution is in correlation with a reduction in the diameter in the 90% of the total particle count (D 0.9). In accordance, the statistical significance can be attributed to the overall change in the values describing the surface area ratio of smaller diameter particles (D 3.2) compared to the volume ratio of larger diameter particles (D 4.3). From previous research by Bebek Markovinović et al. [2], it is evident that smaller diameter particles contribute to more excellent stability and durability of printed samples. Table 9 shows the influence of the printing process parameters on the particle diameters. The significance analysis confirmed that the starch content was the most important factor influencing the particle size distribution of the 3D-printed snacks.

Table 9. Influence of 3DP processing parameters on particle diameters in 3D-printed snacks.

Sample	3DP Program	Starch Content (%)	D [3.2] (μm)	D [4.3] (μm)	d [0.1] (μm)	d [0.5] (μm)	d [0.9] (μm)
1	1	0	120.24	429.11	62.18	297.58	1012.46
2	2	0	117.87	418.75	61.57	290.21	985.83
3	1	6	54.63	234.50	24.05	92.45	682.84
4	2	6	54.02	216.56	24.23	88.52	622.87
5	1	8	40.07	167.06	20.05	59.94	501.01
6	2	8	39.41	174.85	19.28	59.94	530.68
7	1	10	35.30	145.02	17.82	53.20	437.02
8	2	10	36.72	143.65	19.13	54.41	419.94

D (3.2)—surface weighted mean diameter (Sauter mean diameter); D (4.3)—volume weighted mean diameter (De Brouckere mean diameter); d (0.1)—10% of the volume distribution is below the observed diameter; d (0.5)—median diameter, 50% of the volume distribution is below and 50% is above the observed diameter; and d (0.9)—90% of the volume distribution is below the observed diameter.

Comparing the control sample with the samples containing 6% and 10% starch, an increase in the volume fraction (relative frequencies) of the particles in the distribution interval of 17.38–120.22 μm is evident (Figure 2). The addition of starch contributed to the uniformity of the particle distribution curves compared to the control sample, which is characterized by a bimodal distribution. This indicates a greater stability of the samples with added starch in all ratios.

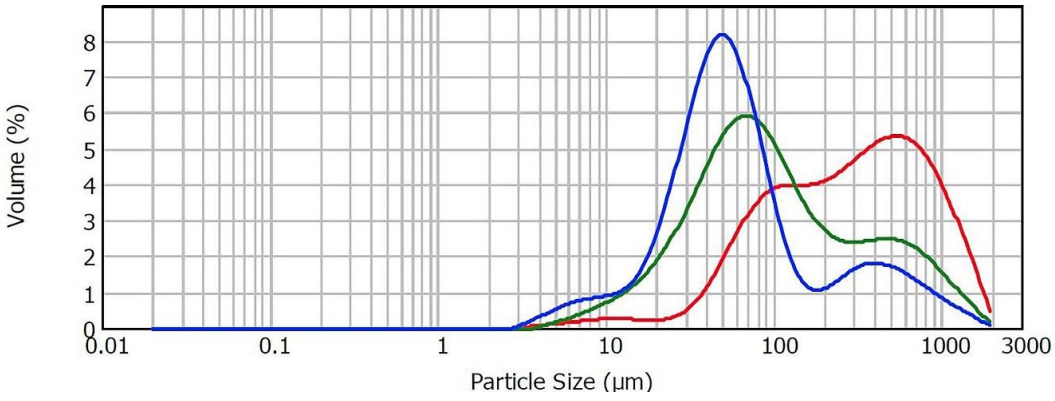


Figure 2. Volume size distribution of control sample (red), sample 4 with 6% (green) and sample 8 with 10% of starch (blue).

3.3. Characterization of Color Properties in 3D-Printed Snacks

Color plays an important role in the consumer's perception of a particular product [40]. Table 10 shows the CIELab color parameters for the 3D-printed samples as a function of the starch content (0, 6, 8 and 10%) and printing program (P1 and P2).

The starch content significantly influenced the lightness value (L*) of the 3D-printed samples. The samples without added starch had the lowest L* value, i.e., they were the brightest. The highest L* value was recorded for the samples with a starch content of 6%, whereupon a further increase in the starch content to 8% led to a decrease. When the starch content was further increased to 10%, no significant difference was observed in the L* parameter. Considering that there was a significant difference in the L* values between the samples without and with the addition of starch, this darkening of the samples with the addition of starch could be due to the short-term exposure to an elevated temperature by heating the fruit mixture to achieve the appropriate consistency for 3DP. During this brief heating, it is possible that a slight non-enzymatic degradation of the mixture by Maillard reactions occurred due to the elevated temperature [41].

The change in starch content (0–10%) in the 3D-printed samples had no significant effect on the color parameter a*. Because the parameter a* refers to the red color, the results obtained indicate that the addition of starch has no significant effect on the expression of the red color, which is a highly desirable sensory characteristic. In contrast, a significant influence of the starch content on the color parameter b* was found. The samples without added starch had the lowest b* value, which was close to the value of the samples with 10% added starch, i.e., the samples without starch and with 10% added starch did not differ significantly. The highest b* value was found in the samples with a starch content of 6%. Increasing the starch content from 6% to 8% decreased the b* value significantly but not less than the samples without starch and with the addition of 10% starch. In our previous studies on 3D-printed strawberry products, the a* and b* parameters ranged from 22.54 to 27.52 and 7.50 to 12.98, respectively [19], while they ranged from 19.76 to 32.78 and 18.24 to 27.90 for 3D-printed strawberry products [2]. As expected, our results of the mean a* and b* values of strawberry and strawberry tree fruit 3D-printed snacks were approximately at the mean values of the a* and b* color parameters of the strawberry and strawberry tree fruit 3D-printed snacks examined separately in the two previous studies (23.40 ± 0.25 and 15.49 ± 0.29, respectively).

The C* and H* values follow almost the same trend with the change in starch content. The addition of 6% starch led to a statistically significant increase in the C* and H* values, while a further addition of 8% starch caused a significant decrease. A further 10% increase in the starch content resulted in a significant decrease in the C* value, while there was no effect on the H* value. The C* and H values of 3DP strawberry snacks from previous studies ranged from 23.85 to 29.93 and 18.33 to 25.96, respectively [19], while the values of 3DP strawberry tree fruit snacks ranged from 27.09 to 41.89 and 35.82 to 44.63, respectively [2]. Like the a* and b* values, the mean C* and H* values of the 3DP strawberry and strawberry tree fruit snacks (28.07 ± 0.35 and 33.46 ± 0.31, respectively) were similar to the mean values of the C* and H* color parameters of the 3DP strawberry and strawberry tree fruit snacks examined separately in the two previous studies.

The color change (ΔE) in the 3D-printed products ranged from 0.00 ± 0.41 to 6.17 ± 0.41 depending on the added starch content. The 3D product with a starch content of 6% showed the highest color change, while further increasing the starch content by 8% caused a significant decrease in the ΔE. No significant difference in the ΔE value was observed when the starch content was further increased by 10%. When we compare the L* and ΔE values, we find that they follow the same trend. In both cases, the darkest samples were those with the addition of 6% starch. It is possible that here the color of the fruit pulp was degraded by the short-term heating due to Maillard reactions [41]. Except for the samples with 6% added starch, all the others had a ΔE value of less than 6, which defines these differences as appreciable differences. Only the addition of 6% starch causes ΔE values above 6, which defines them as large differences [42]. The average ΔE value was 3.26 ± 0.60, and in general, the color differences in the 3D-printed strawberry and strawberry tree fruit products were acceptable.

Table 10. Relationship between 3DP parameters and the color parameters in the 3D-printed snacks.

Variable	n	L*	a*	b*	C*	H*	ΔE
Starch level		$p \leq 0.01$ †	$p = 0.05$ ‡	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †	$p \leq 0.01$ †
0%	4	41.43 ± 0.19 [c]	22.92 ± 0.39 [a]	14.44 ± 0.30 [b]	27.09 ± 0.46 [b]	32.23 ± 0.37 [b]	0.00 ± 0.41 [c]
6%	4	46.74 ± 0.19 [a]	24.48 ± 0.39 [a]	16.98 ± 0.30 [a]	29.80 ± 0.46 [a]	34.74 ± 0.37 [a]	6.17 ± 0.41 [a]
8%	4	44.79 ± 0.19 [b]	23.45 ± 0.39 [a]	15.70 ± 0.30 [a,b]	28.22 ± 0.46 [a,b]	33.80 ± 0.37 [a,b]	3.89 ± 0.41 [b]
10%	4	44.19 ± 0.19 [b]	22.77 ± 0.39 [a]	14.82 ± 0.30 [b]	27.17 ± 0.46 [b]	33.06 ± 0.37 [a,b]	3.00 ± 0.41 [b]
3DP Program		$p = 0.29$ ‡	$p = 0.71$ ‡	$p = 0.35$ ‡	$p = 0.93$ ‡	$p = 0.11$ ‡	$p = 0.15$ ‡
Program 1	8	44.18 ± 0.13 [a]	23.48 ± 0.27 [a]	15.34 ± 0.21 [a]	28.05 ± 0.32 [a]	33.13 ± 0.26 [a]	3.59 ± 0.29 [a]
Program 2	8	44.39 ± 0.13 [a]	23.33 ± 0.27 [a]	15.64 ± 0.21 [a]	28.09 ± 0.32 [a]	33.79 ± 0.26 [a]	2.94 ± 0.29 [a]
Dataset average	16	44.29 ± 0.50	23.40 ± 0.25	15.49 ± 0.29	28.07 ± 0.35	33.46 ± 0.31	3.26 ± 0.60

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; and ‡ not significant factor in multifactor analysis. L*—lightness; a*—redness; b*—yellowness; C*—chroma; H*—hue; and ΔE—color change.

The 3DP programs had no statistically significant effect on any color parameter (L*, a*, b*, C*, H and ΔE). Because the choice of program had no statistically significant influence on the content of the bioactive compounds (with the exception of HCA), including the content of anthocyanins, it is to be expected that no significant changes in the color parameters were observed under the influence of the different program parameters.

3.4. Characterization of the Sensory Properties of 3D-Printed Snacks

Sample 3, which contained 6% starch and was prepared with program 1, was selected for the sensory evaluation as it proved to be the best in terms of the stability of the bioactive compounds and antioxidant capacity. The 3D-printed snacks prepared with eight different sweeteners in two different concentrations (16 samples) and the control samples (without sweeteners) were evaluated using twelve sensory descriptors (Table 11). In the first part of the table, all the samples are compared with regard to the type and amount of added sweetener, while in the second part of the table, the samples are grouped according to the type of added sweetener.

The type of sweetener added and the level of concentration (lower vs. higher concentration) had no significant influence on the sensory properties with the exception of sweetness and harmonious taste. Therefore, no specific correlation was found between the color, taste and odor characteristics compared to the control samples. Significant differences were only found with regard to sweetness and harmonious taste. The perception of harmonious taste implies the relationship between sweetness and acidity [43] and represents a sensory descriptor that encompasses the general preference for the food product [44]. The samples C2, D2, E2, F2, H2 and I2 with a higher content of sweeteners showed a higher sweetness than their counterparts with a lower addition of sweeteners C1, D1, E1, F1, H1 and I1. As expected, the control sample had the lowest intensity of sweetness and differed significantly from the sweetened samples in almost all the comparisons. In addition, the greatest differences in harmonious taste were observed between samples D1 and D2 with the addition of birch sugar, E1 and E2 with the addition of erythritol and H1 and H2 with the addition of agave syrup. The aforementioned samples with a higher sweetener content had a higher harmonicity than their parallel samples with a lower sweetener content.

In the second part of Table 11, the samples are observed according to the type of added sweetener, without observation at the two concentration levels in which they were

added. Statistically significant differences between the samples were recorded in the sensory descriptors, such as strawberry odor, sweet taste, harmony taste and glossy appearance.

Sample E with the addition of erythritol had the least strawberry odor, while samples B, F and H had the strongest intensity of strawberry odor. The addition of saccharose, maple syrup and agave syrup in the mentioned samples had a certain positive effect on the strawberry odor.

Table 11. Sensory comparison results of 3D-printed snacks with the addition of different sweeteners in two different concentrations.

Variable	n	Intensity of Orange Color	Strawberry Odor	Off-Odor	Strawberry Flavor	Strawberry Tree Fruit Flavor	Off-Flavor	Sweet Taste	Sour Taste	Harmony Taste	Off-Taste	Homogeneity	Glossy Appearance
Sample		$p = 0.81$ ‡	$p = 0.76$ ‡	$p = 0.99$ ‡	$p = 0.07$ ‡	$p = 0.99$ ‡	$p = 0.41$ ‡	$p \leq 0.01$ †	$p = 0.14$ ‡	$p \leq 0.01$ †	$p = 0.67$ ‡	$p = 0.71$ ‡	$p = 0.09$ ‡
A	13	5.85 ± 0.32 [a]	5.38 ± 0.37 [a]	1.23 ± 0.19 [a]	4.46 ± 0.38 [a]	4.15 ± 0.45 [a]	1.15 ± 0.18 [a]	2.46 ± 0.36 [d]	4.69 ± 0.38 [a]	3.69 ± 0.34 [a,b]	1.15 ± 0.18 [a]	5.15 ± 0.41 [a]	5.77 ± 0.35 [a]
B1	13	5.92 ± 0.32 [a]	5.08 ± 0.37 [a]	1.08 ± 0.19 [a]	4.92 ± 0.38 [a]	3.54 ± 0.45 [a]	1.08 ± 0.18 [a]	4.46 ± 0.36 [a,b]	3.23 ± 0.38 [a]	4.85 ± 0.34 [a,b]	1.08 ± 0.18 [a]	4.77 ± 0.41 [a]	5.77 ± 0.35 [a]
B2	13	6.08 ± 0.32 [a]	5.00 ± 0.37 [a]	1.23 ± 0.19 [a]	4.85 ± 0.38 [a]	3.46 ± 0.45 [a]	1.08 ± 0.18 [a]	5.00 ± 0.36 [a,b]	2.92 ± 0.38 [a]	4.85 ± 0.34 [a,b]	1.08 ± 0.18 [a]	5.15 ± 0.41 [a]	6.08 ± 0.35 [a]
C1	13	6.15 ± 0.32 [a]	4.85 ± 0.37 [a]	1.31 ± 0.19 [a]	4.31 ± 0.38 [a]	3.46 ± 0.45 [a]	1.23 ± 0.18 [a]	3.92 ± 0.36 [a,b,c,d]	3.31 ± 0.38 [a]	4.54 ± 0.34 [a,b]	1.23 ± 0.18 [a]	5.38 ± 0.41 [a]	5.92 ± 0.35 [a]
C2	13	5.92 ± 0.32 [a]	4.77 ± 0.37 [a]	1.23 ± 0.19 [a]	4.31 ± 0.38 [a]	3.23 ± 0.45 [a]	1.23 ± 0.18 [a]	4.77 ± 0.36 [a,b]	2.92 ± 0.38 [a]	4.85 ± 0.34 [a,b]	1.08 ± 0.18 [a]	5.31 ± 0.41 [a]	6.15 ± 0.35 [a]
D1	13	6.08 ± 0.32 [a]	4.69 ± 0.37 [a]	1.38 ± 0.19 [a]	3.92 ± 0.38 [a]	3.46 ± 0.45 [a]	1.38 ± 0.18 [a]	3.38 ± 0.36 [b,c,d]	3.92 ± 0.38 [a]	3.54 ± 0.34 [b]	1.38 ± 0.18 [a]	5.54 ± 0.41 [a]	5.38 ± 0.35 [a]
D2	13	6.23 ± 0.32 [a]	4.92 ± 0.37 [a]	1.08 ± 0.19 [a]	4.15 ± 0.38 [a]	3.46 ± 0.45 [a]	1.08 ± 0.18 [a]	4.00 ± 0.36 [a,b,c,d]	3.54 ± 0.38 [a]	4.31 ± 0.34 [a,b]	1.23 ± 0.18 [a]	5.54 ± 0.41 [a]	5.46 ± 0.35[a]
E1	13	6.08 ± 0.32 [a]	4.23 ± 0.37 [a]	1.31 ± 0.19 [a]	3.54 ± 0.38 [a]	3.69 ± 0.45 [a]	1.23 ± 0.18 [a]	2.69 ± 0.36 [c,d]	4.15 ± 0.38 [a]	3.23 ± 0.34 [b]	1.46 ± 0.18 [a]	5.69 ± 0.41 [a]	4.69 ± 0.35 [a]
E2	13	6.15 ± 0.32 [a]	4.15 ± 0.37 [a]	1.23 ± 0.19 [a]	3.46 ± 0.38 [a]	3.54 ± 0.45 [a]	1.31 ± 0.18 [a]	3.54 ± 0.36 [a,b,c,d]	4.08 ± 0.38 [a]	3.85 ± 0.34 [a,b]	1.23 ± 0.18 [a]	5.46 ± 0.41 [a]	4.62 ± 0.35 [a]
F1	13	5.54 ± 0.32 [a]	5.15 ± 0.37 [a]	1.31 ± 0.19 [a]	4.62 ± 0.38 [a]	3.38 ± 0.45 [a]	1.15 ± 0.18 [a]	4.15 ± 0.36 [a,b,c,d]	3.62 ± 0.38 [a]	4.38 ± 0.34 [a,b]	1.08 ± 0.18 [a]	5.00 ± 0.41 [a]	5.77 ± 0.35 [a]
F2	13	5.38 ± 0.32 [a]	4.92 ± 0.37 [a]	1.15 ± 0.19 [a]	5.15 ± 0.38 [a]	3.38 ± 0.45 [a]	1.15 ± 0.18 [a]	4.62 ± 0.36 [a,b]	3.31 ± 0.38 [a]	4.69 ± 0.34 [a,b]	1.23 ± 0.18 [a]	5.38 ± 0.41 [a]	5.69 ± 0.35 [a]
G1	13	5.62 ± 0.32 [a]	4.69 ± 0.37 [a]	1.31 ± 0.19 [a]	4.00 ± 0.38 [a]	3.62 ± 0.45 [a]	1.54 ± 0.18 [a]	3.62 ± 0.36 [a,b,c,d]	3.92 ± 0.38 [a]	3.92 ± 0.34 [a,b]	1.62 ± 0.18 [a]	5.54 ± 0.41 [a]	5.31 ± 0.35 [a]
G2	13	5.62 ± 0.32 [a]	5.00 ± 0.37 [a]	1.38 ± 0.19 [a]	4.46 ± 0.38 [a]	3.38 ± 0.45 [a]	1.38 ± 0.18 [a]	4.00 ± 0.36 [a,b,c,d]	3.54 ± 0.38 [a]	4.31 ± 0.34 [a,b]	1.38 ± 0.18 [a]	5.69 ± 0.41 [a]	5.54 ± 0.35 [a]
H1	13	5.54 ± 0.32 [a]	4.85 ± 0.37 [a]	1.31 ± 0.19 [a]	4.62 ± 0.38 [a]	3.62 ± 0.45 [a]	1.15 ± 0.18 [a]	4.38 ± 0.36 [a,b,c]	3.62 ± 0.38 [a]	4.38 ± 0.34 [a,b]	1.38 ± 0.18 [a]	6.23 ± 0.41 [a]	5.46 ± 0.35 [a]
H2	13	5.54 ± 0.32 [a]	5.08 ± 0.37 [a]	1.23 ± 0.19 [a]	5.08 ± 0.38 [a]	3.69 ± 0.45 [a]	1.15 ± 0.18 [a]	5.15 ± 0.36 [a]	3.38 ± 0.38 [a]	5.23 ± 0.34 [a]	1.23 ± 0.18 [a]	5.85 ± 0.41 [a]	5.54 ± 0.35 [a]
I1	13	5.69 ± 0.32 [a]	5.15 ± 0.37 [a]	1.31 ± 0.19 [a]	4.38 ± 0.38 [a]	3.54 ± 0.45 [a]	1.62 ± 0.18 [a]	4.00 ± 0.36 [a,b,c,d]	3.85 ± 0.38 [a]	4.15 ± 0.34 [a,b]	1.46 ± 0.18 [a]	5.62 ± 0.41 [a]	5.08 ± 0.35 [a]
I2	13	5.77 ± 0.32 [a]	5.15 ± 0.37 [a]	1.15 ± 0.19 [a]	4.69 ± 0.38 [a]	3.54 ± 0.45 [a]	1.62 ± 0.18 [a]	4.53 ± 0.36 [a,b]	3.85 ± 0.38 [a]	4.38 ± 0.34 [a,b]	1.38 ± 0.18 [a]	6.00 ± 0.41 [a]	5.08 ± 0.35 [a]

Sample grouped		$p = 0.23$ ‡	$p = 0.20$ ‡	$p = 0.99$ ‡	$p \leq 0.01$ †	$p = 0.94$ ‡	$p = 0.06$ ‡	$p \leq 0.01$ †	$p = 0.01$ †	$p \leq 0.01$ †	$p = 0.27$ ‡	$p = 0.23$ ‡	$p \leq 0.01$ †
A	13	5.85 ± 0.32 ^{a}	5.38 ± 0.37 ^{a}	1.23 ± 0.19 ^{a}	4.46 ± 0.38 ^{a,b}	4.15 ± 0.45 ^{a}	1.15 ± 0.18 ^{a}	2.46 ± 0.36 ^{b}	4.69 ± 0.38 ^{a}	3.69 ± 0.34 ^{a,b}	1.15 ± 0.18 ^{a}	5.15 ± 0.41 ^{a}	5.77 ± 0.35 ^{a,b}
B	26	6.00 ± 0.23 ^{a}	5.04 ± 0.26 ^{a}	1.15 ± 0.13 ^{a}	4.88 ± 0.27 ^{a}	3.50 ± 0.32 ^{a}	1.08 ± 0.12 ^{a}	4.73 ± 0.25 ^{a}	3.08 ± 0.27 ^{b}	4.85 ± 0.24 ^{a}	1.08 ± 0.13 ^{a}	4.96 ± 0.29 ^{a}	5.92 ± 0.25 ^{a}
C	26	6.04 ± 0.23 ^{a}	4.81 ± 0.26 ^{a}	1.27 ± 0.13 ^{a}	4.31 ± 0.27 ^{a,b}	3.35 ± 0.32 ^{a}	1.23 ± 0.12 ^{a}	4.35 ± 0.25 ^{a}	3.12 ± 0.27 ^{b}	4.69 ± 0.24 ^{a}	1.15 ± 0.13 ^{a}	5.35 ± 0.29 ^{a}	6.03 ± 0.25 ^{a}
D	26	6.15 ± 0.23 ^{a}	4.81 ± 0.26 ^{a}	1.23 ± 0.13 ^{a}	4.04 ± 0.27 ^{a,b}	3.46 ± 0.32 ^{a}	1.23 ± 0.12 ^{a}	3.69 ± 0.25 ^{a,b}	3.73 ± 0.27 ^{a,b}	3.92 ± 0.24 ^{a,b}	1.31 ± 0.13 ^{a}	5.54 ± 0.29 ^{a}	5.42 ± 0.25 ^{a,b}
E	26	6.12 ± 0.23 ^{a}	4.19 ± 0.26 ^{a}	1.27 ± 0.13 ^{a}	3.50 ± 0.27 ^{b}	3.62 ± 0.32 ^{a}	1.27 ± 0.12 ^{a}	3.12 ± 0.25 ^{b}	4.12 ± 0.27 ^{a,b}	3.54 ± 0.24 ^{b}	1.35 ± 0.13 ^{a}	5.58 ± 0.29 ^{a}	4.65 ± 0.25 ^{b}
F	26	5.46 ± 0.23 ^{a}	5.04 ± 0.26 ^{a}	1.23 ± 0.13 ^{a}	4.88 ± 0.27 ^{a}	3.38 ± 0.32 ^{a}	1.15 ± 0.12 ^{a}	4.38 ± 0.25 ^{a}	3.46 ± 0.27 ^{a,b}	4.54 ± 0.24 ^{a,b}	1.15 ± 0.13 ^{a}	5.19 ± 0.29 ^{a}	5.73 ± 0.25 ^{a,b}
G	26	5.62 ± 0.23 ^{a}	4.85 ± 0.26 ^{a}	1.35 ± 0.13 ^{a}	4.23 ± 0.27 ^{a,b}	3.50 ± 0.32 ^{a}	1.46 ± 0.12 ^{a}	3.81 ± 0.25 ^{a,b}	3.73 ± 0.27 ^{a,b}	4.12 ± 0.24 ^{a,b}	1.50 ± 0.13 ^{a}	5.62 ± 0.29 ^{a}	5.42 ± 0.25 ^{a,b}
H	26	5.54 ± 0.23 ^{a}	4.96 ± 0.26 ^{a}	1.27 ± 0.13 ^{a}	4.85 ± 0.27 ^{a}	3.65 ± 0.32 ^{a}	1.15 ± 0.12 ^{a}	4.77 ± 0.25 ^{a}	3.50 ± 0.27 ^{a,b}	4.81 ± 0.24 ^{a}	1.31 ± 0.13 ^{a}	6.04 ± 0.29 ^{a}	5.50 ± 0.25 ^{a,b}
I	26	5.73 ± 0.23 ^{a}	5.15 ± 0.26 ^{a}	1.23 ± 0.13 ^{a}	4.54 ± 0.27 ^{a,b}	3.54 ± 0.32 ^{a}	1.62 ± 0.12 ^{a}	4.27 ± 0.25 ^{a}	3.85 ± 0.27 ^{a,b}	4.27 ± 0.24 ^{a,b}	1.42 ± 0.13 ^{a}	5.81 ± 0.29 ^{a}	5.08 ± 0.25 ^{a,b}
Dataset average	221	5.83 ± 0.08	4.89 ± 0.09	1.25 ± 0.04	4.41 ± 0.10	3.54 ± 0.11	1.27 ± 0.04	4.04 ± 0.10	3.64 ± 0.09	4.30 ± 0.09	1.28 ± 0.04	5.49 ± 0.10	5.49 ± 0.09

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis; and ‡ not significant factor in multifactor analysis. A—control sample; 3DP fruit snacks with the addition of: B—saccharose, C—fructose, D—birch sugar (xylitol), E—erythritol, F— maple syrup, G—date syrup, H—agave syrup, I—stevia and erythritol; 1—lower level of sweeteners, 2—higher level of sweeteners.

The control sample without added sweetener had the lowest sweetness, while the samples with added saccharose (B), fructose (C), maple syrup (F), agave syrup (H) and stevia and erythritol (I) had the highest sweetness. It is not surprising that after the sample with added saccharose (B), the sample with added stevia and erythritol (I) had the highest sweetness. The sweetness of stevia comes from the steviol glycosides stevioside and rebaudioside A, which are 250 to 400 times sweeter than saccharose and do not respond to temperature and pH changes during processing [45]. The main disadvantage of using stevia in the production of functional foods is undesirable sensory properties such as a bitter and/or metallic taste [46], which was not the case here, most likely because stevia was chosen in combination with erythritol. Furthermore, the addition of sweeteners led not only to an increase in sweetness but also to an increase in the harmony of the taste, so that samples B, C and H showed the most pronounced harmonious characteristics.

The samples with the addition of saccharose and fructose, i.e., samples B and C, had the most expressive characteristic of a glossy appearance, while the other samples, with the exception of sample E, were most similar to control sample A. Ultimately, the results obtained show that functional 3D-printed snack products can be sensory-enhanced by the addition of appropriate sweeteners, which opens a perspective for exploring new formulations of 3D-printed fruit-based functional foods.

4. Conclusions

This study investigated the effects of starch content and 3D printing programs on the stability of polyphenolic compounds, pigments, antioxidant activity, color and rheological properties as well as sensory characteristics of 3D-printed snacks based on strawberries and strawberry tree fruits. The results showed a decrease in the total phenolic compounds (19.86%), hydroxycinnamic acids (23.65%) and flavonols (23.91%) with increasing starch content, while total flavonoids and condensed tannins showed different trends. The 3D printing programs showed no significant influence on most bioactive compounds, with the exception of hydroxycinnamic acids, whose content was 4.35% higher in the samples printed with program 1. This study also highlighted the influence of starch content on the pigments and antioxidant capacity, with different effects observed depending on the starch content. The color parameters were significantly affected by variations in the starch content, while the 3D printing programs had no significant effect.

This study investigated the rheological properties of 3D-printed snacks, focusing on texture, dimensions and particle size distribution. A higher starch content led to a higher extrusion force and flow resistance, enhancing sample firmness. However, the penetration tests showed only minimal effects on the mechanical properties. The dimensional measurements remained stable at different starch contents and printing programs. The particle size distribution shifted toward smaller particles with higher starch content, which improved the rheological stability of the sample.

Although process parameters such as the addition of starch and the variation in 3D programs significantly affect the CIEL*a*b* color parameters, the color change was generally satisfactory with no noticeable and/or appreciable difference. The sensory evaluation of 3D-printed snacks with different sweeteners provided interesting results. The differences in the sweetener content had no significant effect on the color, taste or odor descriptors, with the exception of sweetness and harmony. The addition of sweeteners not only increased the sweetness but also had a positive effect on the harmony of the flavor, which was particularly evident in the samples with sucrose, fructose and agave syrup. These results underline the potential of different sweeteners to improve the sensory properties of functional 3D-printed snacks.

In summary, the results obtained indicate the great potential of using fruit bases in the production of functional 3D-printed snacks, which, thanks to their good nutritional and biological potential and their rheological properties when using natural sweeteners, represent an excellent basis for the further development of functional personalized nutrition.

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Data Availability Statement: The data used to support the findings of this study can be made available by the corresponding author upon request.

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References

1. Alsubhi, M.; Blake, M.; Nguyen, T.; Majmudar, I.; Moodie, M.; Ananthapavan, J. Consumer willingness to pay for healthier food products: A systematic review. *Obes. Rev.* **2022**, *24*, e13525. https://doi.org/10.1111/obr.13525.
2. Bebek Markovinović, A.; Brdar, D.; Putnik, P.; Bosiljkov, T.; Durgo, K.; Huđek Turković, A.; Brčić Karačonji, I.; Jurica, K.; Pavlić, B.; Granato, D.; et al. Strawberry tree fruits (*Arbutus unedo* L.): Bioactive composition, cellular antioxidant activity, and 3D printing of functional foods. *Food Chem.* **2024**, *433*, 137287. https://doi.org/10.1016/j.foodchem.2023.137287.
3. Zhang, Y.-J.; Gan, R.-Y.; Li, S.; Zhou, Y.; Li, A.-N.; Xu, D.-P.; Li, H.-B. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules* **2015**, *20*, 21138–21156. https://doi.org/10.3390/molecules201219753.
4. Sagbas, H.I.; Ilhan, G.; Zitouni, H.; Anjum, M.A.; Hanine, H.; Necas, T.; Ondrasek, I.; Ercisli, S. Morphological and biochemical characterization of diverse strawberry tree (*Arbutus unedo* L.) genotypes from northern Turkey. *Agronomy* **2020**, *10*, 1581. https://doi.org/10.3390/agronomy10101581.
5. Colak, A.M. Morphological and biochemical diversity in fruits of *Arbutus unedo* L. from east aegean region in Turkey. *Erwerbs-Obstbau* **2019**, *61*, 379–383. https://doi.org/10.1007/s10341-019-00450-4.
6. Boussalah, N.; Boussalah, D.; Cebadera-Miranda, L.; Fernández-Ruiz, V.; Barros, L.; Ferreira, I.C.F.R.; Cortes Sanchez Mata, M.; Madani, K. Nutrient composition of Algerian strawberry-tree fruits (*Arbutus unedo* L.). *Fruits* **2018**, *73*, 283–297. https://doi.org/10.17660/th2018/73.5.4.
7. Rodríguez-Delgado, M.A.; Malovaná, S.; Pérez, J.P.; Borges, T.; García Montelongo, F.J. Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. *J. Chromatogr. A* **2001**, *912*, 249–257. https://doi.org/10.1016/s0021-9673(01)00598-2.
8. Djekić, I.; Velebit, B.; Pavlić, B.; Putnik, P.; Šojić Merkulov, D.; Bebek Markovinović, A.; Bursać Kovačević, D. Food quality 4.0: Sustainable food manufacturing for the twenty-first century. *Food Eng. Rev.* **2023**, *15*, 577–608. https://doi.org/10.1007/s12393-023-09354-2.
9. Sundarsingh, A.; Zhang, M.; Mujumdar, A.S.; Li, J. Research progress in printing formulation for 3D printing of healthy future foods. *Food Bioprocess Technol.* **2023.** https://doi.org/10.1007/s11947-023-03265-0.
10. Ahmadzadeh, S.; Clary, T.; Rosales, A.; Ubeyitogullari, A. Upcycling imperfect broccoli and carrots into healthy snacks using an innovative 3D food printing approach. *Food Sci. Nutr.* **2023**, *12*, 84–93. https://doi.org/10.1002/fsn3.3820.
11. Zhu, S.; Vazquez Ramos, P.; Heckert, O.R.; Stieger, M.; van der Goot, A.J.; Schutyser, M. Creating protein-rich snack foods using binder jet 3D printing. *J. Food Eng.* **2022**, *332*, 111124. https://doi.org/10.1016/j.jfoodeng.2022.111124.
12. Radoš, K.; Pastor, K.; Kojić, J.; Drakula, S.; Dujmić, F.; Novotni, D.; Čukelj Mustač, N. Influence of infill level and post-processing on physical parameters and betaine content of enriched 3D-printed sweet snacks. *Foods* **2023**, *12*, 4417. https://doi.org/10.3390/foods12244417.
13. Molina-Montero, C.; Vicente-Jurado, D.; Igual, M.; Martínez-Monzó, J.; García-Segovia, P. Fiber enrichment of 3D printed apricot gel snacks with orange by-products. *Gels* **2023**, *9*, 569. https://doi.org/10.3390/gels9070569.
14. Tan, J.D.; Lee, C.P.; Foo, S.Y.; Tan, J.C.W.; Tan, S.S.Y.; Ong, E.S.; Leo, C.H.; Hashimoto, M. 3D printability and biochemical analysis of revalorized orange peel waste. *Int. J. Bioprinting* **2023**, *9*, 510–521. https://doi.org/10.18063/ijb.776.
15. Radoš, K.; Benković, M.; Čukelj Mustač, N.; Habuš, M.; Voučko, B.; Pavičić, T.V.; Ćurić, D.; Ježek, D.; Novotni, D. Powder properties, rheology and 3D printing quality of gluten-free blends. *J. Food Eng.* **2023**, *338*, 111251. https://doi.org/10.1016/j.jfoodeng.2022.111251.
16. Letras, P.; Oliveira, S.; Varela, J.; Nunes, M.C.; Raymundo, A. 3D printed gluten-free cereal snack with incorporation of Spirulina (*Arthrospira platensis*) and/or *Chlorella vulgaris*. *Algal Res.* **2022**, *68*, 102863. https://doi.org/10.1016/j.algal.2022.102863.

17. Chen, X.-h.; Zhang, M.; Teng, X.-x.; Mujumdar, A.S. Internal structure design for improved shape fidelity and crispness of 3D printed pumpkin-based snacks after freeze-drying. *Food Res. Int.* **2022**, *157*, 111220. https://doi.org/10.1016/j.foodres.2022.111220.
18. Tomašević, I.; Putnik, P.; Valjak, F.; Pavlić, B.; Šojić, B.; Bebek Markovinović, A.; Bursać Kovačević, D. 3D printing as novel tool for fruit-based functional food production. *Curr. Opin. Food Sci.* **2021**, *41*, 138–145. https://doi.org/10.1016/j.cofs.2021.03.015.
19. Bebek Markovinović, A.; Putnik, P.; Bosiljkov, T.; Kostelac, D.; Frece, J.; Markov, K.; Žigolić, A.; Kaurinović, J.; Pavlić, B.; Duralija, B.; et al. 3D printing of functional strawberry snacks: Food design, texture, antioxidant bioactive compounds, and microbial stability. *Antioxidants* **2023**, *12*, 436. https://doi.org/10.3390/antiox12020436.
20. Bursać Kovačević, D.; Putnik, P.; Dragović-Uzelac, V.; Pedisić, S.; Režek Jambrak, A.; Herceg, Z. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chem.* **2016**, *190*, 317–323. https://doi.org/10.1016/j.foodchem.2015.05.099.
21. Yuan, B.; Danao, M.-G.C.; Stratton, J.E.; Weier, S.A.; Weller, C.L.; Lu, M. High pressure processing (HPP) of aronia berry purée: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. *Innov. Food Sci. Emerg. Technol.* **2018**, *47*, 249–255. https://doi.org/10.1016/j.ifset.2018.03.009.
22. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colometric methods. *J. Food Drug Anal.* **2020**, *10*, 3. https://doi.org/10.38212/2224-6614.2748.
23. Howard, L.R.; Clark, J.R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238–1247. https://doi.org/10.1002/jsfa.1532.
24. Sun, B.; Ricardo-da-Silva, J.M.; Spranger, I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. https://doi.org/10.1021/jf980366j.
25. Lee, J.; Durst, R.W.; Wrolstad, R.E.; Eisele, T.; Giusti, M.M.; Hach, J.; Hofsommer, H.; Koswig, S.; Krueger, D.A.; Kupina; et al. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the ph differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278. https://doi.org/10.1093/jaoac/88.5.1269.
26. Lichtenthaler, H.K.; Buschmann, C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr. Protoc. Food Anal. Chem.* **2001**, *1*, F4.3.1–F4.3.8. https://doi.org/10.1002/0471142913.faf0403s01.
27. Shortle, E.; O'Grady, M.N.; Gilroy, D.; Furey, A.; Quinn, N.; Kerry, J.P. Influence of extraction technique on the anti-oxidative potential of hawthorn (*Crataegus monogyna*) extracts in bovine muscle homogenates. *Meat Sci.* **2014**, *98*, 828–834. https://doi.org/10.1016/j.meatsci.2014.07.001.
28. Benzie, I.F.F. An automated, specific, spectrophotometric method for measuring ascorbic acid in plasma (EFTSA). *Clin. Biochem.* **1996**, *29*, 111–116. https://doi.org/10.1016/0009-9120(95)02013-6.
29. Vahčić, N.; Hruškar, M.; Marković, K. Quantitative descriptive analysis method in sensory evaluation of yoghurt. *Mljekarstvo* **2000**, *50*, 279–296.
30. Cloud Software Group, I. Data Science Workbench, Version 14.1. Available online: http://tibco.com (accessed on 5 March 2024).
31. Bebek Markovinović, A.; Putnik, P.; Duralija, B.; Krivohlavek, A.; Ivešić, M.; Mandić Andačić, I.; Palac Bešlić, I.; Pavlić, B.; Lorenzo, J.M.; Bursać Kovačević, D. Chemometric Valorization of Strawberry (*Fragaria x ananassa* Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. *Foods* **2022**, *11*, 640. https://doi.org/10.3390/foods11050640.
32. Rong, L.; Chen, X.; Shen, M.; Yang, J.; Qi, X.; Li, Y.; Xie, J. The application of 3D printing technology on starch-based product: A review. *Trends Food Sci. Technol.* **2023**, *134*, 149–161. https://doi.org/10.1016/j.tifs.2023.02.015.
33. Zhang, J.; Li, Y.; Cai, Y.; Ahmad, I.; Zhang, A.; Ding, Y.; Qiu, Y.; Zhang, G.; Tang, W.; Lyu, F. Hot extrusion 3D printing technologies based on starchy food: A review. *Carbohydr. Polym.* **2022**, *294*, 119763. https://doi.org/10.1016/j.carbpol.2022.119763.
34. Ngo, T.V.; Kusumawardani, S.; Kunyanee, K.; Luangsakul, N. Polyphenol-modified starches and their applications in the food industry: Recent updates and future directions. *Foods* **2022**, *11*, 3384. https://doi.org/10.3390/foods11213384.
35. Andrés, V.; Villanueva, M.J.; Tenorio, M.D. The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chem.* **2016**, *192*, 328–335. https://doi.org/10.1016/j.foodchem.2015.07.031.
36. Feng, C.; Zhang, M.; Bhandari, B. Controlling the three-dimensional printing mechanical properties of *Nostoc Sphaeroides* system. *Food Biophys.* **2019**, *15*, 240–248. https://doi.org/10.1007/s11483-019-09611-0.
37. Dong, X.; Huang, Y.; Pan, Y.; Wang, K.; Prakash, S.; Zhu, B. Investigation of sweet potato starch as a structural enhancer for three-dimensional printing of *Scomberomorus niphonius* surimi. *J. Texture Stud.* **2019**, *50*, 316–324. https://doi.org/10.1111/jtxs.12398.
38. Yang, F.; Zhang, M.; Bhandari, B.; Liu, Y. Investigation on lemon juice gel as food material for 3D printing and optimization of printing parameters. *Lwt* **2018**, *87*, 67–76. https://doi.org/10.1016/j.lwt.2017.08.054.
39. Yang, F.; Zhang, M.; Bhandari, B. Recent development in 3D food printing. *Crit. Rev. Food Sci. Nutr.* **2015**, *57*, 3145–3153. https://doi.org/10.1080/10408398.2015.1094732.
40. Bhat, R.; Geppert, J.; Funken, E.; Stamminger, R. Consumers perceptions and preference for strawberries—A case study from Germany. *Int. J. Fruit Sci.* **2015**, *15*, 405–424. https://doi.org/10.1080/15538362.2015.1021408.

41. Buvé, C.; Kebede, B.T.; De Batselier, C.; Carrillo, C.; Pham, H.T.T.; Hendrickx, M.; Grauwet, T.; Van Loey, A. Kinetics of colour changes in pasteurised strawberry juice during storage. *J. Food Eng.* **2018**, *216*, 42–51. https://doi.org/10.1016/j.jfoodeng.2017.08.002.
42. Chen, X.D. Food drying fundamentals. In *Drying Technologies in Food Processing*; Chen, X.D., Mujumdar, A.S., Eds.; Blackwell Publishing: Singapore, 2008; pp. 1–55.
43. Krebs, G.; Müller, M.; Becker, T.; Gastl, M. Characterization of the macromolecular and sensory profile of non-alcoholic beers produced with various methods. *Food Res. Int.* **2019**, *116*, 508–517. https://doi.org/10.1016/j.foodres.2018.08.067.
44. Muto, A.; Christofides, S.R.; Sirangelo, T.M.; Bartella, L.; Muller, C.; Di Donna, L.; Muzzalupo, I.; Bruno, L.; Ferrante, A.; Chiappetta, A.A.C.; et al. Fruitomics: The Importance of Combining Sensory and Chemical Analyses in Assessing Cold Storage Responses of Six Peach (Prunus persica L. Batsch) Cultivars. *Foods* **2022**, *11*, 2554. https://doi.org/10.3390/foods11172554.
45. Bursać Kovačević, D.; Barba, F.J.; Granato, D.; Galanakis, C.M.; Herceg, Z.; Dragović-Uzelac, V.; Putnik, P. Pressurized hot water extraction (PHWE) for the green recovery of bioactive compounds and steviol glycosides from *Stevia rebaudiana* Bertoni leaves. *Food Chem.* **2018**, *254*, 150–157. https://doi.org/10.1016/j.foodchem.2018.01.192.
46. Rocha, I.F.d.O.; Bolini, H.M.A. Passion fruit juice with different sweeteners: Sensory profile by descriptive analysis and acceptance. *Food Sci. Nutr.* **2015**, *3*, 129–139. https://doi.org/10.1002/fsn3.195.

General discussion

1. Investigation of the quality of strawberry fruits and the influence of processing on the quality of their juices

As a raw material, strawberries (*Fragaria x ananassa* Duch.) align well with the growing demand for functional foods. Consumers are increasingly favoring products that offer exceptional quality and added value (Basu et al., 2013). However, the processing of strawberry fruit into juice leads to a considerable reduction in phenolic compounds (Oszmiański and Wojdyło, 2008; Skrovankova et al., 2015). Considering that strawberry fruits and their juices differ in terms of quality parameters, one of the objectives of this dissertation was to evaluate the quality of strawberries and analyze the effects of processing parameters on the quality of processed juices. In *Publication No.3*, the physicochemical, toxicological, sensory, and biological properties of strawberries cv. 'Albion', harvested at two ripeness stages (75% *vs.* 100%), along with their juices and by-products produced using cold pressing technology, were investigated.

Table 2 of the publication details the effects of ripeness and storage on the **physicochemical properties** of strawberries. Fully ripe strawberries had significantly greater mass compared to less ripe fruit, with this mass remaining stable over 4 days of storage at 4 °C. Although the observed weight loss during storage was not statistically significant, it was likely due to moisture loss (Kelly et al., 2016). Less ripe fruit exhibited 35% higher firmness than fully ripe fruit. During storage, firmness decreased significantly, making the fruit softer and more susceptible to spoilage, which is consistent with other studies (Hwang et al., 2019; Ornelas-Paz et al., 2013; Nunes et al., 2005). In addition, fully ripe strawberries had about 5% higher soluble solids content, which is consistent with previous findings, although this content decreased during storage. The pH of fully ripe strawberries was slightly higher but also decreased during storage, likely due to the stabilizing effect of low temperatures (Olsson et al., 2004). Conversely, total acidity was about 21% lower in fully ripe samples, and storage significantly reduced total acidity. Overall, these results suggest that both ripeness and storage significantly affect the quality of fresh strawberries intended for processing, and that these factors must be carefully controlled to produce high-quality, functional strawberry juice. The color of fresh strawberries was monitored on the day of harvest and during a four-day storage period at 4°C (Table 3, *Publication No.3*). The analysis showed that strawberries with a lower degree of ripeness were 14% lighter in color than fully ripe fruit. Throughout the storage, the fruit darkened, although other CIELab color parameters remained stable. This darkening can be attributed to the degradation of hexoses during storage, which is more pronounced in riper

fruit due to Maillard reactions, leading to increased color intensity (Concha-Meyer et al., 2016). Notably, fully ripe strawberries exhibited more pronounced color changes, while the L*, a* and H* values of 75% ripe strawberries remained constant during storage. This observation suggests that strawberries with a lower degree of ripeness, provided they meet other quality criteria, could be advantageous for processing (Hwang et al., 2019; Nunes et al., 2005).

Strawberries from both ripening stages were stored and subsequently processed into juice, and the yield and physicochemical properties were analyzed for all samples. Cold pressing was chosen as the production technology for functional strawberry juices because it produces pulpy and cloudy juices without raising the temperature during the process. The results showed that the juice yield was similar for strawberries at both ripening stages (Table 4, *Publication No.3*), confirming the suitability of strawberries from both ripeness levels for juice processing.

The toxicological analyzes included the examination of heavy metals (Cu, Zn, Ni, As, Cd, Pb) and pesticides in strawberry fruit samples, juices and by-products. The analysis of heavy metals revealed that the concentrations of Ni and Cd were below the detection limit, while Cu, Zn, As and Pb were detected (Table 5, *Publication No.3*). These findings are consistent with those of other studies (Shao et al., 2021; Bystricka et al., 2015). Lead (Pb) was not found in the samples of fresh strawberries; however, in sample J2, which consisted of the juice of 100% ripe strawberries, the Pb content was above the maximum permissible concentration (MDK) for juice. The concentration in sample BP2, derived from 100% ripe strawberries, was even higher than in sample J2, but remained below the MDK for strawberry fruit.

The strawberry samples were further analyzed with GC-MS/MS for a total of 261 pesticides and with LC-MS/MS for 305 pesticides. Due to the perishable nature of strawberries, they are frequently treated with fungicides, which cyprodinil, pyrimethanil and fludioxonil are the most commonly used (Fernández-Ortuño et al., 2012; Krieger, 2010). In the analysis, cyprodinil and pyrimethanil were the only pesticides detected in the strawberry samples (Table 6, *Publication No.3*). Cyprodinil was only detected in the by-product BP1. The European Union's maximum residue limits (MRL) for cyprodinil and pyrimethanil in strawberries are 5 mg kg^{-1}, and the detected levels were below these limits. Thus, the processed strawberries and their juices can be considered toxicologically safe.

Sensory evaluations of the fresh strawberry samples and their corresponding juices were carried out using 13 different sensory descriptors (Table 7, *Publication No.3*). The analysis revealed no significant differences in sensory characteristics between the fresh fruit and the juices, indicating that sensory perception remained consistent during the processing of the fruit into juice. However, the degree of ripeness was identified as a crucial factor influencing most of the sensory properties. Juices and fruits that were 100% ripe showed almost 20% higher overall sensory quality compared to those that were less ripe. On the other hand, there were no significant sensory differences between juices and fruits with varying ripeness levels, suggesting that consumers could not distinguish between juices made from 75% and 100% ripe strawberries.

The analysis showed that strawberry by-products contained the highest levels of all **BACs**, while fruit juices had the lowest concentrations (Table 8, *Publication No.3*). By-products exhibited either similar or increased levels of BACs compared to raw fruit and juices. Notably, the total flavonol content was significantly higher in by-products than in juices, highlighting the potential benefits of utilizing strawberry by-products in food production. The levels of total polyphenols and anthocyanins in by-products did not differ from those in raw strawberries. Riper fruit had a lower total polyphenol content, higher anthocyanins content, lower hydroxycinnamic acid content, and stable flavonol content. Since the phenolic compounds are mainly concentrated in the skin of the fruit, their concentration tends to decrease with increasing ripeness and mass of the fruit (Wei et al., 2021). During juice extraction, the fruit cells are typically broken by mechanical methods (Weber and Larsen, 2017). As the fruit ripens and softens (Hwang et al., 2019; Ornelas-Paz et al., 2013; Heng Koh and Melton, 2002; Nunes et al., 2005), it becomes easier to break the cells, enhancing the extraction of the water-soluble components such as anthocyanins, which explains the observed results. Interestingly, the effect of ripeness on the hydroxycinnamic acid content was reversed after the fruit was processed into juices. While the hydroxycinnamic acid content increased in juices from fully ripe fruit, this trend was not observed in the by-products. This change can be attributed to the lower tissue firmness of fully ripe fruit compared to 75% ripe fruit, leading to a more efficient extraction of the hydroxycinnamic acid components (Heng Koh and Melton, 2002).

Hierarchical cluster analysis using the standardized Ward method showed that, when evaluating the average values for CIELab color parameters, pH, different groups of measured BACs, and overall sensory quality at different stages of fruit ripeness and their juices, the

results were grouped such that the juices and their corresponding fully ripe (100%) fruit were closely clustered. Juices from 75% ripe fruit formed the next closest group, indicating that even juices made from less ripe strawberries were similar in quality to those made from fully ripe fruit (Figure 3, *Publication No.3*). Fruits with a 75% ripeness were positioned further away from this cluster, although, still relatively close to the juice samples from 75% ripe fruits, but outside the cluster that included both the fully ripe fruit and their juices.

In summary, strawberries with a lower degree of ripeness (75%) produce juice of comparable quality to fully ripe fruit. This finding could be advantageous for industrial growers and juice processors, as it may reduce the time required for strawberries to reach full ripeness from the 75% stage, enabling earlier harvesting without significant loss of quality.

2. Effects of non-thermal pulsed electric field and high-power ultrasound technologies on the quality of strawberry juices

The second objective of this dissertation was to optimize the process parameters of the hurdle technology and assess their effects on strawberry juice quality. The first step involved investigating and optimizing the parameters of each non-thermal technology individually.

The effect of a pulsed electric field (PEF) on the quality of strawberry juices

The study evaluated the following BACs in strawberry juices produced from fruit at two ripeness levels (75% and 100%) using cold pressing technology, followed by treatment with high-intensity pulsed electric field (HIPEF) technology: total phenols, anthocyanins, hydroxycinnamic acids, flavonols and condensed tannins (*Publication No.4*). The HIPEF treatment parameters included: electric field strength (40 and 50 kV cm^{-1}), frequency (100 and 200 Hz) and treatment duration (3 and 6 minutes). Both treated and untreated (control) juices were stored at 4 °C for 7 days. The effects of HIPEF treatment, ripeness, and storage on the BACs of treated samples are presented in Table 5 of the publication. As expected, juices derived from fruit at different ripeness levels varied in BACs content. Notably, only the total phenolic compounds were more abundant in the juices from 75% ripe fruit, while the other BACs were higher in juices from fully ripe strawberries.

Increasing electric field strength significantly increased the content of all BACs, likely due to electroporation and enhanced leakage from damaged cell walls (Luengo et al., 2013; Roobab et al., 2018; Barba et al., 2015). These results align with previous studies investigating

the impact of electric field strengths (1, 2 and 3 kV cm^{-1}, 10 Hz, 100 s) on BACs in date palm fruit (Siddeeg et al., 2019). In contrast, increasing frequency had no effect on total phenolics and anthocyanins content but caused a significant decrease in hydroxycinnamic acids, flavonols and condensed tannins. This suggests that the electric field frequency has an optimal range necessary to maximize the nutritional value, particularly the polyphenol content (Martín-García et al., 2020). Treatment duration had a negligible effect on most BACs, however, extending the treatment from 3 to 6 min negatively impacted flavonol and condensed tannins content, indicating possible degradation with prolonged exposure. This suggests that the HIPEF process achieves optimal extraction efficiency with shorter treatment times. A 7-day storage period at 4° C positively affected most BACs in the treated juices, except for flavonols, which significantly decreased after the storage. The increase in BACs content during storage may be attributed to continued extraction of BACs from previous disintegrated plant tissue due to electroporation during HIPEF treatment.

When comparing the stability of BACs in untreated and HIPEF treated juice samples, it was observed that the untreated samples showed similar stability patterns during storage, regardless of ripeness (Tables 4 and 5, *Publication No.4*). HIPEF treatment led to slightly elevated levels of all measured BACs, indicating that this technology can serve not only as an alternative to pasteurization but also as an effective method for preserving the stability of BACs in strawberry juices. On average, the HIPEF treatment increased BACs content in all treated strawberry juices by 8.41%, independent of ripeness and processing parameters. Interestingly, control samples displayed a more significant increase in anthocyanins (25%) and condensed tannins (94%) during storage compared to HIPEF-treated samples (8% and 46%, respectively). A similar pattern was observed for total phenolic content, with HIPEF treatment reducing the increase in polyphenol levels threefold during storage. This effect could be attributed to the various charged species generated during HIPEF treatment, which can influence electrostatic stability and lead to the degradation of tannins and anthocyanins when present at high concentrations. These findings underscore the importance of optimizing HIPEF treatment parameters to enhance the preservation of BACs.

HIPEF technology is classified as a non-thermal method that operates at or slightly above ambient temperature, making it effective in preserving heat-sensitive BACs (Putnik et al., 2020). The average temperature during HIPEF treatment was 22.93 ± 0.40 °C, with no statistically significant temperature variations observed, indicating that the treatment temperature did not affect the stability of the BACs in the samples. Process optimization was

conducted to determine the optimal HIPEF parameters for preserving BACs in strawberry juice. The optimal HIPEF conditions for each analyzed BACs are shown in Table 7. The highest levels of total polyphenols were achieved under slightly varied conditions for different polyphenol groups. For instance, a total polyphenol content of 113.75 mg 100 mL^{-1} was best obtained after approximately 7 days of storage, using strawberries with a maturity level of around 75% and HIPEF parameters of 49.90 kV cm^{-1} electric strength, 199.74 Hz frequency, and a treatment duration of 3.02 minutes. Both, a seven-day storage period and an electric field strength of 50 kV cm^{-1} positively influenced most BACs. When examining the effect of frequency, it was found that a lower frequency (100 Hz) was more effective in enhancing certain BACs than a higher frequency (200 Hz). This could be attributed to the fact that lower frequencies allow a higher degree of tissue permeabilization, leading to more efficient extraction of the BACs (Asavasanti et al., 2010; Ersus et al., 2010).

In summary, the electric field strength and frequency significantly influenced the content of all phenolic compounds in the strawberry juices. However, the duration of the HIPEF treatment did not have a statistically significant effect on most phenolic compounds, except for flavonols and condensed tannins. The optimization of the HIPEF treatment parameters demonstrated that strawberry samples, regardless of ripeness level, are suitable for HIPEF processing in the production of functional fruit juices.

The effect of a high-power ultrasound (HPU) on the quality of strawberry juices

The other non-thermal technology studied in this dissertation and its impact on the quality of strawberry juice was high-power ultrasound. Similar to the previous experiment with PEF, this study investigated the effects of non-thermal technology on strawberry juices produced from fruits at two ripeness levels (75% and 100%) using cold pressing technology, followed by storage at 4 °C for 7 days. The HPU parameters included amplitude (25%, 50%, 75%, and 100%), pulses (50% and 100%) and treatment time (5 min and 10 min). The results of this study are detailed in *Publication No.5*.

The effects of HPU treatment, maturity and storage parameters on the BACs in the HPU-treated samples are shown in Table 4. As observed in the previous study, juices made from fully ripe fruits had higher levels of all BACs, except for total phenolic compounds, which were higher in juices from 75% ripe fruit. When evaluating the influence of HPU parameters, it was found that increasing the amplitude did not affect flavonol content. However, higher amplitudes negatively impacted anthocyanins, hydroxycinnamic acids and condensed tannins.

Total phenolic content decreased with increasing amplitude up to a 75%, followed by a slight increase at 100% amplitude. These findings align with existing literature, which reports a reduction in total phenolic content with an increase in amplitude from 40% to 80% (Bursać Kovačević et al., 2019). This indicates the importance of optimizing amplitude to maintain total phenolic content.

Additionally, increasing the pulse from 50% to 100% negatively affected the content of most BACs, such as anthocyanins, flavonols and condensed tannins, but had a positive impact on total phenolic content without affecting condensed tannins content. A similar pattern was observed when treatment time increased from 5 to 10 minutes. The negative effect of higher pulse duration and extended treatment time on BACs can be attributed to the collapse of cavitation bubbles and the formation of free radicals (Tiwari et al., 2008). Seven days of storage at 4 °C affected BACs in various ways: total phenolic content and anthocyanin levels decreased, hydroxycinnamic acids and flavonols increased, and condensed tannins remain unchanged.

When examining the influence of ripeness on the BACs content in untreated samples (Table 3, *Publication No.5*), a similar trend was observed as in the HPU-treated samples. Juices from riper fruits showed higher levels of anthocyanins, hydroxycinnamic acids, flavonols, and condensed tannins compared to less ripe fruits, while the opposite trend was noted for total phenolic content. This can be attributed to the fact that unripe fruits generally contain higher total phenolic content than ripe fruits (Wei et al., 2021). Storage had almost the same effect on BACs in untreated juices as in treated ones, except for total phenolic content, where storage had no effect on total phenolic content in untreated juices. These results clearly underscore the need to optimize the HPU treatment process to produce a product that preserves the maximum biological value.

Numerous studies have demonstrated the detrimental effects of temperature on BACs, such as total phenolic content, anthocyanins, flavonoids, vitamin C and others (Hartmann et al., 2008; Nadeem et al., 2018; Igual et al., 2010). Since temperature variation is critical for maintaining the nutrient content and bioactive value of juices, the relationship between temperature change (ΔT) and HPU parameters was investigated. In contrast to the PEF treatment of strawberry juices, where no significant temperature changes were recorded during processing, the HPU treatment resulted in temperature increases from 4 °C to 54 °C. Table 6 shows that ΔT has a strong positive correlation with all HPU parameters: energy, power, pulse,

tannins, antioxidant activity (DPPH and FRAP), CIELab color parameters, rheological properties, and microbiological analysis. The 3D printing parameters tested included: type of starch (wheat *vs*. corn), starch content (10%, 15%, and 20%), and the 3D printing program (Program 1 *vs.* Program 2), which differed in nozzle speed, extrusion speed of the mixture, printing line thickness, and the distance of the nozzle from the surface during the first layer.

Among the physicochemical parameters (Table 2, *Publication No.8*), all 3D printing parameters (type and starch content as well as the type of program) significantly affected pH values. Higher pH values were observed in products containing higher amounts of wheat starch when printed using Program 2. Literature suggests that interactions between proteins and starch can significantly influence pH (Bravo-Núñez et al., 2019), which may explain these results. Water activity values were not affected by the printing program, but higher water activity values were recorded for products containing wheat starch and higher starch content. This can be attributed to the different swelling capacities of the starch types used (Dogan et al., 2011) and the competition between glucose and fructose in water binding (Evans and Haisman, 2006).

The type of starch significantly influenced the content of the analyzed BACs. Wheat starch resulted in higher yields of total phenolic content, anthocyanins, and condensed tannins, while corn starch yielded higher amounts of hydroxycinnamic acids, flavonols, and total flavonoids. An interesting divergence was observed in the impact of starch type on DPPH and FRAP values, likely due to the different mechanisms of action of these antioxidant assays.

Starch content also significantly affected the BACs, except for total phenolic content and hydroxycinnamic acids. A parabolic trend was noted for most compounds, with a starch content of 15% providing the best yield of BACs. This result aligns with expectations, as higher starch content results in a lower proportion of the fruit component, which is the source of BACs. Increased starch content led to a reduction in DPPH and FRAP levels, likely due to the lower proportion of BACs-rich strawberry pulp. Additionally, the printing program significantly influenced most BACs (total phenolic content, total flavonoids, anthocyanins, and condensed tannins) and DPPH antioxidant capacity, with Program 2 yielding higher values for all these parameters. No significant effect was observed on hydroxycinnamic acids, flavonols and FRAP. Regarding the CIELab color parameters, the 3D printing parameters had minimal impact, with the only significant effect being on L* values. As starch content increased, L* values rose, indicating a lighter color of the products.

In terms of textural properties (Table 5-8, *Publication No.8*), changes in starch content notably affected the work (Wp) and force (Fp) required to penetrate the samples. Both texture

analysis techniques (penetration test and forward extrusion test) showed that force (Fp, F) and work (Wp, W) increased with higher starch content, indicating a firmer texture. The type and content of starch significantly influenced the extrusion properties, with increased starch content requiring more force and work for extrusion. The printing programs did not influence texture significantly. Changes in starch concentration also resulted in significant variations in particle size diameter of the 3D printed samples. A reduction in particle diameter was observed with increased starch content, which contributed to the increased force and work needed for extrusion. Overall, the samples exhibited high stability and minimal geometric deviations.

Microbial analysis of 3D printed samples was conducted over 10 days of storage at 4 °C. The study evaluated the effects of vanillin (1 g L^{-1} and 2 g L^{-1}) and citral (75 mg L^{-1} and 150 mg L^{-1}) as natural antimicrobial agents, in addition to control samples without these additives. Results showed that none of the samples contained pathogenic bacteria, yeasts, or molds, even after 10 days of storage. However, the total bacterial count exceeded the desired limit in samples stored for 2 and 4 days, which might suggest insufficient homogenization of the antimicrobial agents in the strawberry matrix, leading to reduced effectiveness in those areas. Among the treatments, the 3D-printed product with 75 mg L^{-1} citral demonstrated the best microbiological quality.

In summary, the type of starch influenced different BAC groups variably. Starch content had a significant effect on almost all BACs, with 15% content proving to be optimal for yield and antioxidant capacity. Most samples printed using Program 2 showed higher BAC yields and antioxidant activity. Importantly, no pathogenic bacteria were detected in any of the 3D-printed samples during the 10-day storage period at 4°C, confirming the product's health integrity.

The impact of 3D printing on the quality of strawberry tree fruit products

In *Publication No.9*, the influence of 3D printing parameters on the quality of 3D printed strawberry tree fruit products was investigated. The study assessed various factors, including physicochemical properties (pH and water activity), stability of BACs such as total phenolic content, anthocyanins, total flavonoids, hydroxycinnamic acids, flavonols, and condensed tannins, as well as pigments (carotenoids, chlorophyll a, and chlorophyll b). Additionally, antioxidant activity (DPPH and FRAP), CIELab color parameters, and rheological properties were evaluated. The 3D printing parameters tested included: the type of starch (wheat *vs.* corn), starch content (4%, 6% and 8%), the 3D printing program (Program 1

vs. Program 2). The latter differed in nozzle speed, extrusion speed, line thickness, and the distance of the nozzle from the surface during the first layer of printing.

When investigating the influence of printing parameters (type and content of starch and type of program) on the physicochemical parameters of the 3D printed strawberry tree fruit samples, it was found that the pH value was solely affected by the type of starch. Unlike the previous experiment with 3D-printed strawberry products, the strawberry tree fruit samples exhibited a higher pH value with the addition of corn starch. Consistent with the earlier findings for 3D-printed strawberry products, the water activity was significantly higher in samples containing wheat starch. This is likely due to the differing swelling properties of the starch types (Dogan et al., 2011). Increasing the starch content resulted in a decrease in water activity, while the printing program had no significant effect.

The type of starch had no significant effect on the content of total flavonoids or chlorophyll a and chlorophyll b. However, it significantly influenced all other BACs and antioxidant capacity. Samples with wheat starch had higher levels of most BACs, whereas antioxidant capacity was greater with the addition of corn starch. Starch content did not significantly affect hydroxycinnamic acids and flavonols. A parabolic trend was observed for total phenolic content, total flavonoids, anthocyanins, and FRAP antioxidant capacity, with the best yields obtained with 6% starch. For all other BACs and DPPH antioxidant capacity, increasing starch content led to a decrease in values, consistent with the proportion of fruit pulp in the 3D-printed mixture. The type of printing program did not significantly impact most BACs, except for flavonols, chlorophyll a and b, carotenoids, and FRAP antioxidant capacity. In these cases, Program 1 yielded better results.

Of the color parameters, only the b* value, which reflects yellow coloration, and the hue angle (H*) were significantly influenced. Samples with wheat starch exhibited higher b* values, indicating a more intense yellow color. The hue angle (H*) reached its maximum at a starch content of 4%.

The penetration force (Fp) and work (Wp) required to penetrate the 3D-printed samples were significantly affected by the type of starch. Samples with corn starch required higher penetration force and work compared to those with wheat starch. This increased hardness and resistance to external forces are attributed to the higher starch content per unit volume (Dong et al., 2019; Yang et al., 2018). Textural properties were not influenced by the type of 3D printing program. Additionally, the specific particle size distribution decreased with increasing

starch content. Samples with corn starch had a smaller particle size distribution compared to those with wheat starch. Consequently, the 3D-printed samples with a higher proportion of smaller particles exhibited greater strength.

In summary, samples with wheat starch exhibited a higher yield of all BACs. The best BAC yields were achieved with a starch content of 4% and 6%, depending on the BAC group. Program 1 outperformed Program 2 in terms of BAC yield. Most color parameters were unaffected by the 3D printing parameters. The rheological properties of the 3D-printed products were strongly influenced by the process parameters.

The strawberry tree fruit demonstrates potential for producing functional 3D-printed products. Notably, the required starch content to achieve acceptable textural and rheological properties for 3D products made from strawberry tree fruit (4-8%) is significantly lower than that needed for strawberries (10-20%). This suggests that combining strawberry tree fruits with other fruits could substantially reduce the amount of thickening agent (hydrocolloid) needed. The next chapter will show the results of producing 3D products from strawberries and strawberry tree fruits in more detail.

The impact of 3D printing on the quality of strawberry and strawberry tree fruit products

In the last part of the study, based on the previous results, strawberries and strawberry tree fruits were used in a 1:1 ratio with the addition of wheat starch in a proportion of 6%, 8% and 10% to prepare the mixture for 3D printing. The added starch content is significantly lower than that of the mixture with strawberries alone (10%, 15%, 20%) and yet slightly higher than that of the mixture with strawberry tree fruit alone (4%, 6%, 8%). The printing was carried out with Program 1 and Program 2, which differed in the speed of the nozzle, the speed of extrusion of the mixture, the thickness of the printing line and the distance of the nozzle from the surface when printing the first layer. The results of this research are presented in *Publication No.10*, namely the influence of 3D printing parameters on the quality of 3D strawberry-strawberry tree fruit products. The following quality parameters were tested: stability of BACs (total phenolic content, hydroxycinnamic acids, flavonols, total flavonoids, and condensed tannins), pigments (anthocyanins, carotenoids, chlorophyll a and b), antioxidant activity (DPPH and FRAP), CIELab color parameters, rheological properties and sensory properties.

Table 3 (*Publication No.10*) illustrates the impact of starch content and 3D printing program type on the BACs. Generally, an increase in starch content led to a decrease in BACs (total phenolic content, hydroxycinnamic acids, flavonols, and condensed tannins), which aligns with the expectation that a higher starch proportion reduces the pulp content in the mixture. The type of 3D printing program did not significantly affect most BACs, except for hydroxycinnamic acids. Table 4 (*Publication No.10*) details how starch content and 3D printing program type influence pigments and antioxidant capacity measured by DPPH and FRAP methods. Samples without added starch exhibited the highest concentrations of anthocyanins and chlorophyll. A starch content of 6% proved most favorable for carotenoid stability. No significant differences were observed in anthocyanin, chlorophyll a, and chlorophyll b content when starch content increased from 6% to 10%. However, higher starch content negatively impacted carotenoid stability. While increasing starch content from 0% to 10% did not significantly affect DPPH values, samples with 0% to 8% starch showed higher FRAP values compared to those with 10% starch, which had the lowest FRAP values. The type of 3D printing program did not significantly influence pigment content or antioxidant capacity.

Table 10 (*Publication No.10*) presents the impact of starch content and program type on CIELab color parameters. Starch content significantly affected all color parameters except the a* value. A parabolic trend was observed for most color parameters: values increased up to a 6% starch addition and then decreased. The largest color change (ΔE_{ab}) was also noted with 6% starch, measuring 6.17, which is classified as a large color difference (Chen, 2008). The average ΔE_{ab} value was 3.26 ± 0.60, indicating appreciable color differences in the 3D-printed strawberry-strawberry tree fruit products. The type of program had no significant effect on color parameters.

Tables 6-9. (*Publication No.10*) detail the rheological properties of 3D-printed products made from strawberry-strawberry tree fruit mixtures with added wheat starch at levels of 6%, 8%, and 10%, using Program 1 and Program 2. Increasing starch content resulted in a more uniform sample structure. Variations in printing speed and starch content did not lead to significant deviations in extrusion work values, as indicated by the extrusion force measurements. The forward extrusion test results revealed that the penetration test method did not cause significant deviations in the characterization of the mechanical properties. Additionally, higher starch content influenced all parameters of the particle size distribution. Consistent with previous studies, the analysis confirmed that starch content had the greatest impact on the particle size distribution of the 3D printed products.

For the sensory evaluation, 16 samples were prepared with various sweeteners added at two different concentrations (higher and lower), in addition to a control sample prepared without any sweeteners. The sweeteners used were: sucrose (Agragold, Zagreb, Croatia), fructose (Diasan, Germany), birch sugar (xylitol) (GreenLab, Finland), erythritol (GreenLab, France), maple syrup (Alnatura Bio, Darmstadt, Germany), date syrup (Alnatura Bio, Darmstadt, Germany), agave syrup (Alnatura Bio, Darmstadt, Germany), and mixture of stevia and erythritol (Silavit, Germany). The results of the sensory evaluation are presented in Table 11 in *Publication no.10*.

The sensory properties of the 3D printed products were generally not significantly affected by the type of sweetener or its concentration level (lower vs. higher), except for the perceived sweetness and harmonious taste. Samples with higher concentrations of fructose, birch sugar, erythritol, maple syrup, agave syrup, stevia, and erythritol exhibited greater sweetness compared to their counterparts with lower concentrations of the same sweeteners. As expected, the control sample, which had no added sweeteners, was the least sweet and showed significant differences in sweetness from the sweetened samples.

Notably, the greatest differences in harmonious taste were observed between samples with birch sugar, erythritol, and agave syrup. These samples, with higher sweetener concentrations, also had a greater consistency compared to those with lower sweetener concentrations. In the second part of Table 11, which considers the type of added sweetener, statistically significant differences were found in the sensory descriptors of strawberry odor, sweet taste, harmonious taste, and glossy appearance. The control sample, without added sweetener, had the lowest sweetness, while those with sucrose, fructose, maple syrup, agave syrup, stevia, and erythritol were the sweetest. In summary, the findings suggest that the sensory quality of functional 3D-printed products can be enhanced by incorporating appropriate sweeteners.

In conclusion, the observed effects of starch content on BACs, pigments, and antioxidant capacity indicate that a starch content of 6% yields the highest levels of most BACs. The type of 3D printing program had no significant impact on BACs (except for hydroxycinnamic acids), antioxidant capacity, pigments, or color parameters. Thus, both printing programs are suitable for creating functional products from strawberries and strawberry tree fruits. Overall, these fruits demonstrate significant potential for producing functional 3D-printed snacks. Their excellent nutritional and biological properties, combined

with favorable rheological characteristics when using natural sweeteners, make them a promising foundation for the development of personalized nutrition solutions.

Conclusions and prospects

- The results of this study demonstrate that strawberries (*Fragaria x ananassa* Duch.) at both ripeness levels - 75% and 100% - are suitable for processing into functional fruit juices based on the investigated quality parameters including CIELab color parameters, pH, different groups of measured bioactive compounds and overall sensory quality at various ripeness stages of the fruits and their juices.
- The quality of the produced juices significantly differed from that of the by-products; strawberry by-products contained the highest levels of all bioactive compounds, while the juices had the lowest concentrations.
- The pulsed electric field (PEF) process parameters significantly affected the stability of the bioactive compounds. Higher electric field strength positively influenced the yield of all bioactive compounds, whereas higher frequency negatively impacted most bioactive compounds (total hydroxycinnamic acids, flavonols and condensed tannins), and longer process duration led to a decrease in certain bioactive compounds (flavonols and condensed tannins).
- Optimization of the PEF process parameters revealed that the highest yield of most examined bioactive compounds was achieved with higher electric field strength (50 kV cm^{-1}), lower frequency (100 Hz, except for the total phenolic compounds), and a shorter treatment duration (3 minutes, except for the total hydroxycinnamic acids).
- High power ultrasound (HPU) process parameters - amplitude, pulse and treatment time - significantly influenced the stability of the bioactive compounds. Temperature changes during HPU treatment were positively correlated with nearly all HPU parameters.
- HPU optimization showed that the highest yield of bioactive compounds (total phenolic content, flavonols, condensed tannins) was obtained with parameters that minimized temperature change, except for anthocyanins and total hydroxycinnamic acids, whose yields were higher by greater temperature changes.
- The optimization of hurdle technology combining PEF + HPU indicated that shorter PEF treatment time combined with longer HPU treatment resulted in higher yields of most bioactive compounds and antioxidant capacity.
- The optimization of hurdle technology combining HPU + PEF revealed that shorter treatment times for both processes led to higher yields of most bioactive compounds, except for antioxidant capacity.

- 3D printing parameters - type of starch carrier, its content and the 3D printing program – significantly impacted most bioactive compounds and antioxidant capacity in 3D printed strawberry products. The highest yields of most bioactive compounds were achieved with 15% starch and Program 2, while specific starch types (wheat, corn) affected the yield of certain bioactive compounds.
- Similar 3D printing parameters influenced bioactive compounds and antioxidant capacity in 3D printed strawberry tree fruit products. Optimization yielded the highest levels of bioactive compounds with lower amounts (4%, 6%) of wheat starch using Program 1.
- Starch content significantly affected most bioactive compounds and antioxidant capacity (measured by FRAP method), while the type of program had no effect on the bioactive compounds (except total hydroxycinnamic acids) and antioxidant capacity in 3D printed strawberry products with the added strawberry tree fruit. The highest yield of bioactive compounds was obtained with 6% starch content.
- The rheological properties of the 3D-printed samples based on strawberries and strawberry tree fruits showed that samples with the highest starch content lead to an increased sample firmness. Smaller particles with a higher starch content improved the stability of the 3D-printed samples.
- The findings of this dissertation contribute significantly to understanding the impact of sustainable non-thermal technologies (PEF and HPU) within the hurdle technology concept and additive 3D printing technology on the quality and stability of strawberry-based functional products with added strawberry tree fruit.

References

Aaby, K., Mazur, S., Nes, A. and Skrede, G. (2012) Phenolic compounds in strawberry (*Fragaria x ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening, *Food Chemistry*, **132**(1), 86-97. https://doi.org/10.1016/j.foodchem.2011.10.037

Aadil, R. M., Zeng, X. A., Han, Z., Sahar, A., Khalil, A. A., Rahman, U. U., Khan, M. and Mehmood, T. (2017) Combined effects of pulsed electric field and ultrasound on bioactive compounds and microbial quality of grapefruit juice, *Journal of Food Processing and Preservation*, **42**(2). https://doi.org/10.1111/jfpp.13507

Afrin, S., Gasparrini, M., Forbes-Hernandez, T. Y., Reboredo-Rodriguez, P., Mezzetti, B., Varela-López, A., Giampieri, F. and Battino, M. (2016) Promising health benefits of the strawberry: A focus on clinical studies, *Journal of Agricultural and Food Chemistry*, **64**(22), 4435-4449. https://doi.org/10.1021/acs.jafc.6b00857

Aguiló-Aguayo, I., Oms-Oliu, G., Soliva-Fortuny, R. and Martín-Belloso, O. (2009) Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments, *LWT - Food Science and Technology*, **42**(4), 813-818. https://doi.org/10.1016/j.lwt.2008.11.008

Alarcão-E-Silva, M. L. C. M. M., Leitão, A. E. B., Azinheira, H. G. and Leitão, M. C. A. (2001) The Arbutus berry: Studies on its color and chemical characteristics at two mature stages, *Journal of Food Composition and Analysis*, **14**(1), 27-35. https://doi.org/10.1006/jfca.2000.0962

Ali, M. R., Mohamed, R. M. and Abedelmaksoud, T. G. (2021) Functional strawberry and red beetroot jelly candies rich in fibers and phenolic compounds, *Food systems*, **4**(2), 82-88. https://doi.org/10.21323/2618-9771-2021-4-1-82-88

Alvarez-Suarez, J. M., Giampieri, F., Tulipani, S., Casoli, T., Di Stefano, G., González-Paramás, A. M., Santos-Buelga, C., Busco, F., Quiles, J. L., Cordero, M. D., Bompadre, S., Mezzetti, B. and Battino, M. (2014) One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans, *The Journal of Nutritional Biochemistry*, **25**(3), 289-294. https://doi.org/10.1016/j.jnutbio.2013.11.002

Alves Filho, E. G., Almeida, F. D. L., Cavalcante, R. S., de Brito, E. S., Cullen, P. J., Frias, J. M., Bourke, P., Fernandes, F. A. N. and Rodrigues, S. (2016) 1H NMR spectroscopy and chemometrics evaluation of non-thermal processing of orange juice, *Food Chemistry*, **204**, 102-107. https://doi.org/10.1016/j.foodchem.2016.02.121

Asavasanti, S., Ristenpart, W., Stroeve, P. and Barrett, D. M. (2010) Permeabilization of plant tissues by monopolar pulsed electric fields: Effect of frequency, *Journal of Food Science,* **76**(1). https://doi.org/10.1111/j.1750-3841.2010.01940.x

Assiry, A. (2003) Degradation kinetics of ascorbic acid during ohmic heating with stainless steel electrodes, *Journal of Applied Electrochemistry*, **33**(2), 187-196. https://doi.org/10.1023/a:1024076721332

Ayaz, F. A., Kucukislamoglu, M. and Reunanen, M. (2000) Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var.ellipsoidea) fruits, *Journal of Food Composition and Analysis,* **13**(2), 171-177. https://doi.org/10.1006/jfca.1999.0868

Azam, S. M. R., Zhang, M., Mujumdar, A. S. and Yang, C. (2018) Study on 3D printing of orange concentrate and material characteristics, *Journal of Food Process Engineering,* **41**(5), e12689. https://doi.org/10.1111/jfpe.12689

Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., Saraiva, J. A., Raso, J., Martin-Belloso, O., Witrowa-Rajchert, D., Lebovka, N. and Vorobiev, E. (2015) Current applications and new opportunities for the use of pulsed electric fields in food science and industry, *Food Research International,* **77**, 773-798. https://doi.org/10.1016/j.foodres.2015.09.015

Barbosa, B. T., Rodrigues, J. F. and Bastos, S. C. (2017) Sensory optimization of nutritionally enriched strawberry yogurt, *British Food Journal,* **119**(2), 301-310. https://doi.org/10.1108/bfj-08-2016-0370

Barros, L., Carvalho, A. M., Morais, J. S. and Ferreira, I. C. F. R. (2010) Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties, *Food Chemistry*, **120**(1), 247-254. https://doi.org/10.1016/j.foodchem.2009.10.016

Basu, A., Nguyen, A., Betts, N. M. and Lyons, T. J. (2013) Strawberry as a functional food: An evidence-based review, *Critical Reviews in Food Science and Nutrition,* **54**(6), 790-806. https://doi.org/10.1080/10408398.2011.608174

Bebek Markovinović, A., Brčić Karačonji, I., Jurica, K., Lasić, D., Skendrović Babojelić, M., Duralija, B., Šic Žlabur, J., Putnik, P. and Bursać Kovačević, D. (2022a) Strawberry Tree Fruits and Leaves (*Arbutus unedo* L.) as Raw Material for Sustainable Functional Food Processing: A Review, *Horticulturae,* **8**(10), 881. https://doi.org/10.3390/horticulturae8100881

Bebek Markovinović, A., Putnik, P., Duralija, B., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., Palac Bešlić, I., Pavlić, B., Lorenzo, J. M. and Bursać Kovačević, D. (2022b) Chemometric valorization of strawberry (*Fragaria x ananassa* Duch.) cv. 'Albion' for the production of functional juice: The impact of physicochemical, toxicological, sensory, and bioactive value, *Foods,* **11**(5). https://doi.org/10.3390/foods11050640

Benton, D. and Young, H. A. (2020) Role of fruit juice in achieving the 5-a-day recommendation for fruit and vegetable intake, *Nutrition Reviews,* **78**(3), 260-260. https://doi.org/10.1093/nutrit/nuz084

Bi, X., Hemar, Y., Balaban, M. O. and Liao, X. (2015) The effect of ultrasound on particle size, color, viscosity and polyphenol oxidase activity of diluted avocado puree, *Ultrasonics Sonochemistry,* **27**, 567-575. https://doi.org/10.1016/j.ultsonch.2015.04.011

Bordignon-Luiz, M. T., Gauche, C., Gris, E. F. and Falcão, L. D. (2007) Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems, *LWT - Food Science and Technology,* **40**(4), 594-599. https://doi.org/10.1016/j.lwt.2006.02.022

Bosiljkov, T., Tripalo, B., Brnčić, M., Ježek, D., Karlović, S. and Jagušt, I. (2011) Influence of high intensity ultrasound with different probe diameter on the degree of homogenization (variance) and physical properties of cow milk *African journal of biotechnology,* **10**(1), 34-41.

Boussalah, N., Boussalah, D., Cebadera-Miranda, L., Fernández-Ruiz, V., Barros, L., Ferreira, I. C. F. R., Cortes Sanchez Mata, M. and Madani, K. (2018) Nutrient composition of Algerian strawberry-tree fruits (*Arbutus unedo* L.), *Fruits,* **73**(5), 283-297. https://doi.org/10.17660/th2018/73.5.4

Bravo-Núñez, Á., Garzón, R., Rosell, C. M. and Gómez, M. (2019) Evaluation of starch–protein interactions as a function of pH, *Foods,* **8**(5). https://doi.org/10.3390/foods8050155

Bursać Kovačević, D., Bilobrk, J., Buntić, B., Bosiljkov, T., Karlović, S., Rocchetti, G., Lucini, L., Barba, F. J., Lorenzo, J. M. and Putnik, P. (2019) High-power ultrasound altered the polyphenolic content and antioxidant capacity in cloudy apple juice during storage, *Journal of Food Processing and Preservation,* **43**(8). https://doi.org/10.1111/jfpp.14023

Bursać Kovačević, D., Brdar, D., Fabečić, P., Barba, F. J., Lorenzo, J. M. and Putnik, P. (2020) 'Strategies to achieve a healthy and balanced diet: Fruits and vegetables as a natural

source of bioactive compounds', *Agri-Food Industry Strategies for Healthy Diets and Sustainability*, pp. 51-88.

Bystricka, J., Musilova, J., Trebichalsky, P., Tomas, J., Stanovic, R., Bajcan, D. and Kavalcova, P. (2015) The relationships between content of heavy metals in soil and in strawberries, *International Journal of Phytoremediation,* **18**(6), 553-558. https://doi.org/10.1080/15226514.2015.1086304

Caudullo, G., Welk, E. and San-Miguel-Ayanz, J. (2017) Chorological maps for the main European woody species, *Data in Brief,* **12**, 662-666. https://doi.org/10.1016/j.dib.2017.05.007

Cervera-Chiner, L., Barrera, C., Betoret, N. and Seguí, L. (2021) Impact of sugar replacement by non-centrifugal sugar on physicochemical, antioxidant and sensory properties of strawberry and kiwifruit functional jams, *Heliyon,* **7**(1). https://doi.org/10.1016/j.heliyon.2021.e05963

Chen, X. D. (2008) 'Food drying fundamentals.', in Chen, X.D. & Mujumdar, A.S. (eds.) *Drying Technologies in Food Processing*. Singapore: Blackwell Publishing, pp. 1-55.

Cheng, M., Li, F., Han, T., Yu, A. C. H. and Qin, P. (2019) Effects of ultrasound pulse parameters on cavitation properties of flowing microbubbles under physiologically relevant conditions, *Ultrasonics Sonochemistry*, **52**, 512-521. https://doi.org/10.1016/j.ultsonch.2018.12.031

Colak, A. M. (2019) Morphological and biochemical diversity in fruits of *Arbutus unedo* L. from East Aegean Region in Turkey, *Erwerbs-Obstbau,* **61**(4), 379-383. https://doi.org/10.1007/s10341-019-00450-4

Concha-Meyer, A. A., D'Ignoti, V., Saez, B., Diaz, R. I. and Torres, C. A. (2016) Effect of storage on the physico-chemical and antioxidant properties of strawberry and kiwi leathers, *Journal of Food Science,* **81**(3). https://doi.org/10.1111/1750-3841.13214

Dai, J. and Mumper, R. J. (2010) Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties, *Molecules,* **15**(10), 7313-7352. https://doi.org/10.3390/molecules15107313

Dick, A., Bhandari, B. and Prakash, S. (2019) 3D printing of meat, *Meat Science,* **153**, 35-44. https://doi.org/10.1016/j.meatsci.2019.03.005

Dogan, M., Toker, O. S. and Goksel, M. (2011) Rheological behaviour of instant hot chocolate beverage: Part 1. optimization of the effect of different starches and gums, *Food Biophysics,* **6**(4), 512-518. https://doi.org/10.1007/s11483-011-9233-0

Dong, X., Huang, Y., Pan, Y., Wang, K., Prakash, S. and Zhu, B. (2019) Investigation of sweet potato starch as a structural enhancer for three-dimensional printing of *Scomberomorus niphonius* surimi, *Journal of Texture Studies,* **50**(4), 316-324. https://doi.org/10.1111/jtxs.12398

Dündar, B., Ağçam, E. and Akyıldız, A. (2019) Optimization of thermosonication conditions for cloudy strawberry nectar with using of critical quality parameters, *Food Chemistry,* **276**, 494-502. https://doi.org/10.1016/j.foodchem.2018.10.028

Durazzo, A., Lucarini, M., Souto, E. B., Cicala, C., Caiazzo, E., Izzo, A. A., Novellino, E. and Santini, A. (2019) Polyphenols: A concise overview on the chemistry, occurrence, and human health, *Phytotherapy Research,* **33**(9), 2221-2243. https://doi.org/10.1002/ptr.6419

Dziadek, K., Kopeć, A., Dróżdż, T., Kiełbasa, P., Ostafin, M., Bulski, K. and Oziembłowski, M. (2019) Effect of pulsed electric field treatment on shelf life and nutritional value of apple juice, *Journal of Food Science and Technology,* **56**(3), 1184-1191. https://doi.org/10.1007/s13197-019-03581-4

Elez-Martínez, P., Odriozola-Serrano, I., Oms-Oliu, G., Soliva-Fortuny, R. and Martín-Belloso, O. (2017) Effects of pulsed electric fields processing strategies on health-related compounds of plant-based foods, *Food Engineering Reviews,* **9**(3), 213-225. https://doi.org/10.1007/s12393-017-9162-x

Emamifar, A. and Mohamadizadeh, M. (2020) Influence of sonication and antimicrobial packaging-based nano-ZnO on the quality of fresh strawberry juice during cold storage, *Journal of Food Measurement and Characterization,* **14**(6), 3280-3290. https://doi.org/10.1007/s11694-020-00570-6

Ersus, S., Oztop, M. H., McCarthy, M. J. and Barrett, D. M. (2010) Disintegration efficiency of pulsed electric field induced effects on onion (*Allium cepa* L.) tissues as a function of pulse protocol and determination of cell integrity by 1H-NMR relaxometry, *Journal of Food Science,* **75**(7). https://doi.org/10.1111/j.1750-3841.2010.01769.x

Es-Said, O. S., Pitayachaval, P., Sanklong, N., Thongrak, A. and Huang, C. A. (2018) A Review of 3D food printing technology, *MATEC Web of Conferences,* **213**. https://doi.org/10.1051/matecconf/201821301012

Escalante-Aburto, A., Trujillo-de Santiago, G., Álvarez, M. M. and Chuck-Hernández, C. (2021) Advances and prospective applications of 3D food printing for health improvement and personalized nutrition, *Comprehensive Reviews in Food Science and Food Safety,* **20**(6), 5722-5741. https://doi.org/10.1111/1541-4337.12849

Evans, I. D. and Haisman, D. R. (2006) The effect of solutes on the gelatinization temperature range of potato starch, *Starch - Stärke,* **34**(7), 224-231. https://doi.org/10.1002/star.19820340704

Faisal Manzoor, M., Ahmed, Z., Ahmad, N., Karrar, E., Rehman, A., Muhammad Aadil, R., Al-Farga, A., Waheed Iqbal, M., Rahaman, A. and Zeng, X. A. (2021) Probing the combined impact of pulsed electric field and ultra-sonication on the quality of spinach juice, *Journal of Food Processing and Preservation,* **45**(5). https://doi.org/10.1111/jfpp.15475

FAO (2022) *FAOSTAT statistical database, FAO-Food and Agriculture Organization of the United Nations*. Available at: https://www.fao.org/faostat/en/#data/QCL (Accessed: 8 December 2023.

Feng, C., Zhang, M. and Bhandari, B. (2018) Materials properties of printable edible inks and printing parameters optimization during 3D printing: A review, *Critical Reviews in Food Science and Nutrition,* **59**(19), 3074-3081. https://doi.org/10.1080/10408398.2018.1481823

Fernández-Ortuño, D., Chen, F. and Schnabel, G. (2012) Resistance to pyraclostrobin and boscalid inbotrytis cinereaisolates from strawberry fields in the Carolinas, *Plant Disease*, **96**(8), 1198-1203. https://doi.org/10.1094/pdis-12-11-1049-re

Fierascu, R. C., Temocico, G., Fierascu, I., Ortan, A. and Babeanu, N. E. (2020) *Fragaria genus*: Chemical composition and biological activities, *Molecules,* **25**(3). https://doi.org/10.3390/molecules25030498

Forbes-Hernández, T. Y., Giampieri, F., Gasparrini, M., Mazzoni, L., Quiles, J. L., Alvarez-Suarez, J. M. and Battino, M. (2014) The effects of bioactive compounds from plant foods on mitochondrial function: A focus on apoptotic mechanisms, *Food and Chemical Toxicology*, **68**, 154-182. https://doi.org/10.1016/j.fct.2014.03.017

Fortalezas, S., Tavares, L., Pimpão, R., Tyagi, M., Pontes, V., Alves, P. M., McDougall, G., Stewart, D., Ferreira, R. B. and Santos, C. N. (2010) Antioxidant properties and neuroprotective capacity of strawberry tree fruit (*Arbutus unedo*), *Nutrients,* **2**(2), 214-229. https://doi.org/10.3390/nu2020214

Gabrić, D., Barba, F., Roohinejad, S., Gharibzahedi, S. M. T., Radojčin, M., Putnik, P. and Bursać Kovačević, D. (2017) Pulsed electric fields as an alternative to thermal processing for preservation of nutritive and physicochemical properties of beverages: A review, *Journal of Food Process Engineering,* **41**(1). https://doi.org/10.1111/jfpe.12638

Gaikwad, V., Ghose, A., Cholake, S., Rawal, A., Iwato, M. and Sahajwalla, V. (2018) Transformation of E-waste plastics into sustainable filaments for 3D printing, *ACS Sustainable Chemistry & Engineering*, **6**(11), 14432-14440. https://doi.org/10.1021/acssuschemeng.8b03105

Gambardella, M. and Sánchez, S. (2016) 'Genetic resources of the strawberry', *Strawberry: growth, development and diseases*, pp. 10-25.

García-García, R., Escobedo-Avellaneda, Z., Tejada-Ortigoza, V., Martín-Belloso, O., Valdez-Fragoso, A. and Welti-Chanes, J. (2015) Hurdle technology applied to prickly pear beverages for inhibiting *Saccharomyces cerevisiae* and *Escherichia coli*, *Letters in Applied Microbiology*, **60**(6), 558-564. https://doi.org/10.1111/lam.12406

Giampieri, F., Alvarez-Suarez, J. M., Mazzoni, L., Romandini, S., Bompadre, S., Diamanti, J., Capocasa, F., Mezzetti, B., Quiles, J. L., Ferreiro, M. S., Tulipani, S. and Battino, M. (2013) The potential impact of strawberry on human health, *Natural Product Research*, **27**(4-5), 448-455. https://doi.org/10.1080/14786419.2012.706294

Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S., Bompadre, S., Quiles, J. L., Mezzetti, B. and Battino, M. (2015) Strawberry as a health promoter: an evidence based review, *Food & Function*, **6**(5), 1386-1398. https://doi.org/10.1039/c5fo00147a

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B. and Battino, M. (2012) The strawberry: Composition, nutritional quality, and impact on human health, *Nutrition*, **28**(1), 9-19. https://doi.org/10.1016/j.nut.2011.08.009

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M. and Kader, A. A. (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing, *Journal of Agricultural and Food Chemistry*, **48**(10), 4581-4589. https://doi.org/10.1021/jf000404a

Granato, D., Barba, F. J., Bursać Kovačević, D., Lorenzo, J. M., Cruz, A. G. and Putnik, P. (2020) Functional foods: Product development, technological trends, efficacy testing, and safety, *Annual Review of Food Science and Technology*, **11**(1), 93-118. https://doi.org/10.1146/annurev-food-032519-051708

Granato, D., Nunes, D. S. and Barba, F. J. (2017) An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal, *Trends in Food Science & Technology*, **62**, 13-22. https://doi.org/10.1016/j.tifs.2016.12.010

Guimarães, R., Barros, L., Calhelha, R. C., Carvalho, A. M., Queiroz, M. J. R. P. and Ferreira, I. C. F. R. (2013a) Bioactivity of different enriched phenolic extracts of wild fruits from Northeastern Portugal: A comparative study, *Plant Foods for Human Nutrition*, **69**(1), 37-42. https://doi.org/10.1007/s11130-013-0394-5

Guimarães, R., Barros, L., Dueñas, M., Carvalho, A. M., Queiroz, M. J. R. P., Santos-Buelga, C. and Ferreira, I. C. F. R. (2013b) Characterisation of phenolic compounds in wild fruits from Northeastern Portugal, *Food Chemistry*, **141**(4), 3721-3730. https://doi.org/10.1016/j.foodchem.2013.06.071

Gündoğdu, M., Ercisli, S., Canan, I., Orman, E., Sameeullah, M., Naeem, M. and Ben Ayed, R. (2018) Diversity in phenolic compounds, biochemical and pomological characteristics of *Arbutus unedo* fruits, *Folia Horticulturae*, **30**(1), 139-146. https://doi.org/10.2478/fhort-2018-0014

Hao, L., Li, Y., Gong, P. and Xiong, W. (2019) 'Material, process and business development for 3D chocolate printing', *Fundamentals of 3D Food Printing and Applications*, pp. 207-255.

Hartmann, A., Patz, C.-D., Andlauer, W., Dietrich, H. and Ludwig, M. (2008) Influence of processing on quality parameters of strawberries, *Journal of Agricultural and Food Chemistry*, **56**(20), 9484-9489. https://doi.org/10.1021/jf801555q

Heng Koh, T. and Melton, L. D. (2002) Ripening-related changes in cell wall polysaccharides of strawberry cortical and pith tissues, *Postharvest Biology and Technology*, **26**(1), 23-33. https://doi.org/10.1016/s0925-5214(02)00003-0

Holzwarth, M., Korhummel, S., Carle, R. and Kammerer, D. R. (2011) Impact of enzymatic mash maceration and storage on anthocyanin and color retention of pasteurized strawberry purées, *European Food Research and Technology*, **234**(2), 207-222. https://doi.org/10.1007/s00217-011-1601-y

Holzwarth, M., Korhummel, S., Kammerer, D. R. and Carle, R. (2012) Thermal inactivation of strawberry polyphenoloxidase and its impact on anthocyanin and color retention in strawberry (*Fragaria x ananassa* Duch.) purées, *European Food Research and Technology*, **235**(6), 1171-1180. https://doi.org/10.1007/s00217-012-1852-2

Hwang, H., Kim, Y.-J. and Shin, Y. (2019) Influence of ripening stage and cultivar on physicochemical properties, sugar and organic acid profiles, and antioxidant compositions of strawberries, *Food Science and Biotechnology*, **28**(6), 1659-1667. https://doi.org/10.1007/s10068-019-00610-y

Igual, M., García-Martínez, E., Camacho, M. M. and Martínez-Navarrete, N. (2010) Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice, *Food Chemistry*, **118**(2), 291-299. https://doi.org/10.1016/j.foodchem.2009.04.118

Iqbal, A., Murtaza, A., Hu, W., Ahmad, I., Ahmed, A. and Xu, X. (2019) Activation and inactivation mechanisms of polyphenol oxidase during thermal and non-thermal methods of food processing, *Food and Bioproducts Processing*, **117**, 170-182. https://doi.org/10.1016/j.fbp.2019.07.006

Jiang, H., Zheng, L., Zou, Y., Tong, Z., Han, S. and Wang, S. (2018) 3D food printing: main components selection by considering rheological properties, *Critical Reviews in Food Science and Nutrition*, **59**(14), 2335-2347. https://doi.org/10.1080/10408398.2018.1514363

Jurica, K. and Brčić Karačonji, I. (2016) 'Maginja – neotkriveno mediteransko blago', in Stipičević, S. (ed.) *Znanstveni dalekozor - Zbirka radova za popularizaciju znanosti*. Zagreb: Institut za medicinska istraživanja i medicinu rada, pp. 21-26.

Kader, A. A. (1999) Fruit maturity, ripening, and quality relationships, *Acta Horticulturae*, (485), 203-208. https://doi.org/10.17660/ActaHortic.1999.485.27

Kammerer, D. R., Schillmöller, S., Maier, O., Schieber, A. and Carle, R. (2006) Colour stability of canned strawberries using black carrot and elderberry juice concentrates as natural colourants, *European Food Research and Technology*, **224**(6), 667-679. https://doi.org/10.1007/s00217-006-0356-3

Karasawa, M. M. G. and Mohan, C. (2018) Fruits as prospective reserves of bioactive compounds: A review, *Natural Products and Bioprospecting*, **8**(5), 335-346. https://doi.org/10.1007/s13659-018-0186-6

Kelly, K., Whitaker, V. M. and Nunes, M. C. d. N. (2016) Physicochemical characterization and postharvest performance of the new Sensation® 'Florida127' strawberry compared to commercial standards, *Scientia Horticulturae*, **211**, 283-294. https://doi.org/10.1016/j.scienta.2016.09.012

Kempkes, M. A. (2017) 'Industrial pulsed electric field systems', *Handbook of Electroporation*, pp. 1-21.

Khan, S. A., Dar, A. H., Bhat, S. A., Fayaz, J., Makroo, H. A. and Dwivedi, M. (2020) High intensity ultrasound processing in liquid foods, *Food Reviews International*, **38**(6), 1123-1148. https://doi.org/10.1080/87559129.2020.1768404

Koubaa, M., Barba, F. J., Bursać Kovačević, D., Putnik, P., Santos, M. D., Queirós, R. P., Moreira, S. A., Inácio, R. S., Fidalgo, L. G. and Saraiva, J. A. (2018) 'Pulsed electric field processing of fruit juices', *Fruit Juices*, pp. 437-449.

Koutchma, T., Popović, V., Ros-Polski, V. and Popielarz, A. (2016) Effects of ultraviolet light and high-pressure processing on quality and health-related constituents of fresh juice products, *Comprehensive Reviews in Food Science and Food Safety*, **15**(5), 844-867. https://doi.org/10.1111/1541-4337.12214

Kouzani, A. Z., Adams, S., J. Whyte, D., Oliver, R., Hemsley, B., Palmer, S. and Balandin, S. (2017) 3D printing of food for people with swallowing difficulties, *KnE Engineering*, **2**(2). https://doi.org/10.18502/keg.v2i2.591

Kowaleski, J., Quast, L. B., Steffens, J., Lovato, F., Rodrigues dos Santos, L., Zambiazi da Silva, S., Maschio de Souza, D. and Felicetti, M. A. (2020) Functional yogurt with strawberries and chia seeds, *Food Bioscience*, **37**. https://doi.org/10.1016/j.fbio.2020.100726

Krieger, R. (2010) *Hayes' Handbook of Pesticide Toxicology.* Cambridge, MA, USA: Academic Press.

Lafarga, T., Rodríguez-Roque, M. J., Bobo, G., Villaró, S. and Aguiló-Aguayo, I. (2019) Effect of ultrasound processing on the bioaccessibility of phenolic compounds and antioxidant capacity of selected vegetables, *Food Science and Biotechnology*, **28**(6), 1713-1721. https://doi.org/10.1007/s10068-019-00618-4

Lebovka, N. I., Bazhal, M. I. and Vorobiev, E. (2001) Pulsed electric field breakage of cellular tissues: visualisation of percolative properties, *Innovative Food Science & Emerging Technologies*, **2**(2), 113-125. https://doi.org/10.1016/s1466-8564(01)00024-8

Lebovka, N. I., Bazhal, M. I. and Vorobiev, E. (2002) Estimation of characteristic damage time of food materials in pulsed-electric fields, *Journal of Food Engineering*, **54**(4), 337-346. https://doi.org/10.1016/s0260-8774(01)00220-5

Liston, A., Cronn, R. and Ashman, T. L. (2014) Fragaria: A genus with deep historical roots and ripe for evolutionary and ecological insights, *American Journal of Botany*, **101**(10), 1686-1699. https://doi.org/10.3732/ajb.1400140

Liu, Q., Tang, G.-Y., Zhao, C.-N., Feng, X.-L., Xu, X.-Y., Cao, S.-Y., Meng, X., Li, S., Gan, R.-Y. and Li, H.-B. (2018) Comparison of antioxidant activities of different grape varieties, *Molecules*, **23**(10). https://doi.org/10.3390/molecules23102432

Luengo, E., Álvarez, I. and Raso, J. (2013) Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields, *Innovative Food Science & Emerging Technologies*, **17**, 79-84. https://doi.org/10.1016/j.ifset.2012.10.005

Mahmoud, M. Z., Fagiry, M. A., Davidson, R. and Abdelbasset, W. K. (2022) The benefits, drawbacks, and potential future challenges of the most commonly used ultrasound-based hurdle combinations technologies in food preservation, *Journal of Radiation Research and Applied Sciences*, **15**(1), 206-212. https://doi.org/10.1016/j.jrras.2022.03.006

Manstan, T. and McSweeney, M. B. (2019) Consumers' attitudes towards and acceptance of 3D printed foods in comparison with conventional food products, *International Journal of Food Science & Technology*, **55**(1), 323-331. https://doi.org/10.1111/ijfs.14292

Mantihal, S., Prakash, S., Godoi, F. C. and Bhandari, B. (2019) Effect of additives on thermal, rheological and tribological properties of 3D printed dark chocolate, *Food Research International*, **119**, 161-169. https://doi.org/10.1016/j.foodres.2019.01.056

Maoka, T. (2019) Carotenoids as natural functional pigments, *Journal of Natural Medicines*, **74**(1), 1-16. https://doi.org/10.1007/s11418-019-01364-x

Maragò, E., Iacopini, P., Camangi, F., Scattino, C., Ranieri, A., Stefani, A. and Sebastiani, L. (2015) Phenolic profile and antioxidant activity in apple juice and pomace: effects of different storage conditions, *Fruits*, **70**(4), 213-223. https://doi.org/10.1051/fruits/2015015

Martín-García, B., Tylewicz, U., Verardo, V., Pasini, F., Gómez-Caravaca, A. M., Caboni, M. F. and Dalla Rosa, M. (2020) Pulsed electric field (PEF) as pre-treatment to improve the phenolic compounds recovery from brewers' spent grains, *Innovative Food Science & Emerging Technologies*, **64**. https://doi.org/10.1016/j.ifset.2020.102402

Miguel, M., Faleiro, M., Guerreiro, A. and Antunes, M. (2014) *Arbutus unedo* L.: Chemical and biological properties, *Molecules*, **19**(10), 15799-15823. https://doi.org/10.3390/molecules191015799

Milosavljević, D. M., Mutavdžić, D. R., Radotić, K., Milivojević, J. M., Maksimović, V. M. and Dragišić Maksimović, J. J. (2020) Phenolic profiling of 12 strawberry cultivars using different spectroscopic methods, *Journal of Agricultural and Food Chemistry*, **68**(15), 4346-4354. https://doi.org/10.1021/acs.jafc.9b07331

Morgado, S., Morgado, M., Plácido, A. I., Roque, F. and Duarte, A. P. (2018) *Arbutus unedo* L.: From traditional medicine to potential uses in modern pharmacotherapy, *Journal of Ethnopharmacology*, **225**, 90-102. https://doi.org/10.1016/j.jep.2018.07.004

Nadeem, M., Ubaid, N., Qureshi, T. M., Munir, M. and Mehmood, A. (2018) Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage, *Ultrasonics Sonochemistry,* **45**, 1-6. https://doi.org/10.1016/j.ultsonch.2018.02.034

Narendar, A., Hanani, N. and Syafrial, S. (2018) Factors that influence technical efficiency of organic rice in Sumberngepoh Village, Lawang District, Malang Regency, *Agricultural Social Economic Journal,* **18**(2), 79-85. https://doi.org/10.21776/ub.agrise.2018.018.2.5

Newerli-Guz, J., Śmiechowska, M., Drzewiecka, A. and Tylingo, R. (2023) Bioactive ingredients with health-promoting properties of strawberry fruit (*Fragaria x ananassa* Duchesne), *Molecules,* **28**(6). https://doi.org/10.3390/molecules28062711

Nowosad, K., Sujka, M., Pankiewicz, U. and Kowalski, R. (2020) The application of PEF technology in food processing and human nutrition, *Journal of Food Science and Technology,* **58**(2), 397-411. https://doi.org/10.1007/s13197-020-04512-4

Nunes, M. C. N., Brecht, J. K., Morais, A. M. M. B. and Sargent, S. A. (2005) Physicochemical changes during strawberry development in the field compared with those that occur in harvested fruit during storage, *Journal of the Science of Food and Agriculture,* **86**(2), 180-190. https://doi.org/10.1002/jsfa.2314

Odriozola-Serrano, I., Soliva-Fortuny, R. and Martín-Belloso, O. (2009) Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice, *LWT - Food Science and Technology,* **42**(1), 93-100. https://doi.org/10.1016/j.lwt.2008.05.008

Olsson, M. E., Ekvall, J., Gustavsson, K.-E., Nilsson, J., Pillai, D., Sjöholm, I., Svensson, U., Åkesson, B. and Nyman, M. G. L. (2004) Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (*Fragaria* × *ananassa*): Effects of cultivar, ripening, and storage, *Journal of Agricultural and Food Chemistry,* **52**(9), 2490-2498. https://doi.org/10.1021/jf030461e

Ornelas-Paz, J. d. J., Yahia, E. M., Ramírez-Bustamante, N., Pérez-Martínez, J. D., Escalante-Minakata, M. d. P., Ibarra-Junquera, V., Acosta-Muñiz, C., Guerrero-Prieto, V. and Ochoa-Reyes, E. (2013) Physical attributes and chemical composition of organic strawberry fruit (*Fragaria x ananassa* Duch, Cv. Albion) at six stages of ripening, *Food Chemistry,* **138**(1), 372-381. https://doi.org/10.1016/j.foodchem.2012.11.006

Oszmiański, J. and Wojdyło, A. (2008) Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices, *European Food Research and Technology*, **228**(4), 623-631. https://doi.org/10.1007/s00217-008-0971-2

Özcan, M. M. and Hacıseferoğulları, H. (2007) The strawberry (*Arbutus unedo* L.) fruits: Chemical composition, physical properties and mineral contents, *Journal of Food Engineering*, **78**(3), 1022-1028. https://doi.org/10.1016/j.jfoodeng.2005.12.014

Patil, S., Bourke, P., Kelly, B., Frías, J. M. and Cullen, P. J. (2009) The effects of acid adaptation on *Escherichia coli* inactivation using power ultrasound, *Innovative Food Science & Emerging Technologies*, **10**(4), 486-490. https://doi.org/10.1016/j.ifset.2009.06.005

Piatti, C., Graeff-Hönninger, S. and Khajehei, F. (2019) *Food tech transitions.*

Portanguen, S., Tournayre, P., Sicard, J., Astruc, T. and Mirade, P.-S. (2019) Toward the design of functional foods and biobased products by 3D printing: A review, *Trends in Food Science & Technology*, **86**, 188-198. https://doi.org/10.1016/j.tifs.2019.02.023

Putnik, P. and Bursać Kovačević, D. (2021) Sustainable functional food processing, *Foods*, **10**(7). https://doi.org/10.3390/foods10071438

Putnik, P., Pavlić, B., Šojić, B., Zavadlav, S., Žuntar, I., Kao, L., Kitonić, D. and Kovačević, D. B. (2020) Innovative hurdle technologies for the preservation of functional fruit juices, *Foods*, **9**(6). https://doi.org/10.3390/foods9060699

Rahman, M. S. (2015) 'Hurdle technology in food preservation', *Minimally Processed Foods Food Engineering Series*, pp. 17-33.

Rojas, M. L., Miano, A. C. and Augusto, P. E. D. (2017) 'Ultrasound processing of fruit and vegetable juices', *Ultrasound: Advances for Food Processing and Preservation*, pp. 181-199.

Roman, T., Tonidandel, L., Nicolini, G., Bellantuono, E., Barp, L., Larcher, R. and Celotti, E. (2020) Evidence of the possible interaction between ultrasound and thiol precursors, *Foods*, **9**(1). https://doi.org/10.3390/foods9010104

Roobab, U., Aadil, R. M., Madni, G. M. and Bekhit, A. E. D. (2018) The impact of nonthermal technologies on the microbiological quality of juices: A review, *Comprehensive Reviews in Food Science and Food Safety*, **17**(2), 437-457. https://doi.org/10.1111/1541-4337.12336

Ruiz-Rodríguez, B.-M., Morales, P., Fernández-Ruiz, V., Sánchez-Mata, M.-C., Cámara, M., Díez-Marqués, C., Pardo-de-Santayana, M., Molina, M. and Tardío, J. (2011) Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional

assessment and natural production data, *Food Research International*, **44**(5), 1244-1253. https://doi.org/10.1016/j.foodres.2010.11.015

Sagbas, H. I., Ilhan, G., Zitouni, H., Anjum, M. A., Hanine, H., Necas, T., Ondrasek, I. and Ercisli, S. (2020) Morphological and biochemical characterization of diverse strawberry tree (*Arbutus unedo* L.) genotypes from Northern Turkey, *Agronomy*, **10**(10). https://doi.org/10.3390/agronomy10101581

Sala, F. J. B., J.; Condón, S.; Lopez, P.; Raso, J. (1995) Effect of heat and ultrasound on microorganisms and enzymes, *New Methods of Food Preservation*, 176-204.

Salehi, F. (2020a) Physico-chemical properties of fruit and vegetable juices as affected by pulsed electric field: a review, *International Journal of Food Properties*, **23**(1), 1036-1050. https://doi.org/10.1080/10942912.2020.1775250

Salehi, F. (2020b) Physico-chemical properties of fruit and vegetable juices as affected by ultrasound: a review, *International Journal of Food Properties*, **23**(1), 1748-1765. https://doi.org/10.1080/10942912.2020.1825486

Salem, I. B., Ouesleti, S., Mabrouk, Y., Landolsi, A., Saidi, M. and Boulilla, A. (2018) Exploring the nutraceutical potential and biological activities of *Arbutus unedo* L. (Ericaceae) fruits, *Industrial Crops and Products*, **122**, 726-731. https://doi.org/10.1016/j.indcrop.2018.06.024

Schaffer, S., Heinrich, M., Leonti, M., Nebel, S., Peschel, W., Pieroni, A., Smith, F., Rivera, D., Obón, C., Inocencio, C., Verde, A., Fajardo, J., Llorach, R., Müller, W. E., Eckert, G. P., Schmitt-Schillig, S., Antonopoulou, S., Kypriotakis, Z., Manios, Y., Nomikos, T., Kaliora, A., Sidossis, L., Galli, C., Visioli, F., Grande, S., Bogani, P., de Saizieu, A., Flühmann, B., D'Orazio, D., Fowler, A., Koj, A., Bereta, J., Dulak, J., Guzdek, A. and Kapiszewska, M. (2005) Understanding local Mediterranean diets: A multidisciplinary pharmacological and ethnobotanical approach, *Pharmacological Research*, **52**(4), 353-366. https://doi.org/10.1016/j.phrs.2005.06.005

Serçe, S., Özgen, M., Torun, A. A. and Ercişli, S. (2010) Chemical composition, antioxidant activities and total phenolic content of *Arbutus andrachne* L. (Fam. Ericaceae) (the Greek strawberry tree) fruits from Turkey, *Journal of Food Composition and Analysis*, **23**(6), 619-623. https://doi.org/10.1016/j.jfca.2009.12.007

Severini, C. and Derossi, A. (2016) Could the 3D printing technology be a useful strategy to obtain customized nutrition?, *Journal of Clinical Gastroenterology*, **50**(Supplement 2), S175-S178. https://doi.org/10.1097/mcg.0000000000000705

Severini, C., Derossi, A., Ricci, I., Caporizzi, R. and Fiore, A. (2018) Printing a blend of fruit and vegetables. New advances on critical variables and shelf life of 3D edible objects, *Journal of Food Engineering,* **220**, 89-100. https://doi.org/10.1016/j.jfoodeng.2017.08.025

Shao, W.-C., Zang, Y.-Y., Ma, H.-Y., Ling, Y. and Kai, Z.-P. (2021) Concentrations and related health risk assessment of pesticides, phthalates, and heavy metals in strawberries from Shanghai, China, *Journal of Food Protection,* **84**(12), 2116-2122. https://doi.org/10.4315/jfp-21-165

Sharma, R. J., Gupta, R. C., Singh, S., Bansal, A. K. and Singh, I. P. (2016) Stability of anthocyanins- and anthocyanidins-enriched extracts, and formulations of fruit pulp of *Eugenia jambolana* ('jamun'), *Food Chemistry,* **190**, 808-817. https://doi.org/10.1016/j.foodchem.2015.06.029

Siddeeg, A., Faisal Manzoor, M., Haseeb Ahmad, M., Ahmad, N., Ahmed, Z., Kashif Iqbal Khan, M., Aslam Maan, A., Mahr Un, N., Zeng, X.-A. and Ammar, A.-F. (2019) Pulsed electric field-assisted ethanolic extraction of date palm fruits: Bioactive compounds, antioxidant activity and physicochemical properties, *Processes,* **7**(9). https://doi.org/10.3390/pr7090585

Singh, S. K. and Sharma, M. (2017) Review on biochemical changes associated with storage of fruit juice, *International Journal of Current Microbiology and Applied Sciences,* **6**(8), 236-245. https://doi.org/10.20546/ijcmas.2017.608.032

Skendrović Babojelić, M., Bogdanović, S., Dlačić, I., Duralija, B., Prgomet, Ž., Prgomet, I., Šic Žlabur, J. and Voća, S. 2020. Obična planika (Arbutus unedo L.) - Biološka, kemijska i gospodarska svojstva. *In:* Sveučilište u Zagrebu, A.f. (ed.). Zagreb.

Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T. and Sochor, J. (2015) Bioactive compounds and antioxidant activity in different types of berries, *International Journal of Molecular Sciences,* **16**(10), 24673-24706. https://doi.org/10.3390/ijms161024673

Skupien, K. and Oszmianski, J. (2004) Comparison of six cultivars of strawberries (*Fragaria x ananassa* Duch.) grown in northwest Poland, *European Food Research and Technology,* **219**(1), 66-70. https://doi.org/10.1007/s00217-004-0918-1

Solomon, O., Svanberg, U. and Sahlström, A. (1995) Effect of oxygen and fluorescent light on the quality of orange juice during storage at 8°C, *Food Chemistry,* **53**(4), 363-368. https://doi.org/10.1016/0308-8146(95)99828-n

Soltani Firouz, M., Farahmandi, A. and Hosseinpour, S. (2019) Recent advances in ultrasound application as a novel technique in analysis, processing and quality control of fruits,

juices and dairy products industries: A review, *Ultrasonics Sonochemistry,* **57**, 73-88. https://doi.org/10.1016/j.ultsonch.2019.05.014

Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I. and Bahorun, T. (2005) Phenolics as potential antioxidant therapeutic agents: Mechanism and actions, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* **579**(1-2), 200-213. https://doi.org/10.1016/j.mrfmmm.2005.03.023

Sood, M. and Dogra Bandral, J. (2019) 'Composition, Quality and Uses', in M.R., S., R., Y., A.K., D. & V., P. (eds.) *Strawberries - Production, Postharvest, Management and Protection*: CRC Press,, pp. 23-30.

Sulaiman, A., Farid, M. and Silva, F. V. M. (2016) Quality stability and sensory attributes of apple juice processed by thermosonication, pulsed electric field and thermal processing, *Food Science and Technology International,* **23**(3), 265-276. https://doi.org/10.1177/1082013216685484

Sun, J., Peng, Z., Zhou, W., Fuh, J. Y. H., Hong, G. S. and Chiu, A. (2015) A review on 3D printing for customized food fabrication, *Procedia Manufacturing,* **1**, 308-319. https://doi.org/10.1016/j.promfg.2015.09.057

Tardío, J., Pardo-De-Santayana, M. and Morales, R. (2006) Ethnobotanical review of wild edible plants in Spain, *Botanical Journal of the Linnean Society,* **152**(1), 27-71. https://doi.org/10.1111/j.1095-8339.2006.00549.x

Tardío, J., Sánchez-Mata, M. d. C., Morales, R., Molina, M., García-Herrera, P., Morales, P., Díez-Marqués, C., Fernández-Ruiz, V., Cámara, M., Pardo-de-Santayana, M., Matallana-González, M. C., Ruiz-Rodríguez, B. M., Sánchez-Mata, D., Torija-Isasa, M. E., Guil-Guerrero, J. L. and Boussalah, N. (2016) 'Ethnobotanical and food composition monographs of selected Mediterranean wild edible plants', *Mediterranean Wild Edible Plants*, pp. 273-470.

Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (No 2073/2005).

Tiwari, B. K., O' Donnell, C. P., Muthukumarappan, K. and Cullen, P. J. (2009) Effect of sonication on orange juice quality parameters during storage, *International Journal of Food Science & Technology,* **44**(3), 586-595. https://doi.org/10.1111/j.1365-2621.2008.01858.x

Tiwari, B. K., O'Donnell, C. P., Patras, A., Brunton, N. and Cullen, P. J. (2008) Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage, *European*

Food Research and Technology, **228**(5), 717-724. https://doi.org/10.1007/s00217-008-0982-z

Tomadoni, B., Cassani, L., Moreira, M. D. R., Ponce, A. and Agüero, M. V. (2019) Natural antimicrobials combined with ultrasound treatments to enhance quality parameters and safety of unpasteurized strawberry juice, *International Journal of Fruit Science*, **20**(sup2), S178-S197. https://doi.org/10.1080/15538362.2019.1709115

Tomadoni, B., Cassani, L., Viacava, G., Moreira, M. D. R. and Ponce, A. (2017) Effect of ultrasound and storage time on quality attributes of strawberry juice, *Journal of Food Process Engineering*, **40**(5). https://doi.org/10.1111/jfpe.12533

Tomašević, I., Putnik, P., Valjak, F., Pavlić, B., Šojić, B., Bebek Markovinović, A. and Bursać Kovačević, D. (2021) 3D printing as novel tool for fruit-based functional food production, *Current Opinion in Food Science*, **41**, 138-145. https://doi.org/10.1016/j.cofs.2021.03.015

Trumbo, P., Schlicker, S., Yates, A. A. and Poos, M. (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids, *Journal of the American Dietetic Association*, **102**(11), 1621-1630. https://doi.org/10.1016/s0002-8223(02)90346-9

Voon, S. L., An, J., Wong, G., Zhang, Y. and Chua, C. K. (2019) 3D food printing: A categorised review of inks and their development, *Virtual and Physical Prototyping*, **14**(3), 203-218. https://doi.org/10.1080/17452759.2019.1603508

Weber, F. and Larsen, L. R. (2017) Influence of fruit juice processing on anthocyanin stability, *Food Research International*, **100**, 354-365. https://doi.org/10.1016/j.foodres.2017.06.033

Wei, M., Wang, H., Ma, T., Ge, Q., Fang, Y. and Sun, X. (2021) Comprehensive utilization of thinned unripe fruits from horticultural crops, *Foods*, **10**(9). https://doi.org/10.3390/foods10092043

Yang, F., Zhang, M., Bhandari, B. and Liu, Y. (2018) Investigation on lemon juice gel as food material for 3D printing and optimization of printing parameters, *Lwt*, **87**, 67-76. https://doi.org/10.1016/j.lwt.2017.08.054

Yıkmış, S. (2020) Sensory, physicochemical, microbiological and bioactive properties of red watermelon juice and yellow watermelon juice after ultrasound treatment, *Journal of Food Measurement and Characterization*, **14**(3), 1417-1426. https://doi.org/10.1007/s11694-020-00391-7

Yu, X., Bals, O., Grimi, N. and Vorobiev, E. (2015) A new way for the oil plant biomass valorization: Polyphenols and proteins extraction from rapeseed stems and leaves assisted by pulsed electric fields, *Industrial Crops and Products,* **74**, 309-318. https://doi.org/10.1016/j.indcrop.2015.03.045

Zhao, Z., Wu, X., Chen, H., Liu, Y., Xiao, Y., Chen, H., Tang, Z., Li, Q. and Yao, H. (2021) Evaluation of a strawberry fermented beverage with potential health benefits, *PeerJ*, **9**. https://doi.org/10.7717/peerj.11974

Zitouni, H., Hssaini, L., Messaoudi, Z., Ourradi, H., Viuda-Martos, M., Hernández, F., Ercisli, S. and Hanine, H. (2020a) Phytochemical components and bioactivity assessment among twelve strawberry (*Arbutus unedo* L.) genotypes growing in Morocco using chemometrics, *Foods,* **9**(10). https://doi.org/10.3390/foods9101345

Zitouni, H., Hssaini, L., Ouaabou, R., Viuda-Martos, M., Hernández, F., Ercisli, S., Ennahli, S., Messaoudi, Z. and Hanine, H. (2020b) Exploring antioxidant activity, organic acid, and phenolic composition in strawberry tree fruits (*Arbutus unedo* L.) growing in Morocco, *Plants,* **9**(12). https://doi.org/10.3390/plants9121677

Zou, Y. and Jiang, A. (2016) Effect of ultrasound treatment on quality and microbial load of carrot juice, *Food Science and Technology,* **36**(1), 111-115. https://doi.org/10.1590/1678-457x.0061

Autobiography

Anica Bebek Markovinović, MSc in Food Engineering, graduated from the Faculty of Food and Biotechnology at the University of Zagreb (PBF) in 2014. That same year, she began working at the Ministry of Agriculture as an expert in the registration and protection of special quality marks within the Special Food Quality Marks Department of the Directorate for Quality and Plant Protection. In 2019, she transitioned to the PBF Laboratory of Water Technology as a senior technical assistant, contributing to the teaching and experimental part of the project "Mitigation of the negative effects of climate change on the water treatment of surface waters in the production of water for human consumption by flocculation and ozonation (KK .05.1.1.02.0003)". In 2020, she joined the Laboratory of Chemistry and Technology of Fruits and Vegetables as an assistant. Since 2021, she has been pursuing in a PhD in Biotechnology and Bioprocess Engineering, Food Technology and Nutrition at PBF, under the supervision of Full Professor Dr. Danijela Bursać Kovačević. Her PhD research is part of the scientific project "Hurdle technology and 3D printing for sustainable fruit juice processing and preservation (IP-2019-04-2105)", funded by the Croatian Science Foundation. Her current research focuses on non-thermal sustainable technologies for plant material processing and extraction, with an emphasis on 3D printing of functional foods.

Over the past four years, she has published over fifteen scientific papers in Web of Science journals, predominantly Q1, often as the first author. She has also presented her work at more than 20 international scientific conferences, both through posters or oral presentations. Notably, she was awarded for the best poster on "Strawberry Tree Fruit (*Arbutus unedo* L.) as a Valuable Ingredient for Functional Food Production" at the XXXIV Scientific-Professional Conference-Processing and Energy in Agriculture (PTEP 2022) in Sokobanja, Serbia. At PBF, she has been involved in assisting the development and execution of 13 graduate and final theses and she is a member of the Postgraduate Studies Committee at PBF. She actively participates in science popularization, presenting her research at various events such as the PBF Open Days (2022, 2023) and the Science Festival (2021, 2022, 2023, 2024). Additionally, she has shared her expertise on 3D food printing through public media appearances, including the HRT program *Prometej* (2023) and the radio show *Divni Novi Svijet* (HRT, 2022).

List of authors publications

Original scientific papers indexed in Web of Science (Current Contents Connect)

- Kovač, V., Radošević, K., **Bebek Markovinović, A.,** Makarević, J., Štefanić, Z., Barišić, L., Žinić, M., Rapić, V. (2016) The first oxalamide-bridged ferrocene: facile synthesis, preliminary conformational analysis and biological evaluation. *Applied organometallic chemistry,* **31** (2016), DOI:10.1002/aoc.3653.
- Duralija, B., Putnik, P., Brdar, D., **Bebek Markovinović, A.,** Zavadlav, S., Pateiro, M., Domínguez, R., Lorenzo, J.M., Bursać Kovačević, D. (2021) The Perspective of Croatian Old Apple Cultivars in Extensive Farming for the Production of Functional Foods. *Foods,* **10**, 708, DOI:10.3390/foods10040708.
- Tomašević, I., Putnik, P., Valjak, F., Pavlić, B., Šojić, B., **Bebek Markovinović, A**., Bursać Kovačević, D. (2021) 3D printing as novel tool for fruit-based functional food production. *Current Opinion in Food Science,* **41**, 138–145. DOI: 10.1016/j.cofs.2021.03.015.
- **Bebek Markovinović, A**., Putnik, P., Duralija, B., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., Palac Bešlić, I., Pavlić, B., Manuel Lorenzo, J., Bursać Kovačević, D. (2022) Chemometric Valorization of Strawberry (*Fragaria x ananassa* Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. *Foods,* **11**(5), 640. DOI: 10.3390/foods11050640.
- **Bebek Markovinović, A**., Putnik, P., Stulić, V., Batur, L., Duralija, B., Pavlić, B., Vukušić Pavičić, T., Herceg, Z., Bursać Kovačević, D. (2022) The Application and Optimization of HIPEF Technology in the Processing of Juice from Strawberries Harvested at Two Stages of Ripeness. *Foods,* 2022, **11**, 1997. DOI: 10.3390/foods11141997.
- **Bebek Markovinović, A**., Brčić Karačonji, I., Jurica, K., Lasić, D., Skendrović Babojelić, M., Duralija, B., Šic Žlabur, B., Putnik, P., Bursać Kovačević, D. (2022) Strawberry Tree Fruits and Leaves (*Arbutus unedo* L.) as Raw Material for Sustainable Functional Food Processing: A Review. *Horticulturae* 2022, **8**, 881. DOI: 10.3390/horticulturae8100881.
- **Bebek Markovinović, A**., Putnik, P., Bičanić, P., Brdar, D., Duralija, B., Pavlić, B., Milošević, S., Rochetti, G., Lucini, L., Bursać Kovačević, D. (2022) A Chemometric Investigation on the Functional Potential in High Power Ultrasound (HPU) Processed

Strawberry Juice Made from Fruits Harvested at two Stages of Ripeness. *Molecules*, 2023, **28**(1), 138. DOI: 10.3390/molecules28010138.

- **Bebek Markovinović, A**., Putnik, P., Bosiljkov, T., Kostelac, D., Frece, J., Markov, K., Žigolić, A., Kaurinović, J., Pavlić, B., Duralija, B., Zavadlav, S., Bursać Kovačević, D. (2023) 3D Printing of Functional Strawberry Snacks: Food Design, Texture, Antioxidant Bioactive Compounds, and Microbial Stability. *Antioxidants*, 2023, **12**(2), 436. DOI: 10.3390/antiox12020436.
- Pavlić, B., Kaplan, M., Zekovi ć, Z., Canli, O., Jovičić, N., Bursać Kovačević, D., **Bebek Markovinović, A.**, Putnik, P., Bera, O. (2023) Kinetics of Microwave-Assisted Extraction Process Applied on Recovery of Peppermint Polyphenols: Experiments and Modeling. *Plants*, **12**(6), 1391. DOI: 10.3390/plants12061391.
- **Bebek Markovinović, A**., Milošević, S., Teslić, N., Pavlić, B., Putnik, P., Brčić Karačonji, I., Jurica, K., Lasić, D., Bursać Kovačević, D. (2023) Development of a Pressurized Green Liquid Extraction Procedure to Recover Antioxidant Bioactive Compounds from Strawberry Tree Fruit (*Arbutus unedo* L.). *Plants*, 2023, **12**(10), 2006. DOI: 10.3390/plants12102006.
- Tomašević, I., Hambardzumyan, G., Marmaryan, G., Nikolić, A., Mujčinović, A., Sun, W., Liu, X-C., Bursać Kovačević, D., **Bebek Markovinović, A**., Terjung, N., Heinz, V., Papageorgiou, M., Skendi, A., Goel, G., Raghav, M., Dalle Zotte, A., Nakov, D., Velkoska, V., Sołowiej, B., Semenova, A., Kuznetsova, O., Krocko, M., Duckova, V., Lorenzo, J.M., Echegaray, N., Oz, E., Oz, F., Djekic, I. (2023) Eurasian consumers' food safety beliefs and trust issues in the age of COVID-19: evidence from an online survey in 15 countries. *Journal of The Science of Food and Agriculture*, 12815. DOI: 10.1002/jsfa.12815.
- **Bebek Markovinović, A**., Stulić, V., Putnik, P., Birkić, A., Jambrović, M., Šaško, D., Ljubičić, J., Pavlić, B., Herceg, Z., Bursać Kovačević, D. (2023) Pulsed Electric Field (PEF) and High-Power Ultrasound (HPU) in the Hurdle Concept for the Preservation of Antioxidant Bioactive Compounds of Strawberry Juice—A Chemometric Evaluation—Part I. *Foods*, **12**(17), 3172. DOI: 10.3390/foods12173172.
- **Bebek Markovinović, A**., Brdar, D., Putnik, P., Bosiljkov, T., Durgo, K., Huđek Turković, A., Brčić Karačonji, I., Jurica, K., Pavlić, B., Granato, D., Bursać Kovačević, D**.** (2024) Strawberry tree fruits (*Arbutus unedo* L.): Bioactive composition, cellular

antioxidant activity, and 3D printing of functional foods. *Food Chemistry*, **433**, 137287. DOI: 10.1016/j.foodchem.2023.137287.

- **Bebek Markovinović, A**., Stulić, V., Putnik, P., Bekavac, N., Pavlić, B., Milošević, S., Velebit, B., Herceg, Z., Bursać Kovačević, D. (2024) High-Power Ultrasound (HPU) and Pulsed Electric Field (PEF) in the Hurdle Concept for the Preservation of Antioxidant Bioactive Compounds in Strawberry Juice—A Chemometric Evaluation—Part II. *Foods*, **13**(4), 537. DOI: 10.3390/foods13040537.
- Djekić, I., Velebit, B., Pavlić, B., Putnik, P., Šojić Merkulov, D., **Bebek Markovinović, A**., Bursać Kovačević, D. (2023) Food Quality 4.0: Sustainable Food Manufacturing for the Twenty-First Century. *Food Engineering Reviews*, **15** (2023), 2023, 577-608. DOI: 10.1007/s12393-023-09354-2.
- Milošević, S., **Bebek Markovinović, A**., Teslić, N., Mišan, A., Pojić, M., Brčić Karačonji, I., Jurica, K., Lasić, D., Putnik, P., Bursać Kovačević, D. Use of Natural Deep Eutectic Solvent (NADES) as a green extraction of antioxidant polyphenols from strawberry tree fruit (*Arbutus unedo* L.): An optimization study. *Microchemical Journal*, **200**, 110284, DOI: 10.1016/j.microc.2024.110284.
- **Bebek Markovinović, A.**, Bosiljkov, T., Janči, T., Kostić, M., Dedović, N., Lučić, E., Bavrka, K., Pavlić, B., Bursać Kovačević, D. (2024) Characterization of Antioxidant Bioactive Compounds and Rheological, Color and Sensory Properties in 3D-Printed Fruit Snacks. *Foods*, **13**, 1623. DOI: 10.3390/foods13111623.
- Palac Bešlić, I., Ivešić, M., Mandić Andačić, I., Bursać Kovačević, D., Žuntar, I., **Bebek Markovinović, A.**, Oštarić, F., Krivohlavek, A. (2024) Development and Optimization Method for Determination of the Strawberries'Aroma Profile. Molecules, **29**, 3441. DOI: 10.3390/molecules29143441.

Abstracts in Book of abstracts

- **Bebek Markovinović, A**., Putnik, P., Bursać Kovačević, D. (2021) Hurdle technology and 3D printing for environmentally friendly production of functional fruit juices. In: *Book of Abstracts of 1st international scientific conference Food & Climate Change*. Šamec, D., Šarkanj, B., Sviličić Petrić, I. (ur.). Koprivnica, Croatia, 24-24.
- Putnik, P., Palac Bešlić, I., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., Duralija, B., **Bebek Markovinović, A.**, Bursać Kovačević, D. (2021) Valorization of bilberry pomace for potential use in functional food production. In: *Book of Abstracts of 13th International Scientific and Professional Conference WITH FOOD TO HEALTH*. Babić, J., Šubarić, D., Jašić, M. (ur.). Osijek, Croatia, 60-60.
- **Bebek Markovinović, A.**, Duralija, B., Putnik, P., Bursać Kovačević, D. (2021) Changes in physicochemical and sensory properties of strawberries during processing into juice. In: *Book of Abstracts of the 13th International Scientific and Professional Conference WITH FOOD TO HEALTH*. Babić, J., Šubarić, D., Jašić, M. (ur.). Osijek, Croatia 57-57.
- Bursać Kovačević, D., Valjak, F., Žuntar, I., Putnik, P., **Bebek Markovinović, A.** (2021) Danas printam ja, a sutra ćeš ti! – 3D printanje hrane kao kultura nove dimenzije zdravlja. In: *Festival Znanosti 2021,* Zagreb, Hrvatska, 1-1.
- **Bebek Markovinović, A.**, Lasić, D., Jurica, K., Brčić Karačonji, I., Brdar, D., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., Palac Bešlić, I., Putnik, P., Bursać Kovačević, D. (2022) Strawberry tree fruit (*Arbutus unedo* L.) as a valuable ingredient for functional food production. In: *Traditional XXXIV Scientific-Professional Conference - Processing And Energy In Agriculture PTEP 2022*. Sokobanja, Serbia, 1-1.
- **Bebek Markovinović, A.**, Stulić, V., Batur, L., Putnik, P., Vukušić, T., Herceg, Z., Duralija, B., Zavadlav, S., Bursać Kovačević, D. (2022) Effect of pulsed electric field treatment on polyphenols stability in strawberry juices. In: *Traditional XXXIV Scientific-Professional Conference - Processing And Energy In Agriculture PTEP 2022*. Sokobanja, Serbia, 1-1.
- **Bebek Markovinović, A.**, Stulić, V., Batur, L., Putnik, P., Vukušić Pavičić, T., Herceg, Z., Duralija, B., Bursać Kovačević, D. (2022) Effect of pulsed electric field (PEF) treatment on stability of bioactive compounds in strawberry juice. In: *CEFood*

Congress Book. Raspor, P., Vovk, I., Ovca, A., Smole Možina, S., Butinar, B., Jevšnik, M. (ur.)., Čatež ob Savi, Slovenia, 171-171.

- Huđek Turković, A., Butumović, L., **Bebek Markovinović, A.**, Durgo, K., Bursać Kovačević, D. (2022) Biological activity of strawberry tree fruit. In: *CEFood Congress Book*. Raspor, P., Vovk, I., Ovca, A., Smole Možina, S., Butinar, B., Jevšnik, M. (ur.)., Čatež ob Savi, Slovenia, 172-172.
- **Bebek Markovinović, A**., Pavlić, B., Putnik, P., Bursać Kovačević, D. (2022) Green extraction of bioactive compounds from bilberry pomace. In: *Book of Abstracts of the 4th International Congress on "Green Extraction of Natural Products" (GENP2022)*. Karlović, S., Dujmić, F., Ninčević Grassino, A. (ur.). Poreč, Croatia, 42-42.
- Putnik, P., **Bebek Markovinović, A.**, Bičanić, P., Bursać Kovačević, D. (2022) Monitoring the stability of bioactive compounds in strawberry juice treated by high power ultrasound through chemometrics. In: *Book of Abstracts of the 4th International Congress on "Green Extraction of Natural Products" (GENP2022)*. Karlović, S., Dujmić, F., Ninčević Grassino, A. (ur.). Poreč, Croatia, 45-45.
- **Bebek Markovinović, A.**, Valjak, F., Žigolić, A., Putnik, P., Duralija, B., Bursać Kovačević, D. (2022) Application of additive technology in a production of functional strawberry-based product. In: *10th International Congress of Food Technologists, Biotechnologists and Nutritionists*. Komes, D. (ur.). Zagreb, Croatia, 10-10.
- **Bebek Markovinović, A.**, Bičanić, P., Putnik, P., Duralija, B., Bursać Kovačević, D. (2022) Effect of high-power ultrasound (HPU) on stability of bioactive compounds in strawberry juices. In: *Book of Abstracts - 10th International Congress of Food Technologists, Biotechnologists and Nutritionists*. Komes, D. (ur.). Zagreb, Croatia, 9-9.
- Palac Bešlić, I., Krivohlavek, A., Mandić Andačić, I., Ivešić, M**., Bebek Markovinović, A.**, Putnik, P., Bursać Kovačević, D. (2022) Development and optimization of a method for determining the aroma profile of strawberries. In: *4th International Congress on Food Safety and Quality "One Health"*, Dubrovnik, Croatia.
- **Bebek Markovinović, A.**, Brdar, D., Brčić Karačonji, I., Karlo, J., Lasić, D., Putnik, P., Bosiljkov, T., Bursać Kovačević, D. (2023) Strawberry tree fruit (*Arbutus unedo* L.) and 3D printing – Development of an innovative functional food product. In: *Book of Abstracts 58th Croatian & 18th International Symposium on Agriculture*. Carović-Stanko, K., Širić, I. (ur.). Zagreb, Croatia, 193-193.

- **Bebek Markovinović, A.**, Brdar, D., Brčić Karačonji, I., Jurica, K., Lasić, D., Putnik, P., Bosiljkov, T., Duralija, B., Bursać Kovačević, D. (2023) Development of functional strawberry tree fruit-based product by 3D food printing technology. In: *The Book of Abstracts - V Balkan Symposium on Fruit Growing University of Zagreb Faculty of Agriculture*. Skrendrović Babojelić, M.; Duralija, B.; Vuković, M. (ur.). Zagreb, Croatia, 90-90.
- **Bebek Markovinović, A.**, Brdar, D., Brčić Karačonji, I., Jurica, K., Lasić, D., Putnik, P., Bursać Kovačević, D. (2023) Primjena tehnologije 3D ispisa u proizvodnji funkcionalnog snack proizvoda od maginje (*Arbutus unedo* L.). In: *Knjiga sažetaka Dani doktorata biotehničkog područja 2023*. Klarić, M., Beljo Lučić, R., Kos, B., Ivanković, A., Jovanović, J., Lovrinčević, M. (ur.). Zagreb, Hrvatska, 62-64.
- Putnik, P.; Tomiša, M., Zavadlav, S., **Bebek Markovinović, A.**, Bursać Kovačević, D. (2023) 3D food printing: sustainable food manufacturing for the 21st century. In: *IRASA International Scientific Conference SCIENCE, EDUCATION, TECHNOLOGY AND INNOVATION - Book of Proceedings*. 181-189.
- Milošević, S., **Bebek Markovinović, A.**, Teslić, N., Mišan, A., Pojić, M., Brčić Karačonji, I., Jurica, K., Lasić, D., Putnik, P., Bursać Kovačević, D., Pavlić, B. (2023) Screening of natural deep eutectic solvent (NADES) towards improved isolation of polyphenolic antioxidants from strawberry tree fruit (*Arbutus unedo* L.). In: *29th International Symposium on Analytical and Environmental Problems, Book of Abstracts*. Szeged, Hungary, 226-227.
- Milošević, S., **Bebek Markovinović, A.**, Teslić, N., Mišan, A., Pojić, M., Brčić Karačonji, I., Jurica, K., Lasić, D., Putnik, P., Božović, D., Bursać Kovačević, D., Pavlić, B. (2023) Multi-response optimization of natural deep eutectic solvent (NADES) extraction of polyphenols from strawberry tree fruit (*Arbutus unedo* L.) In: *International Conference BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY FOR YOUNG SCIENTISTS, Book of Abstracts*, Belgrade, Serbia, 18-18.
- Bursać Kovačević, D., Valjak, F., Putnik, P., Bavrka, K., Bekavac, N., **Bebek Markovinović, Anica.** (2023) 3D printanje hrane u svrsi očuvanja zdravlja društva i prirode. In: *Festival Znanosti 2023,* Zagreb, Croatia, 1-1.