Utilization of fruit processing by-products and edible films for enhancing nutritional value, bioactive potential and extending the shelf life of cookies

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UTILIZATION OF FRUIT PROCESSING BY-PRODUCTS AND EDIBLE FILMS FOR ENHANCING NUTRITIONAL VALUE, BIOACTIVE POTENTIAL AND EXTENDING THE SHELF-LIFE OF COOKIES

DOCTORAL DISSERTATION

Supervisors:
Mario Ščetar, Ph.D., Associate Professor
Dubravka Novotni, Ph.D., Full Professor

Zagreb, 2024.

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Prehrambeno – biotehnološki fakultet

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PRIMJENA NUSPROIZVODA PRERADE VOĆA I JESTIVIH FILMOVA RADI POVEĆANJA NUTRITIVNE VRIJEDNOSTI, BIOAKTIVNOG POTENCIJALA I PRODULJENJA TRAJNOSTI ČAJNOG PECIVA

DOKTORSKI RAD

Mentori:

izv. prof. dr. sc. Mario Ščetar

prof. dr. sc. Dubravka Novotni

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Dunja Molnar, mag. nutr.

Thesis performed at Faculty of Food Technology and Biotechnology, University of Zagreb
Mentor: *Mario Ščetar*, **Ph.D., Associate professor**, *Dubravka Novotni*, **Ph.D., Full professor**

Abstract: This dissertation explores the substitution of cocoa powder with grape- and aronia pomace in whole grain cookies regarding their quality and consumers acceptance. An edible film made of chitosan, gum arabic, and grape seed extract was tested for its impact on nutrition value, antioxidants, shelf-life, and starch digestibility of cookies. The results showed substitution of 24 % cocoa powder with pomaces an optimum resulting in improved flavonoid content by 22 %, antioxidant potential by 27-73 %, and extended shelf-life by at least 30 days. Cookie consumption did not lead to an increase of oxidized low-density lipoprotein receptors in healthy women. Additionally, the majority of consumers (96 %) in Croatia, France and North Macedonia expressed their interest in purchasing environmentally friendly cookies.

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Mentor: izv. prof. dr. sc. *Mario Ščetar*, **prof. dr. sc.** *Dubravka Novotni*

Sažetak: Ova disertacija istražuje mogućnost zamjene kakao praha u recepturi integralnog čajnog peciva tropom grožđa i tropom aronije s obzirom na njihovu kvalitetu i prihvaćanje kod potrošača. Istražen je i utjecaj jestivog filma na bazi kitozana i gume arabike s ekstraktom sjemenki grožđa na nutritivni sastav, antioksidacijsku aktivnost, produljenje trajnosti čajnog peciva te probavljivost škroba. Rezultati su pokazali da nusproizvodi voća mogu uspješno zamijeniti 24 % kakao praha u čajnom pecivu, a pritom doprinose povećanju sadržaja flavonoida (22 %) i antioksidacijskog potencijala (27-73 %) te produljuju rok trajanja za najmanje 30 dana. Umjerena konzumacija čajnog peciva ne povećava koncentraciju receptora za oksidirani lipoprotein niske gustoće kod zdravih žena. Dodatno, većina potrošača (96 %) u Hrvatskoj, Francuskoj i Sjevernoj Makedoniji je potvrdila interes kupnje održivog čajnog peciva.

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Rad je pohranjen u knjižnici Prehrambeno-biotehnološkog fakulteta u Zagrebu, Kačićeva 23, Nacionalnoj i sveučilišnoj knjižnici u Zagrebu, Hrvatske bratske zajednice bb i Sveučilištu u Zagrebu, Trg Republike Hrvatske 14.

Small ideas can transition into something big if you are surrounded by people who believe in you and encourage you.

Undertaking this doctoral thesis research program was an exciting journey, but a long one filled with lots of ups and downs. It was not an easy win, but despite my great will and effort, it would never have ended if there were not many people along the way who supported and helped me to make it happen. It started as a small idea, a dream, with no financial investment in this project, but in the end, it grew into something bigger with the support of many people who believed in me and helped me during this journey. Each of you was a piece of the puzzle, and together we were able to create an amazing picture! Therefore, it is my great pleasure to express my acknowledgment to those who supported me during this journey.

First of all, I would like to express my sincere appreciation, gratitude, and thanks to my supervisors Mario Ščetar, Ph.D., Associate Professor, and Dubravka Novotni, Ph.D., Full Professor, for their guidance, time, efforts, patience and for sharing their knowledge with me. Thank you for giving me the opportunity to work with you, for guiding me, pushing me when needed and for encouraging me along the way. Thank you for showing me that I have capabilities and for the confidence you gave me. You were my mentors but also friends. I hope we will maintain our friendship and collaboration in the future.

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Special thanks go to Tanja Dinić, Anamarija Ilijaš, and Sample Control for their great help and assistance with physico-chemical analysis. I really appreciate your involvement and willingness to support my Ph.D.

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None of this could have been achieved without the great support, love, and advice from my parents and my brother. You never doubted me, my capabilities, and you gave me strength to go through this journey. You were my greatest support in good and bad days, and you made my life beautiful. I love you so much!

In the end, I would like to thank Hrvoje for being here for me, for supporting me, and for taking part in making packaging for my cookies! You became a real expert in doing it!

I would like to dedicate this dissertation to my family:

my mother, my father, and my brother.

The dissertation topic was accepted at the 11th regular session of the Faculty Council of the Faculty of Food Technology and Biotechnology, of the University of Zagreb in the academic year 2019/2020. held on September 29th,2020, and the University of Zagreb Senate approved the initiation of the procedure for obtaining a doctorate of science within the doctoral study on July 13th, 2021 at the 12th regular session in the 352nd academic year 2020/2021.

Extended Abstract

The increasing demand for greater utilization of food industry by-products has been driven by a growing interest in environmental sustainability. Cocoa powder is one of the most popular ingredients in the bakery industry, and the demand for cocoa is constantly growing, leading to higher prices. Therefore, it is important to find an adequate substitute for cocoa with a reduced impact on the environment. The utilization of edible and biodegradable films is able to reduce the amount of disposable packaging needed, which can further lead to a reduction in environmental pollution and CO_2 emission. The first aim of this dissertation was to investigate the optimization of the whole grain cookie formulation by partial substitution of cocoa powder with powdered grape- and aronia pomace. The second aim of this dissertation was to explore the physico-chemical, morphological, and thermal characteristics of edible films based on chitosan and gum arabic, enriched with grape seed extract. Additionally, the aim was to determine how their application impacts both the extension of the shelf-life of cookies and improvement of their overall quality. The present dissertation also aimed to investigate the effects of replacing cocoa powder with fruit by-products and the application of edible films on the nutritional composition, antioxidant activity, and starch digestibility of cookies. Further the aim was to investigate the effects of cookie consumption on oxidized low-density lipoprotein (oxLDL) receptors in healthy women and to examine the market potential and consumer attitude towards whole grain and environmentally friendly cookies in three Mediterranean countries (Croatia, France, and North Macedonia). This included evaluating the consumers level of sustainability knowledge and nutritional awareness, their willingness to purchase environmentally friendly cookies containing food by-products, and evaluating the differences in answers between countries and generations. The findings suggest that utilizing powdered grape- and aronia pomace as sustainable alternatives could replace up to 24 % of cocoa powder in whole grain cookies. The most significant impact was found regarding the physical properties of the product, where cocoa replacement was leading to larger spread ratios, reduced hardness (-23 %) and toughness (-19 %), while maintaining sensory acceptability comparable to cookies only containing cocoa. Furthermore, edible films based on chitosan and gum arabic with grape seed extract have shown desirable mechanical properties that could improve the quality and that were able to extend the shelf-life for at least 30 days compared to the control cookies. The results show that cookies with grape- and aronia pomace and edible film enriched with grape seed extract had an increased flavonoid content (22 %) and antioxidant potential (27–73 %) compared to the control cookies. Moreover, the content of slowly digestible starch predominated over rapidly digestible starch. In a randomized controlled study on healthy women, a moderate consumption of cookies with grape- and aronia pomace and edible film enriched with grape seed extract was found not to increase the concentration of oxLDL receptors in healthy women, but a potential connection between the concentration of oxLDL receptors and the waist circumference was observed. A cross-sectional study conducted in three Mediterranean countries (Croatia, France and North Macedonia) showed that whole grain cookies are on first or second place when looking into preferability. Also, 96 % of participants expressed their interest in purchasing environmentally friendly cookies. Yet, the participants knowledge about by-products and their health benefits was limited. Nevertheless, market potential for whole grain cookies with by-products exists, but factors such as brand, price and consumer education play a key role in the purchasing decision.

Key words: *fruit by-products, edible films, chitosan, gum arabic, coated cookies, oxidized LDL receptor, starch digestibility, nutritional awareness, sustainability knowledge*

Prošireni sažetak

Porast zanimanja za ekološku održivost povećao je potražnju za korištenjem nusproizvoda u prehrambenoj industriji. Kakao je jedan od najpopularnijih pomoćnih sastojaka u pekarskoj industriji zbog čega potražnja za kakaom neprestano raste te posljedično možemo očekivati porast cijene kakaa. Iz tog je razloga važno pronaći adekvatnu zamjenu za kakao sa smanjenim utjecajem na okoliš. Korištenjem jestivih i biorazgradivih filmova može se smanjiti količina otpada, te posljedično smanjiti onečišćenje okoliša i emisije CO_2. Cilj ove disertacije je istražiti mogućnost zamjene kakao praha nusproizvodima proizvodnje soka grožđa i aronije u recepturi integralnog čajnoga peciva. Drugi cilj ove disertacije bio je ispitati fizikalno-kemijska, morfološka i termalna svojstva jestivog filma na bazi kitozana i gume arabike obogaćenog ekstraktom sjemenki grožđa te ispitati njihov utjecaj na nutritivni sastav, antioksidacijsku aktivnost, probavljivost škroba te produljenje trajnosti gotovog proizvoda. Također, cilj ove disertacije je istražiti utjecaj konzumacije čajnog peciva s nusproizvodima proizvodnje voća, (trop grožđa i aronije), i jestivim filmom na bazi kitozana i gume arabike obogaćenog ekstraktom sjemenki grožđa na receptor za oksidirani lipoprotein niske gustoće (oxLDL) u zdravih ženskih osoba te ispitati tržišni potencijal i stavove potrošača prema funkcionalnom i održivom čajnom pecivu u tri mediteranske zemlje (Hrvatska, Francuska i Sjeverna Makedonija). Istraživanje je uključivalo i procjenu razine znanja o održivosti i nutritivnoj svijesti, spremnosti kupovine čajnog peciva s nusproizvodima hrane i procjenu razlika u odgovorima između zemalja i generacija. Rezultati pokazuju da nusproizvodi proizvodnje soka grožđa i aronije, kao održiva alternativa, mogu uspješno zamijeniti do 24 % kakao praha u proizvodnji integralnog čajnog peciva, smanjujući pritom tvrdoću (-23 %) i žilavost (19 %), što rezultira većim faktorom širenja te jednakom senzorskom prihvatljivošću u usporedbi s čajnim pecivom koje sadrži samo kakao. Nadalje, jestivi filmovi na bazi kitozana i gume arabike s ekstraktom sjemenki grožđa imaju poželjna svojstva koja mogu poboljšati kvalitetu i produljiti rok trajnosti za najmanje 30 dana u odnosu na kontrolno čajno pecivo. Čajno pecivo s tropom grožđa i aronije te s jestivim filmovima obogaćenim ekstraktom sjemenki grožđa ima povećan sadržaj flavonoida (22 %) i antioksidacijski potencijal (27-73 %) u usporedbi s kontrolnim keksom. Također, sadržaj sporo probavljivog škroba prevladava

nad brzo probavljivim škrobom. Randomizirana kontrolirana studija na zdravim ženskim osobama pokazala je da umjerena konzumacija čajnog peciva s tropom grožđa i aronije te s jestivim filmovima obogaćenim ekstraktom sjemenki grožđa ne povećava koncentraciju oksidiranih LDL receptora kod zdravih žena, ali da moguće postoji povezanost između koncentracije oksidiranog LDL receptora i opsega struka. Presječno istraživanje provedeno u trima mediteranskim zemljama (Hrvatska, Francuska i Sjeverna Makedonija), pokazalo je da je čajno pecivo od cjelovitih žitarica na prvom odnosno drugom mjestu kada govorimo o preferenciji. Također, 96 % ispitanika je potvrdilo interes za kupnju održivog čajnog peciva. Ipak, znanje potrošača o nusproizvodima i njihovim blagotvornim učincima koje imaju za zdravlje je ograničeno. Stoga, tržišni potencijal čajnog peciva od cjelovitih žitarica s nusproizvodima postoji, ali brand, cijena i obrazovanje potrošača pritom imaju ključnu ulogu prilikom kupovine.

Ključne riječi: *nusproizvodi voća, jestivi filmovi, kitozan, guma arabika, obloženi keksi, oksidirani LDL receptor, probavljivost škroba, svijest o prehrani, znanje o održivosti*

Information about the Supervisor – Mario Ščetar, Ph.D., Associate Professor

MARIO ŠČETAR, Ph.D., serves as an associate professor at the University of Zagreb Faculty of Food Technology and Biotechnology. He obtained his doctorate in Food Science and Technology from the same institution in 2014. Since 2021, he has held the position of Associate Professor, instructing undergraduate and graduate students in courses focused on food packaging and the preservation of packaged food products. Prof. Dr. Ščetar's research revolves around edible and polymer films for food packaging, exploring their impact on product shelf-life. He underwent scientific training abroad at the University of Burgundy, Dijon, France, focusing on *Water, Active Molecules, Macromolecules, and Activities* (EMMA EA 581). With over 50 peer-reviewed journal articles and numerous book chapters published by reputable publishers such as Elsevier, Springer, and CRC Press, Prof. Dr. Ščetar has made significant contributions to the field. He maintains an active presence in national and international scientific conferences, presenting his findings regularly. Prof. Dr. Ščetar's expertise extends to peer review, as he has evaluated numerous scientific papers for various journals and served as the editor of several special issues. He is affiliated with the Croatian Society of Food Technologists, Biotechnologists, and Nutritionists, and contributes to the working team at the Croatian Agency for Agriculture and Food. Since 2009, Prof. Dr. Ščetar has been involved in numerous national and international research projects. Presently, he serves as an investigator on projects funded by the Croatian Science Foundation, including *Sustainable Concept in Active Edible Coatings Development for Shelf-Life Extension of Fresh Adriatic Fish* and *Laser Synthesis of Nanoparticles and Applications*. Additionally, Prof. Dr. Ščetar participates as the Croatian partner in various international initiatives, such as *Food Packaging Open Courseware for Higher Education and Staff of Companies 2.0* (FitNESS 2.0), COST Action *Rethinking packaging for circular and sustainable food supply chains of the future*, PRIMA *INTelligent, ACTive MicroBIOme-based, biodegradable PACKaging for Mediterranean food* – INTACTBioPack and PRIMA *Design, processing and characterisation of innovative biodegradable and compostable active packaging structures to improve food safety of Mediterranean foods* – EVOLVEPACK (Partnership for Research and Innovation in the Mediterranean Area).

Information about the Supervisor – Dubravka Novotni, Ph.D., Full Professor

DUBRAVKA NOVOTNI is a full professor at the University of Zagreb Faculty of Food Technology and Biotechnology. In 2011 she obtained a Ph.D. in Nutrition from the same faculty. Since 2023, she works there as a full professor, teaching courses related to cereal chemistry and technology, gluten-free food, food extrusion, organic food production, etc. Her scientific work includes researching the different technologies and ingredients (including by-products) for making bakery products of enhanced nutritional value, technological quality, and shelf-life. She had her scientific training abroad at Warsaw University of Life Sciences in Poland, University of Natural Resources and Life Sciences, Vienna in Austria, Institute of Agrochemistry and Food Technology, Valencia, Spain, and with the Cochran Fellowship in USA. Dubravka Novotni has published over 50 peer reviewed journal articles with more than 500 citations (h-index 13) and authored several book chapters with recognized scientific publishers like Elsevier, Springer and CRC Press. She presents her work consistently at international scientific conferences in different countries. She reviewed numerous scientific papers for peer reviewed journals. She is a member of the Technical Committee of International Association for Cereal Science and Technology, Croatian Society of Food Technologists, Biotechnologists and Nutritionists, Croatian Chemical Society, working team Codex Alimentarius, Board for cereals and pulses, Council for implementing a use of Croatian Fields Flour Sign and the Croatian Field Bread Sign, Croatian Agency for Agriculture and Food, committee for the Regulation on cereals and cereal products, and the Expert working group on the Regulation of salt levels in bakery products (Ministry of Agriculture). Since 2006 she has been involved in numerous national and international research projects. Currently, she is the principal investigator of the project *Development of new generation of snack food for consumers with specific dietary needs using 3D printing technologies*, Croatian Science Foundation (HRZZ 3829), as well as the principal investigator of the Croatian partner in *Flat bread of Mediterranean area; innovation & emerging process & technology*, Partnership for Research and Innovation in the Mediterranean Area (PRIMA, 2031).

Authors publications included in the doctoral dissertation:

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Publication No. 2

Molnar, **D**., Gabaj, N.N., Vujić, L., Ščetar, M., Krisch, J., Miler, M., Štefanović, M., Novotni, D., 2023. Application of fruit by-products and edible film to cookies: Antioxidant activity and concentration of oxidized LDL receptor in women—A first approach. *Applied Science,* **13**, 5513. 513. https://doi.org/10.3390/app13095513

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List of Abbreviations

*a**	Lab coordinate
AA	Antioxidant activity
AC	After Christ
ACE	Angiotensin converting enzyme
ALT	Alanine transaminase
ASLT	Accelerated shelf-life testing
AST	Aspartate transaminase
*b**	Lab coordinate
BC	Before Christ
BMI	Body mass index
CAD	Coronary artery disease
CC	Control cookie
CO_2	Carbon dioxide
CRP	C-reactive protein
CVDs	Cardiovascular diseases
DBP	Diastolic blood pressure
DD	Degree of deacetylation
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimetry
EC	Endothelian cells
eNOS	Endothelial nitric oxide synthase
EPC	Endothelial progenitor cells
ET-1	Endothelin-1
FABP1	Fatty acid binding protein 1
FABP4	Fatty acid binding protein 4
FAS	Faty acid synthase
FFS	Film forming solution
FTIR	Fourier transform infrared spectroscopy

GA	Gum arabic
GAE	Gum arabic with extract
GAP	Cookies with grape- and aronia pomace
GAP with KGAE	Cookies with grape- and aronia pomace coated with edible film
GSE	Grape seed extract
GSH-Px	Glutathione peroxidase
HDL	High density lipoprotein
ICAM	Intercellular adhesion molecule
IL-1'	Interleukin 8
IL-6	Interleukin 6
IL-8	Interleukin 8
KGA	Chitosan and gum arabic
KGAE	Chitosan and gum arabic with extract
*L**	Lab coordinate
LDL	Low-density lipoprotein
LOX-1	Lecitin like receptor 1
LPL	Lipoprotein lipase
MCP1	Monocyte chemoattractant protein-1
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated b cells
NO	Nitric oxide
Ox-LDL	Oxidized low-density lipoprotein
PCA	Principal component loadings
PGE2	Prostaglandin e2
pH	Potential of hydrogen
RDS	Rapidly digestible starch
ROS	Reactive oxygen species
SBD	Systolic blood pressure
SEM	Scanning electron microscopy
SDS	Slowly digestible starch
TAG	Triacylglycerol

TBARS	Substances reacting with thiobarbituric acid
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor-α
TPC	Total phenol content
VCAM	Vascular cell adhesion molecule
VSMC	Vascular smooth muscle cell
WPA	Water vapor permeability
WS	Water solubility

Table of Contents

General introduction

In recent years, there has been a growing concern among consumers about the environmental sustainability of the food supply chain (Sörqvist et al., 2015). The global food system encounters a significant challenge related to food waste, with more than one-third of the food intended for human consumption being lost or wasted every year (Yilmaz and Kahveci, 2022).

Cookies are a very popular cereal-based food consumed by both children and adults worldwide (Florence et al., 2014) due to their nutrient-dense profile, diverse flavors and shapes, and due to their long shelf-life (Ajila et al., 2008). Given the growing interest in functional cookies enriched with various nutrients and bioactive compounds such as dietary fiber, β-glucans, polyphenols or vitamins, cookie consumption can be incorporated into global nutrition strategies to address several nutrient deficiencies as well as chronic and nutrition-related diseases (Canalis et al., 2017) such as celiac disease, obesity, diabetes mellitus type II and cardiovascular diseases (Kekalih et al., 2019; Buffière et al., 2020; Puerta et al., 2022; Molnar et al., 2023).

Food industry by-products including peels, remnants of pulp, seeds, and stems, depending on the type of fruit, represent 20 % to 50 % of the original fruit mass (Galanakis, 2017). The rising interest in extracting nutrients from by-products for waste management with economic and environmental benefits (Palocci and Chronopoulou, 2015), makes fruit by-products a relevant source of dietary fiber and polyphenols for cookie production (Prokopov et al., 2015). Previous studies have indicated that increasing the amount of grape pomace in cookies can reduce their acceptance by consumers due to an intense fruity-acidic taste and a darker color. Cookies with maximum of 10 % added grape pomace were the best accepted (Acun et al., 2014). While numerous scientific studies have been conducted, they have primarily focused on exploring the physicochemical and sensory properties of cookies enriched with by-products from individual fruit varieties or other specific sources. An extensive review of the existing literature was not able to identified any published studies regarding the simultaneous enrichment of cookies with by-products from aronia and grape juice production. Therefore, additional research and emphasis are needed to show the acceptance of cookies containing fruit by-products.

Finding a suitable alternative for cocoa powder with reduced environmental impact is becoming imperative (Loullis and Pinakoulaki, 2018) since the main limitation of commercial alternatives, such as carob, starch-based raw materials, legumes, soy flour, chicory, chocolate flavoring, and various additives, is that they differ from natural cocoa powder in one or more properties, such as color, water solubility, and chemical composition (Rikon and del Valle, 1982). Fruit by-products might serve as a partial substitute for cocoa powder in cookies. However, no existing study has explored the potential of substituting cocoa powder with fruit by-products.

Packaging plays an important role in the marketing and shelf-life of cookies. As a result of environmental influences during storage, such as exposure to light and oxygen, the fat content of fat-rich cookies can undergo notable changes. Depending on the level of exposure this can lead to a reduction in both the quality and nutritional value of the product (Lu & Xu, 2010). Hence, the crucial aspect for packaging materials is their capability to establish strong barriers against the infiltration of oxygen and water vapor (Galić et al., 2009). Oriented polypropylene (OPP) and metalized polymers have emerged as the primary selections in the domain of commercial cookie packaging (Galić et al., 2009). Besides, the growing awareness and tightening of regulations on sustainability in product development and industrial practices have increased the interest in innovative packaging options, such as edible and biodegradable films. Developments in this area show potential for decreasing the widespread use of disposable packaging, which can lead to a decrease in environmental impact and carbon emissions. Additionally, their use can significantly improve product quality in several ways: by preventing moisture loss, reducing gas permeability, controlling migration of water-soluble substances, and incorporating functional ingredients such as vitamins, minerals, antioxidants into edible films (Vujković et al., 2017).

Consuming cookies high in sugar and saturated fat in high amounts increases health risks including cardiovascular diseases (CVDs) and type II diabetes mellitus (DiNicolantonio et al., 2016). CVDs are a significant cause of premature death in Europe, according to the World Health Organization (Benjamin et al., 2019). While cookie consumption affects blood glucose and lipid levels, it is the oxidation of low-density lipoprotein (LDL) cholesterol that primarily contributes to the development of CVDs (DiNicolantonio et al., 2016). Research indicates that certain food ingredients like cocoa powder, olive oil, grapes, wine, grape seed extract, and aronia can inhibit LDL cholesterol oxidation, potentially leading to a reduced CVD risk (Taheri et al., 2013). Nevertheless, a gap in the literature has been identified regarding the impact of consuming cookies enriched with fruit by-products on the LDL cholesterol oxidation, highlighting a need for further investigation in this area.

Understanding the consumers motivation behind choosing functional or sustainable (upcycled) food is crucial for the growth of a more sustainable industry (Sukboonyasatit, 2009).

Previous studies explored and clarified consumer behaviour concerning the purchase intention, acceptance, and consumption of cookies (Nystrand and Olsen, 2020; Sielicka-Rózynska et al., 2021), considering factors like sensory appeal, demographics, perceived benefits, and marketing strategies (Dean et al., 2007; Kraus, 2015).

While factors such as availability, quality, brand, novelty, packaging, and pricing were proven to influence consumer habits (Puerta et al., 2022a), only a limited number of studies has examined the market viability of functional and sustainable cookies.

Chapter 1

Theoretical background

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2. Cocoa – composition, health benefits and the need for sustainable alternatives

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1. Ready-to-eat food – Cookies

Cookies have become popular cereal-based products appreciated by both the young and elderly due to their nutrient content, satisfying human cravings for sugar and chocolate, being available in diverse shapes and flavors, convenient ready-to-eat formats, and due to their long shelf-life (Ajila et al., 2008, Crofton and Scannell, 2020). Nowadays, cookies serve various purposes from functioning as luxurious gift or as a convenient snack to being used as pet food for dogs and cats. Additionally, they are used as decorative items, incorporating ingredients such as chocolate, cream, nuts, and a variety of flavors (Arepally et al., 2020).

Within the fast-moving consumer goods category, the cookie market stands out as one of the leading sectors (Apeda, 2020). Projections indicate that European cookie consumption is expected to reach 11 billion kilograms by 2026, which means a 0.2 % year-on-year increase. In 2021, the United Kingdom took the lead in cookie consumption, reaching 2.5 billion kilograms, followed by Spain, Italy, and France (ReportLinker, 2022).

Main ingredients used in cookie formulations include flour, fat or oil, sugar, water, and raising agents such as sodium bicarbonate or ammonium bicarbonate. Secondary ingredients include but are not limited to salt, egg, emulsifier, milk powder, and flavoring compounds. According to the Croatian regulation cookies are baked products made from shaped soft dough with a minimum of 10 % fat and a maximum of 5 % water (Official Gazette no. 73/2010). Cookie consumption is popular although consumers automatically perceive cookies as unhealthy food due to their high fat and sugar content.

However, based on previous studies, cookies can be integrated into public nutrition strategies aimed to tackle several nutrient deficiencies as well as chronic and nutrition–related diseases (Canalis et al., 2017) such as celiac disease, diabetes mellitus type II, obesity and cardiovascular diseases (Kekalih et al., 2019; Buffière et al., 2020; Puerta et al., 2022b; Molnar et al., 2023). An increasing demand for the development of cookies that possess three key characteristics: traditional nutritional elements, health benefits through regular consumption and sustainability are described by Arepally et al., 2020.

2. Cocoa – composition, health benefits and the need for sustainable alternatives

The cocoa fruit, scientifically known as *Theobroma cacao*, is known to originate from the western coast of South America. Its name, derived from the Greek words for *God* and *Food*, reflects its status as the *Food of the Gods* in the Mayan civilization. The discovery of cocoa is credited to the Olmec population during the period of 1500 to 400 BC. Afterwards, it was cultivated by Mesoamericans (600 BC) and later by the Aztecs (400 AC), who esteemed its divine origins, transforming it into what they considered *ancestral* chocolate (Jean-Marie et al., 2021).

Cocoa, an evergreen tree from the *Sterculiaceae* family, naturally thrives in the tropical region of Central and South America, but it is also cultivated in various other regions (Doaré et al., 2020). The plant comprises four primary varieties: *Criollo* (the initial cultivated group), *Forastero* (the most robust and widespread group), and *Trinitario* (a hybrid of *Criollo* and *Forastero*) each with distinct phytochemical content and sensory attributes (Jean-Marie et al., 2021). These trees produce millions of tons of cocoa beans annually, which are then processed to cocoa powder, cocoa butter, and cocoa liquor (Castro-Alayo et al., 2019).

Cocoa beans consist of water, lipids, proteins, fibers, and various other biologically active compounds (Jean-Marie et al., 2021). A total of approximately 380 compounds have been identified in cocoa beans (Andújar et al., 2012), with polyphenols and xanthine alkaloids being predominant, contributing to approximately 14-20 % of the mass of the cocoa bean. Cocoa is particularly abundant in flavanols, especially in their monomeric forms, such as (+)-catechin and (−)-epicatechin, as well as in their oligomeric and polymeric forms, procyanidins. The polyphenolic profile of cocoa beans varies based on cultivar type, crop quality, cultivation location, geographic area, climate, influence of processing and production steps (Jean-Marie et al., 2021).

Cocoa and its derivatives are widely consumed due to their pleasant taste and positive health impacts. The health benefits of cocoa are closely related to its phytochemicals, especially polyphenols. Numerous studies have demonstrated that the intake of cocoa and its products is associated with a reduced risk of cardiovascular diseases and metabolic syndrome. Furthermore, positive effects on the immune- and nervous system, protection against the development of cancer, and systemic and intestinal anti-inflammatory effects are described by Andújar et al., 2012.

Cocoa powder stands out as a highly favored ingredient in the baking industry due to its distinctive flavor and unique sensory characteristics (Manley, 2000). However, the cultivation of cocoa is associated with various sustainability challenges on environmental, social, and economic fronts. In response to these challenges, the European Commission (2022) launched *The Sustainable Cocoa Initiative*, with a primary focus on enhancing the sustainability of the cocoa supply chain, particularly in West African countries, which are major global cocoa producers. The key goals of this initiative are to tackle and eradicate child labor and trafficking in the cocoa supply chain, to strengthen efforts of forest protection and restoration in cocoa-producing regions, and to guarantee that cocoa farmers receive fair and decent incomes. Despite these efforts, the demand for cocoa is consistently growing, potentially leading to production challenges. Consequently, the price of cocoa is increasing, emphasizing the necessity to investigate sustainable alternatives with minimized environmental impacts (Loullis and Pinakoulaki, 2018). Limited research has been conducted on the exploration of substitutes for cocoa powder. Currently, commercially available alternatives include starch-based materials, legumes, soy flour, chicory, chocolate flavoring, and various additives. The main disadvantage of these cocoa substitutes is their deviation from natural cocoa powder in terms of color, water solubility, and chemical composition (Rikon and del Valle, 1982). Carob flour emerges as an economically feasible alternative to cocoa (Loullis and Pinakoulaki, 2018), capable of replacing up to 30 % of the cocoa powder in cookies without substantially altering the flavor profile (Manley, 2000). While carob is an appealing cocoa substitute due to its similar phytochemical content, it contains abundant condensed tannins, contributing to a persistent aftertaste of the product (Silanikove et al., 2006). Nevertheless, no existing study explored the potential of substituting cocoa powder with fruit by-products, emphasizing the need to identify a cocoa replacement that not only preserves but enhances sensory attributes.

3. Fruit by-products – new functional and sustainable ingredients

In developing countries, concerns about sustainability, including food waste and the disposal of industrial residues, are challenging (WHO, 2021). Recognizing these issues, the United Nations' 2030 Agenda emphasizes the necessity of developing sustainable food systems to provide nutritious diets for a growing population (Paucar-Menacho, 2022). Furthermore, the European Union aims to reduce food waste per capita by half by 2030, with implementing innovative approaches for the recycling of food waste (Stenmarck et al., 2016). Given that the agro-industry generates approximately 30 % of organic waste rich in valuable bioactive compounds, has prompted companies to explore the use of food by-products as functional foods with the aim of mitigating the environmental impact of the food production (Comunian et al., 2021).

Fruits and their by-products provide numerous beneficial components beyond their macronutrient- (carbohydrates, proteins, and fats) and micronutrient (vitamins and minerals) composition. These components consist of bioactive compounds like carotenoids, sterols, phenolic compounds (comprising both flavonoids and non-flavonoids), and dietary fiber (Sayago-Ayerdi, et al., 2021). As a result, by-products generated during the production of fruit juice can be utilized as economical sources of dietary fiber and polyphenols in the manufacturing of cookies (Prokopov et al., 2015). Many scientific studies have been conducted, focusing on the partial substitution of wheat flour with fruit by-products (Table 1). Their primary emphasis was to investigate the physicochemical and sensory characteristics of products enriched with by-products from specific fruit varieties or other sources. However, despite an extensive review of the existing literature, no published studies could be identified that address the enrichment of cookies with by-products derived from aronia and grape simultaneously.

Table 1. Summary of studies using fruit by-products for partial substitution of wheat flour

Fruit by-product	Dosage (flour weight)	Major findings	Reference
Strawberries, red currants, and raspberry pomace	10, 15, 20 %	↑ fiber content and hardness	Tarasevičienė et al., 2021
Blueberry pomace powder	3, 6, 9 %	↑ antioxidant activity, total phenolic content, fiber content	Tagliani et al., 2019
Orange peel powder	5, 10, 15, 20 %	↑ antioxidant activity, total phenolic content, total fiber, soluble and insoluble dietary fiber	Rani et al., 2020
Banana peel flour	7.5, 10, 12.5, 15 %	Antioxidant activity and total phenolic content improvement. ↑ hardness, moisture content, ash ↓ amount of protein and fat ↓ brightness and yellowness	Shafi et al., 2022
Passion fruit peel powder	10, 20, 30 %	↑crude fiber and ash content	Garcia et al., 2020
Grape pomace powder	2, 4, 6, 8 %	↑ fiber and protein content Improved maintenance of texture properties throughout storage.	Theagarajan et al., 2019
	19.8 %	High protein content (75 %) and appropriate microbiological quality. Adequate product acceptability and consumer willingness to buy.	Fontana et al., 2022
Goji berry pomace	10, 20, 30, 40 %	↑ fiber content (soluble and insoluble), free polyphenolic compounds and protein	Bora et al., 2019
Apple pomace powder	5, 10, 15, 20, 25 %	↑ fiber and spread factor	Usman et al., 2020
Sea buckthorn fruit pomace powder	5, 10, 15, 20 %	Enhancement of sensory appeal.	Janotková et al., 2021

↑-increase, ↓-decrease

3.1. Aronia and grape – composition, health benefits and food applications

3.1.1. Aronia

Aronia (*Aronia melanocarpa*), a member of the *Rosaceae* family, is native to the eastern regions of North America (Borowska et al., 2016). Indigenous communities highly esteemed aronia and utilized it to make tea for treating colds. Moreover, the bark was used for its astringent properties (Kulling and Ravel, 2008). The Aronia genus consists of two species: *Aronia melanocarpa* (*Michx.*) *Elliot*, commonly known as aronia, and *Aronia arbutifolia* (*L.*) *Pers*. (red aronia) (Kulling and Ravel, 2008), known as *Siberian blueberry*. Aronia bushes typically reach a maximum height of 2-3 meters and produce clusters of 20-30 small white flowers from May to June. These flowers eventually develop into black berries, measuring 6-13 mm in diameter and weighing 0.5-2 grams (Ochmian et al., 2012). Due to their tart flavor and astringency, aronia berries are seldom consumed directly as fresh fruit (Borowska et al., 2016). Aronia has gained popularity primarily through the extensive production of juices, jams, wines, liqueurs, and brandy (Kulling and Ravel, 2008).

Aronia is recognized as one of the berries with the highest polyphenol content (Jakobek et al., 2007). Products derived from *Aronia melanocarpa* have been found to retain substantial amounts of polyphenols even after processing (Tolić et al., 2015), and the total polyphenol content in aronia ranges from 3100 to 6300 mg/100 g of dry matter (Mayer-Miebach et al., 2012). This recognition originates from its elevated levels of procyanidins, anthocyanidins, and phenolic acids, with flavonols presenting in lower quantities (Oszmiański and Wojdylo, 2005). Among these compounds, polymeric procyanidins stand out as the predominant class, representing 66 % of the fruit's polyphenols. Research suggests that almost 40 % of aronia's antioxidant properties can be attributed to procyanidins (Denev et al., 2009). Anthocyanins in aronia contribute to approximately 25 % of the total polyphenols, primarily comprising a blend of four different cyanidin glycosides: 3-O-galactoside (68.9 %), 3-O-glucoside (1.3 %), 3-O-arabinoside (27.5 %), and 3-O-xyloside (2.3 %) (Jurendić and Ščetar, 2021), giving a black, dark purple color to the fruit. Besides, by-products derived from aronia are abundant in dietary fiber, making them valuable ingredients for nutritional supplements and functional foods (Wawer et al., 2006).

The high proportion of polyphenols in aronia contributes to its robust antioxidant properties, evident in both the fruit and its derived products (Tolić et al., 2017). As verified by various *in vivo* and *in vitro* studies, Aronia's primary polyphenolic components have shown diverse benefits including anti-inflammatory, anti-cancerogenic, antimicrobial, and antiviral properties (Borowska and Brzóska, 2016; Szopa et al., 2017; Jurikova et al., 2017). These anti-inflammatory properties are associated with preventing chronic diseases like diabetes, cardiovascular diseases, and autoimmune diseases (Jurikova et al., 2017). *In vitro* studies demonstrate the aronia extracts' bacteriostatic activity against pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Kim and Shin, 2020). Park et al. (2013) reported the antiviral activity of aronia juice, and proanthocyanidins were identified as potent antimicrobial agents against ten different pathogens by Pavlović et al. (2015). Additionally, Sonoda et al. (2013) suggested that *Aronia melanocarpa* extract intake acts as a thermoregulator in healthy women with a cold constitution, potentially regulating body temperature by affecting adrenaline levels and oxidative stress.

Additionally, aronia is not only used as safe and natural food coloring (Ochmian et al., 2012) and as a partial replacement for wheat flour (Raczkowska et al., 2022), but also for increasing the fiber, potassium, calcium, magnesium, and iron content.

3.1. Aronia and grape – composition, health benefits and food applications

3.1.2. Grape

Grapes (*Vitis vinifera*) belong to the *Vitaceae* family and are widely consumed worldwide. The average production of wine, has been between 250-300 million hectoliters per year over the last two decades, reflecting the environmental impact of this activity and the promising utilization of wine-derived by-products in diverse industries and medicine (OIV, 2017). The leading global producers of grapes include the USA, China, Italy, and Europe. Grapes come in various categories based on their use, such as wine grapes, table grapes, seedless grapes, and raisins (Gupta et al., 2020).

About 75 % of cultivated grapes are destined for the production of wine, of which approximately 20–30 % are by-products (García-Lomillo, 2014; Bender, 2017). These by-products, commonly referred to as grape pomace, encompass grape skins, residual pulp, seeds, and stalks (Balbinoti, 2020). The composition of grape pomace as a by-product varies significantly based on factors such as the type of waste, grape variety, cultivation conditions, processing techniques, and numerous other considerations (Garrido, 2011).

Given that grape cultivation (*Vitis vinifera*) is among the most established fruit crops globally, the utilization of post-production waste holds significant industrial importance (Bordiga, 2019). These by-products are either disposed of, used in wine alcohol production, serve as fertilizer, or become animal feed (García-Lomillo, 2014). Waste disposal raises environmental concerns, including water contamination, the attraction of disease vectors, and oxygen depletion in soil and groundwater, potentially impacting wildlife (Dwyer, 2014).

Nevertheless, it is essential to highlight that grape pomace contains significant amounts of health-beneficial substances (Bennato, 2020), such as dietary fiber (up to 85 %, depending on the grape variety) and polyphenolic compounds, retaining about 70 % of these compounds after the winemaking (Yu, 2013). Grape pomace polyphenols include non-flavonoids like hydroxybenzoic acid, hydroxycinnamic acid, and stilbenes while flavonoids include flavanols, anthocyanins (present exclusively in the black grape pomace), and flavonols (García-Lomillo, 2017).

Grape pomace has been successfully integrated into various food products, including cereals, meat, fish, and dairy products (Table 2). The addition of grape pomace resulted in a noticeable rise in the overall polyphenol content in all fortified products. However, it is crucial to highlight that the addition of grape pomace also leads to changes in the color, making the products darker, reddish, or bluish. The increase in the total polyphenol content can significantly enhance the oxidation stability, particularly in meat and fish products, thereby extending their shelf-life. It is important to note that a higher level of enrichment, including a higher concentration of grape pomace has predominantly shown a negative effect on texture and sensory characteristics in various products.

Table 2. Effect of the addition of grape pomace to different product categories

Product	Dosage	Major findings	Reference
Muffins	10, 20 and 25 % of grape pomace (flour weight)	↑ total phenolic content, radical scavenging activity, total dietary fiber, and good sensory acceptability Change in color and textural properties.	Ortega-Heras et al., 2019; Walker et al., 2014
Cookies	2, 4, 6 and 8 % of grape pomace (flour weight)	↑ polyphenolic content and lipid oxidation and textural stability during	Theagarajan et al., 2019

		storage time Change in sensory properties.	
Bread	6, 10 and 15 % of grape pomace (flour weight)	↑ polyphenolic content and antioxidant activity Darker color.	Šporin et al., 2018
Cookies	5 % of grape seed powder (flour weight)	↑ total phenolic content and antioxidant activity Darker color.	Aksoylu et al., 2015
Rice	grape pomace flour : rice ratio 1:2	↑ antioxidant activity Change in color.	Balbinoti et al., 2020
Salmon burger	1 and 2 % of grape pomace flour	↑ dietary fiber content and storage stability ↓ sensory properties	Cilli et al., 2019
Pork loin marinade	Soaking of pork loin in 0.5, 1, 2, 20 and 40 % grape pomace solution	Inhibits the lipid oxidation and microorganisms' growth.	Lee et al., 2017
Pork sausages	0.5 and 1 % of grape pomace into the recipe	↓ lipid oxidation Change in color.	Ryu et al., 2014
Chicken meat	Addition of grape pomace extract to achieve total phenol content of 60 mg/kg in meat	↓ lipid oxidation in raw and cooked meat Change in color and flavor.	Selani et al., 2011
Yogurt	b1, 3 and 5 % of grape pomace to the milk before the fermentation	↑ polyphenolic content and antioxidant activity. Sensory acceptable products. ↓ viscosity	Demirkol et al., 2018
Cheese	0.8 and 1.6 % of grape pomace into cheese formulation	↑ antioxidant activity and total phenolic content	Marchiani et al., 2016
Ice cream	2.5, 5 and 10 % of grape pomace to the ice cream formulation	↑ antioxidant activity and total phenolic content	Vital et al., 2018

↑-increase, ↓-decrease

4. Cardiovascular diseases – the role of oxidized low-density lipoprotein (oxLDL)

Cardiovascular diseases (CVDs) is a major cause of premature mortality and disability across Europe. According to the World Health Organization's 2019 data, CVDs were responsible for 17.9 million deaths, representing 32 % of the global total (WHO, 2021). Approximately 75 % of all deaths related to cardiovascular disease could be prevented by adopting a healthy lifestyle (Benjamin et al., 2019). A health-oriented lifestyle includes not smoking, follow a balanced diet, and to engage in regular physical exercise. The fundamental pathology of CVDs is atherosclerosis, a condition affecting arteries and leading to complications like myocardial infarction and stroke (Libby et al., 2019). Atherosclerosis primarily occurs in the subendothelial space of the middle and large arteries, especially in areas with disturbed or bifurcated blood flow (Kong et al., 2022). Several risk factors, such as hyperlipidemia, hypertension, oxidative stress, inflammation, endothelial dysfunction, and unhealthy habits like smoking, collectively contribute to the development and progression of atherosclerotic CVD (Lee and Cooke, 2011).

Oxidative stress is recognized as a significant risk factor in the pathogenesis of various diseases, including cancer, age-related disorders, neurodegenerative diseases, autoimmune disorders, and CVDs. The influence of oxidative stress on CVD development involves multiple pathways. In the context of atherosclerosis, the oxidation of low-density lipoprotein (LDL) particles in the vascular endothelium is identified as an initial event in the formation of atherosclerotic plaques (Lin et al., 2021). An imbalance between the generation of radicals (production of reactive oxygen species) and their elimination (action of antioxidants) can result in the creation of oxidized low-density lipoprotein (oxLDL) (Leopold and Loscalzo, 2008). The newly generated oxLDL triggers the onset of atherosclerosis through a sequence of events as described by Obermayer et al. (2018):

1. Enhanced expression of adhesion molecules on vascular endothelial cells.
2. Attraction of leukocytes to endothelial cells.
3. Sequestration of leukocytes into the intimal layer, activation of macrophages.
4. Release of cytokines and reactive oxygen species (ROS), and
5. Formation of plaques.

Furthermore, the lectin-like receptor-1 (LOX-1) for oxLDL plays a crucial role in initiating atherosclerosis, as various responses are initiated upon oxLDL binding to this receptor in various cell types (Figure 1). This binding enhances the absorption of oxLDL in both macrophages and vascular smooth muscle cells (VSMCs) and stimulates foam cell formation. Additionally, it induces apoptosis in VSMCs and contributes to endothelial activation (Poznyak et al., 2021).

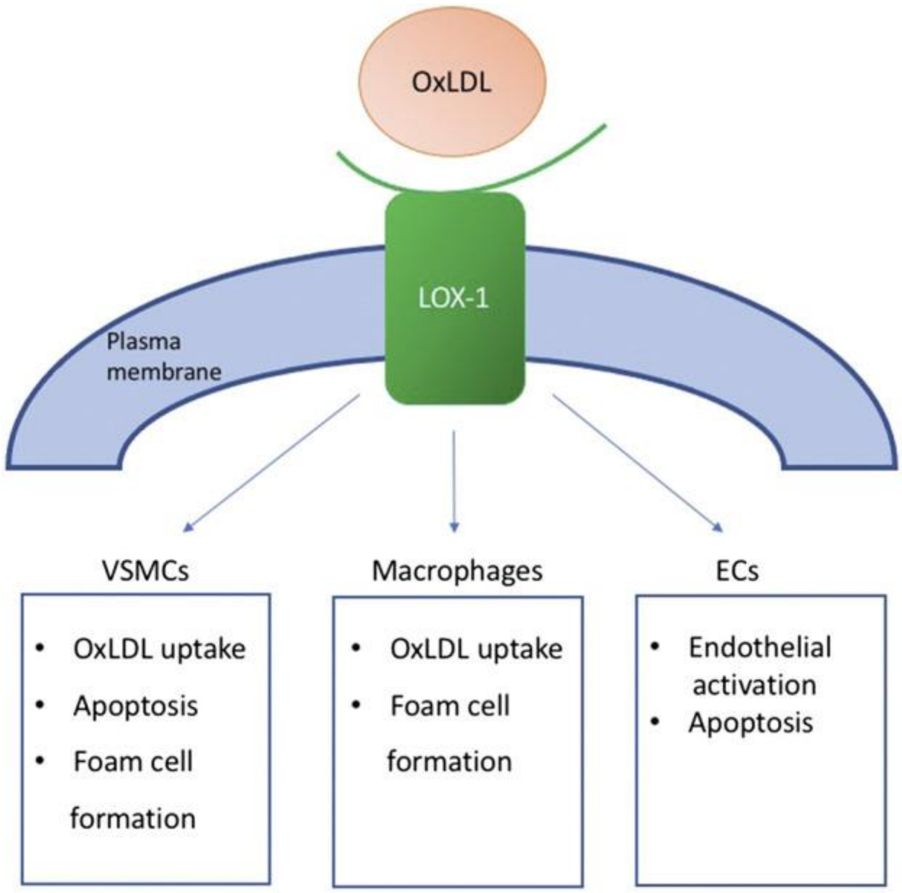


Figure 1. Contribution of LOX-1 to atherosclerosis (Poznyak, et al., 2021)

VSMCs-vascular smooth muscle cell, ECs-endothelian cells, oxLDL-oxidized LDL, LOX-1-lecitin like receptor 1

4.1. Grape and aronia – protective properties against cardiovascular diseases

Numerous studies have confirmed the protective effects of polyphenols from aronia, grapes (in various forms and dosages), and wine against CVDs, with investigations conducted in both human and animal studies. For example, consuming organic aronia juice at a daily dose of 250 mL over a period of three weeks led to reduced triacylglycerol (TAG) levels and increased serum antioxidant capacity in healthy individuals (Nowak et al., 2016). In postmenopausal women with abdominal obesity, supplementation with aronia enriched with stable glucomannan fibers resulted in a decrease of body mass index (BMI), waist circumference, and systolic blood pressure (SBP), accompanied by beneficial changes in fatty acid profiles (Kardum et al., 2014). Animal studies have shown that inclusion of aronia pomace in feed mixtures for Polish Merino lambs reduced various metabolic markers including alanin transaminase, aspartate transaminase, and glucose levels (Lipińska et al., 2018). Similarly, the administration of dried aronia powder to mice with non-alcoholic fatty liver disease resulted in decreased TAG levels and liver weight, accompanied by the downregulation of genes involved in the lipid metabolism (Park et al., 2016). Sterilized aronia juice consumption in male rats increased body weight and BMI while LDL levels decreased (Daskalova et al., 2015). Additionally, freeze-dried aronia fruits in the diet of mice fed a high-fat diet reduced liver fibrosis and lipid levels while increasing total cholesterol (Yamane et al., 2016). In human studies involving grape products, the consumption of grape juice or powder led to improvements in lipid profiles and blood pressure (Zern et al. 2005; Park et al. 2009; Toaldo et al. 2015; Corredor et al. 2016). Overall, these findings highlight the potential health benefits of aronia and grape products associated with various health conditions.

These beneficial effects include the preservation of vascular endothelial function, modulation of blood pressure, regulation of lipid metabolism, reduction of oxidative stress, attenuation of inflammation, inhibition of platelet function and thrombosis, inhibition of platelet aggregation, and the decrease in LDL oxidation (Rasines-Perea and Teissedre, 2017) as presented in Figures 2 and 3. Nevertheless, a gap in the existing literature can be noticed, as no study examining the impact of consuming cookies enriched with fruit by-products on the oxidation of LDL cholesterol is available.

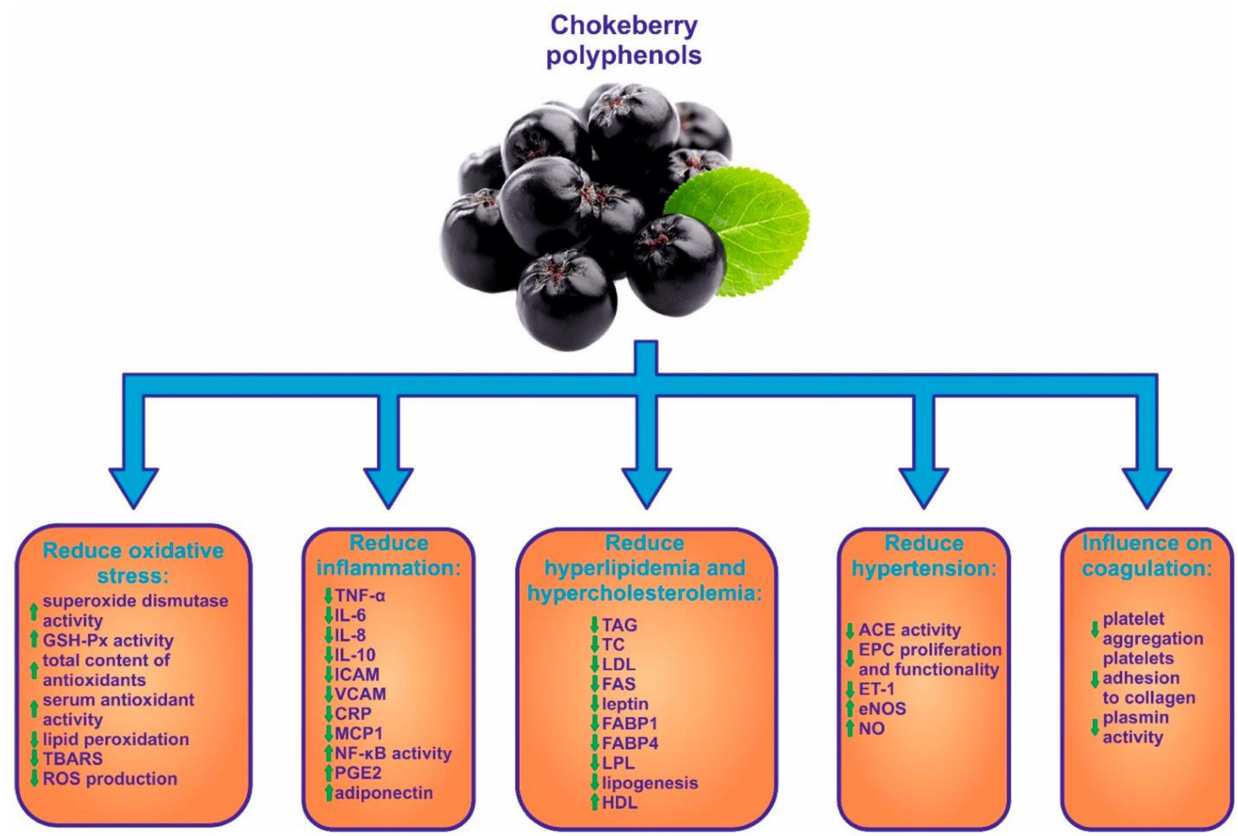


Figure 2. Protective effects of aronia against cardiovascular disease (Kasprzak-Drozd, 2021)

↑value increase; ↓value decrease; ACE-angiotensin converting enzyme, CRP-C-reactive protein, eNOS-endothelial nitric oxide synthase, EPC-endothelial progenitor cells, ET-1-endothelin-1, FABP1-fatty acid binding protein 1, FABP4-fatty acid binding protein 4, FAS-faty acid synthase, GSH-Px-glutathione peroxidase, HDL-high-density lipoprotein cholesterol, ICAM-intercellular adhesion molecule, IL-6-interleukun 6, IL-8-interleukun 8, IL-10-interleukun 10, LDL-low-density lipoprotein cholesterol, LPL-lipoprotein lipase, MCP1-monocyte chemoattractant protein-1, NF-κB-nuclear factor kappa-light-chain-enhancer of activated B cells, NO-nitric oxide, PGE2-prostaglandin E2, ROS-reactive oxygen species, TAG-triacylglycerol, TBARS-substances reacting with thiobarbituric acid, TC-total cholesterol, TNF-α-tumor necrosis factor-α, VCAM-vascular cell adhesion molecule.

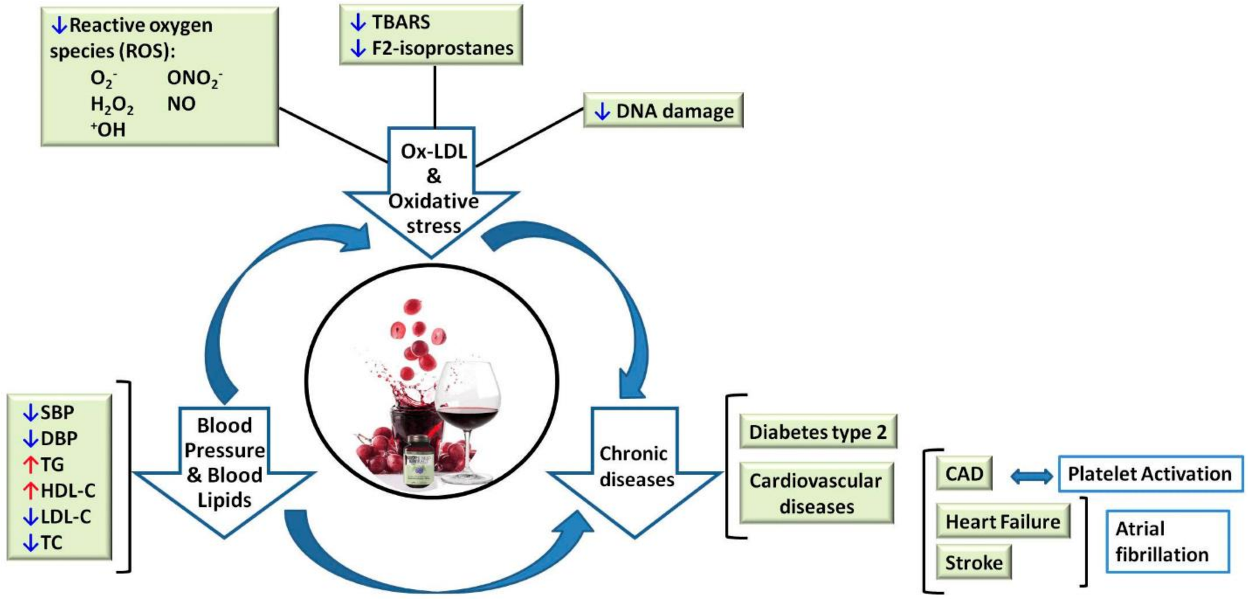


Figure 3. Protective effects of grape against cardiovascular disease (Rasines-Perea and Teissedre, 2017)

SBD-systolic blood pressure, DBP- diastolic blood pressure, TG-triglycerides, HDL-high density cholesterol, LDL-low density cholesterol, TC-total cholesterol, TBARS-substances reacting with thiobarbituric acid, CAD-coronary artery disease

5. Starch digestibility

Starch is one of the three primary polysaccharides found in nature. In contrast to cellulose and chitin, which serve as structural molecules, starch functions as a storage carbohydrate. Starch comprises of two D-glucose polymers: amylose, essentially an unbranched α(1-4) linked glucan, and amylopectin, α(1-4) linked glucose chains form highly branched structures with α(1-6) branching linkages. The proportion of amylose and amylopectin varies based on the botanical source of the starch (Lindeboom et al., 2004). Starches are classified according to their amylose/amylopectin ratio: those containing 25-30 % amylose and 70-75 % amylopectin are commonly known as *normal* starches, while those with high amylopectin levels of 98-99 % are known as *waxy* starches. Another category consists of starches with increased amylose contents of 50-70 % (Bello-Perez et al., 2020). The hydrolysis of starch present in food has been a prominent subject of discussion for over twenty-five years. Initially, it was thought that all starch in food underwent hydrolyses, primarily by the action of salivary α-amylase and later in the small intestine by the action of pancreatic α-amylase. This process generates maltose, maltotriose and other branched oligosaccharides known as α-limit dextrins, which are further converted into glucose with the help of brush border enzymes like maltase-glucoamylase and sucrase-isomaltase (Lee et al., 2013). Following a period of analytical debate, a European Concerted Action (EURESTA) was initiated, providing confirmation that a substantial portion of starch in food resists digestion and absorption in the small intestine, reaching the colon where it undergoes fermentation to varying degrees by the microbiome. This resistant fraction was subsequently termed resistant starch (RS) (Asp NG, 1992).

Since the introduction of a comprehensive definition of RS as *the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals* (Asp and Bjorck, 1992) there has been ongoing interest to better understand this fraction. Recently, RS has been incorporated into the dietary fiber definition, and it has been linked to advantages in weight management and other potential positive effects. Numerous reviews on RS have emerged over the years. In more recent publications, RS is typically categorized into five distinct groups, as outlined by Lockyer and Nugent (2017):

RS1: physically inaccessible starch, i.e., entrapped in a mechanically resistant cellular matrix.

RS2: native starch granules (raw) with particularly organized structure.

RS3: retrograded starch, produced after cooking and cooling of starchy food.

RS4: chemically modified starch, with the formation of new chemical bonds.

RS5: amylose-lipid complexes and resistant maltodextrins.

Englyst et al. (1992) classified starch digestion according to the rate and extent of its hydrolysis after ingestion.

Resistant starch possesses the capability to regulate the postprandial glycemic and insulin sensitivity (Gower et al., 2016), suggesting its potential utility in diabetes management. Research indicates that RS may influence the ratio between the *Bacteroidetes* and *Firmicutes*, in the colonic ecosystem, thereby impacting colonic microbiota and short-chain fatty acid (SCFA) production (Ríos-Covian et al., 2016).

The significance of the slow digestible starch (SDS) lies in its ability to cause a gradual increase in blood glucose levels after a meal, accompanied by an insulin response. Managing the postprandial glycemic and insulin response is crucial for individuals with diabetes as well as those who are generally healthy. Zhang and Hamaker (2009) proposed two primary mechanisms contributing to the delayed digestion of starch: a) modifying the starch structure to reduce enzyme sensitivity, and b) altering the starch structure to restrict the rate of enzyme hydrolysis. The advantages of SDS are linked to a low glycemic index, which is correlated with a decreased risk of various chronic degenerative conditions such as diabetes mellitus type II, overweight, and other obesity-related disorders (Jenkins et al., 2002). The consumption of SDS has the potential to trigger the secretion of incretin hormones, leading to reduced gastric emptying, diminished food intake, and increased satiety (Strader and Woods, 2005). These effects prolong the nutrient delivery to the body, potentially curbing appetite, and enhancing weight management.

Interactions between amylose and lipids can notably alter the attributes and performance of starch, leading to decreased starch solubility in water, enhanced rheological properties of the paste, reduced swelling capacity, elevated gelatinization temperature, lowered gel stiffness, delayed retrogradation, and diminished susceptibility to enzymatic hydrolysis (Holm et al., 1983).

The nutritional value of starch is significantly influenced by the structural characteristics of starch and alterations that occur during processing (Bello-Pereza et al., 2020).

6. Biopolymers as sustainable alternatives in the food packaging industry

Packaging is crucial for preserving products or contents during handling, transportation, and storage (Ibrahim et al., 2022). It can also contribute to maintaining and extending the shelf-life of these products. Predominantly, packaging materials have relied on petroleum-based polymeric substances, with polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) being commonly utilized polymers (Pal et al., 2019). Plastic-based materials stand out as the most frequently used packaging materials, accounting for approximately 26 % of the total polymer usage in packaging and representing the largest application of plastics (Mendes & Pedersen, 2021). The usage of plastics is expected to double over the next 20 years, rapidly replacing other packaging materials. This shift is driven not only by inherent characteristics like excellent barrier properties and lightweight quality (MacArthur et al., 2016), but also by the advantages of low costs and easy processability (Ibrahim et al., 2022).

Plastic, widely used in global packaging, is responsible for almost half of the total plastic waste generated (Walther et al., 2020). Additionally, improper handling, insufficient collection, or inadequate recycling of plastic packaging can result in their inappropriate disposal in landfills and water bodies, causing pollution and contamination of both land and waters (Chatterjee and Sharma, 2019). Many industries are actively exploring alternative sustainable and eco-friendly materials, partly driven by the European Union (EU) Commission's goal to reduce plastic waste by 55 % by 2025 and to ensure that materials are 100 % recyclable or reusable by 2030 (Plastic Europe, 2015). Furthermore, starting from July 3, 2021, in accordance with the Directive (EU) 2019/904, a ban on disposable plastic items, such as ear buds, cutlery, straws, cups, and balloon holders, was implemented across all European Union member states, including Croatia. Recycling plastic materials with multiple layers of different substances poses mechanical challenges and is not cost-effective. As a result, there is an urgent requirement for materials that are both sustainable and environmentally friendly to tackle these issues. (Ibrahim et al., 2022).

Reichert et al. (2020) define sustainable packaging materials as those characterized by a reduction in the use of virgin resources and the ability to recycle or reuse materials after consumer use. Material sustainability considers a range of factors, encompassing economic and environmental aspects, including cost, impact, functional aesthetic properties, production, end-of-life processing, and effects at local and global levels (Reichert et al., 2020). Defruyt et al. (2019) highlight that only 2 % of plastic packaging materials are globally recycled, with the majority being incinerated or ending up in landfills, water bodies, and the environment. According to Defruyt et al. (2019) 30 % of packaging items made from plastic are either too intricate or too compact for recycling. Examples of such items include materials with multiple layers and small sachets or wrappers.

Edible packaging materials are recognized for their suitability for direct human consumption without negative health effects. Edible films are composed entirely of food-grade elements, including the film-forming the polymer matrix, the solvent in which it is dissolved, and the additives used such as fillers, plasticizers, cross-linkers, or biologically active agents (Otoni et al., 2017). Edible films are separately created and applied to food surfaces or used as sealable pouches, while coatings are directly formed on food surfaces through methods like dipping or spraying. Although they cannot fully replace traditional packaging, edible coatings can help extend a foods shelf-life by controlling moisture, aroma, and gas exchange, and protect against contamination and discoloration (Dutta et al., 2009). Biodegradable polymers may originate from either natural or synthetic sources (Figure 4). It is important to distinguish between biopolymer materials and biodegradable materials. While all biopolymer materials are inherently biodegradable, it's important to note that not all biodegradable materials are biopolymers (Ferreira et al., 2016).

Biopolymers can be derived from biomass, microorganisms, or chemical synthesis. Biomass sources involve the extraction of biopolymers directly from polysaccharides (e.g., cellulose and starch) and proteins (e.g., gluten and casein). Microorganism sources involve the biosynthesis of biopolymers such as polyhydroxyalkanoates (PHA) and polysaccharides. Chemical sources encompass the synthesis of bio-based monomers, including polylactic acid (PLA).

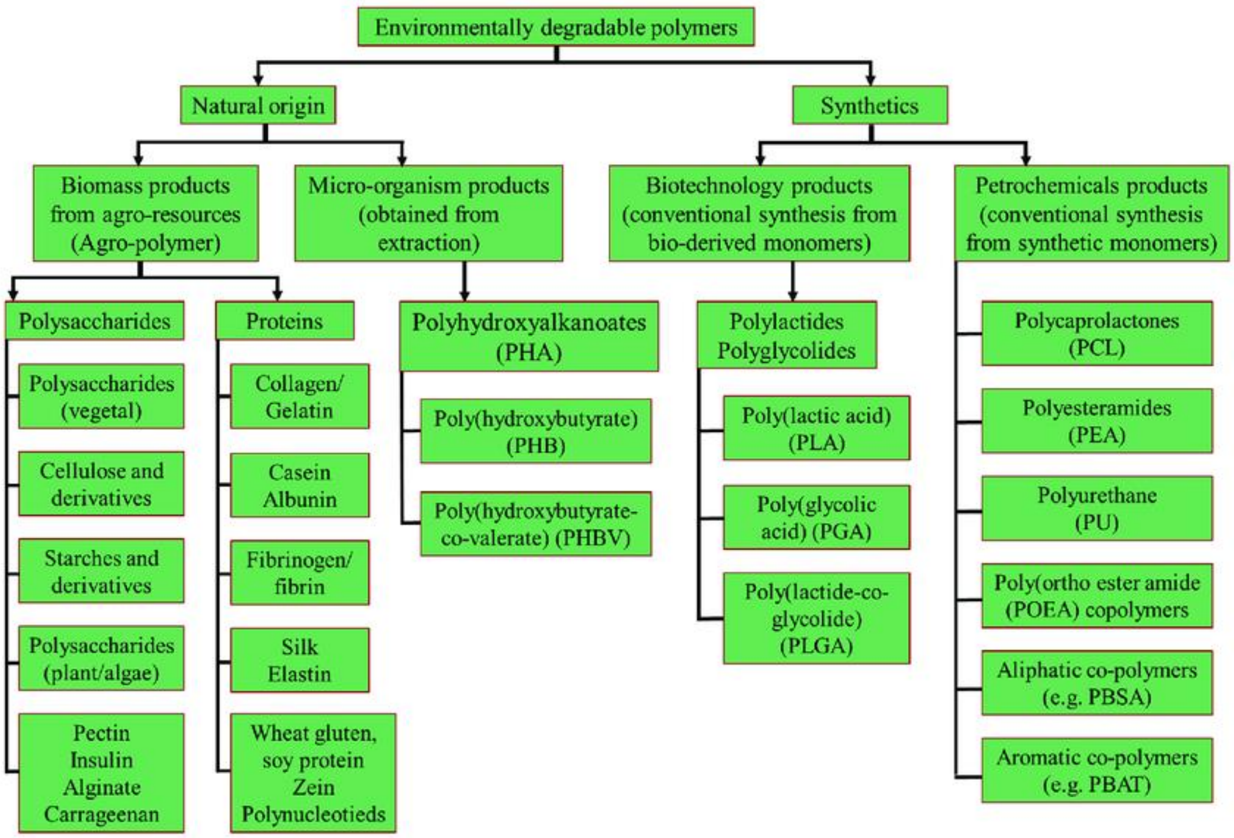


Figure 4. Classifications of biopolymer and biodegradable materials (Ibrahim et al., 2022)

6.1. Chitosan

Chitin and its deacetylated form, chitosan, belong to a group of linear polysaccharides composed of varying amounts of (β1→4) linked residues of N-acetyl-2 amino-2-deoxy-D-glucose (glucosamine) and 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine) residues. Chitin, an abundant biopolymer, is found in the exoskeleton of crustaceans, insect cuticles, algae and fungal cell walls (Figure 5). Traditionally, commercial chitosan has been predominantly derived from the chemical deacetylation of chitin sourced from crustaceans. However, there is growing interest in chitosan sourced from fungi and insect cuticles on the market. These alternative sources offer advantages such as better viscosity control and higher deacetylation levels (Ghormad, 2017).

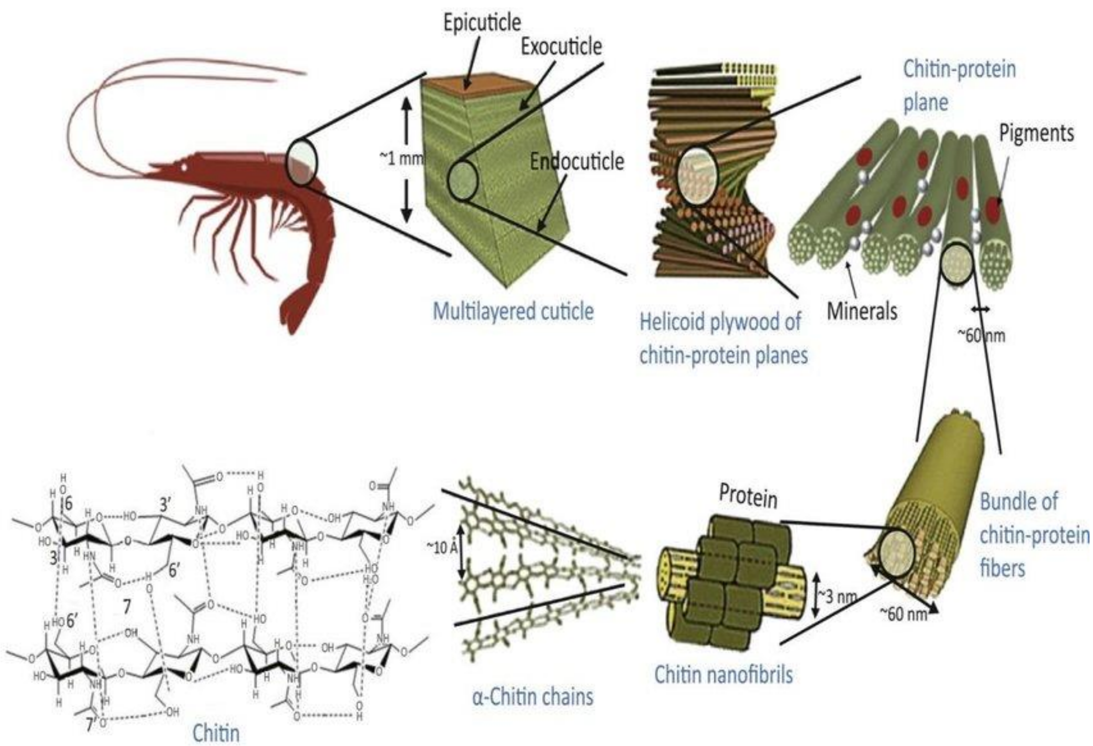


Figure 5. Structural composition and arrangement of chitin in the shell of crustaceans (Chen et al., 2016)

The solubility of chitosan depends on various factors such as the molecular weight of the polymer, the degree of acetylation, the pH level, the temperature and the crystallinity of the polymer. The presence of large amounts of protonated $-NH_2$ groups on the structure of chitosan, as well as the larger molecular weight, explain its solubility in acidic water media, since its pKa value is approximately 6.5 (Desai et al., 2023). The homogeneous deacetylation of chitin, achieved through alkaline treatment at 0 °C, allows the creation of polymers that are soluble in acetic acid solutions with a degree of deacetylation (DD) of only 28 %. In contrast, heterogeneous deacetylation at high temperatures never attains this level. Additionally, samples with a DD of 49 % exhibit water solubility. This phenomenon is attributed to the fact that uniform deacetylation results in a rise in the quantity of glucosamine units and alters the polymer's crystal structure. Depending on the degree of deacetylation of the polymer, these alterations vary from diminishing crystal size and perfection to the emergence of a new crystal structure resembling β-chitin (Cho, 2000).

Chitosan, its derivatives, and chitooligosaccharides exhibit antimicrobial characteristics against a range of microorganisms such as bacteria, fungi, and yeasts (Raafat, 2009). This is attributed to their interaction with $-NH_2$ and -COO- groups on the membranes of microorganism cells, with the effectiveness against microbes being affected by the degree of acetylation. Additionally, chitosan might interfere with RNA transcription by attaching to bacterial DNA, indicating an extra mode of action (Ke, 2021).

Research has shown that chitosan-based coatings, due to their excellent biochemical and filmogenic properties, have potential for a variety of wide applications on various products such as fish (Merlo et al., 2019), fruits (Karagöz and Demirdöven, 2019), cheese (Hani Tabaie Zavareh and Ardestani, 2020) and the meat and meat product preservation (Mahdavi et al., 2018). A summary of chitosan applications, properties and biological activities is given in Figure 6.

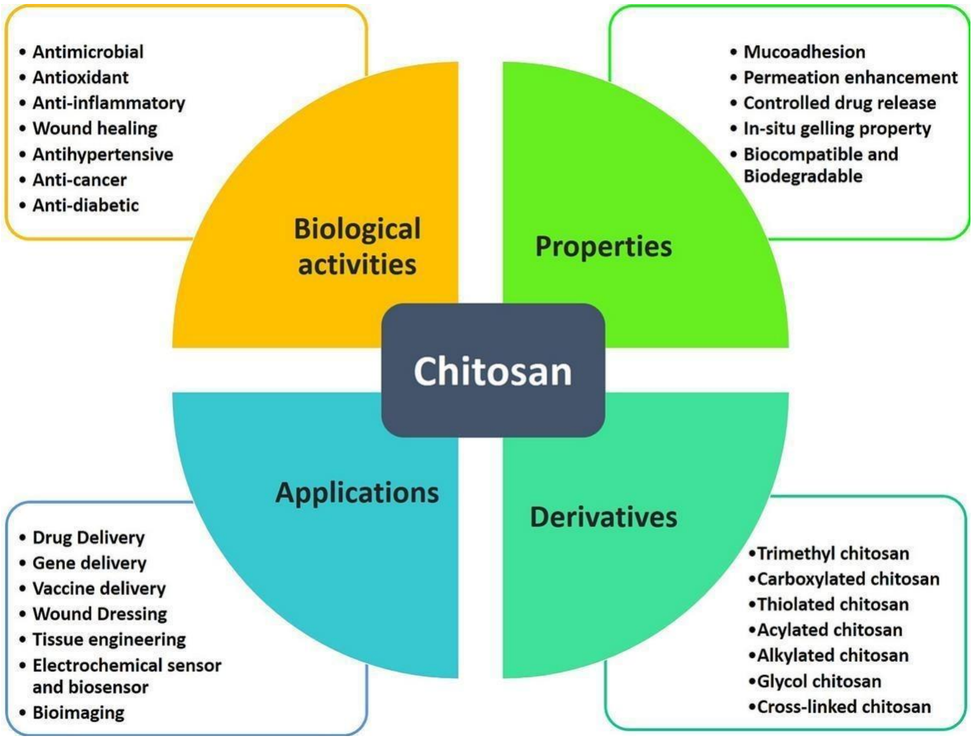


Figure 6. Chitosan applications, properties and biological activities (Harugade at al., 2023)

6.2. Gum arabic

Gum arabic (GA) is a natural polysaccharide obtained from the stems and branches of *Acacia senegal* and *Acacia seyal*, which are rich in non-viscous soluble fibers (Williams and Phillips, 2000). Primarily found in Sudan, Chad, and Nigeria, GA forms an arabinogalactan-protein complex with magnesium, calcium, and potassium salts of arabic acid (Figure 7). Arabic acid consists of 1-3-linked β-D-galactopyranosyl units, along with branches that consist of two to five β-D-galactopyranosyl residues linked together through 1,3-ether linkages and connected to the fundamental β-D-galactopyranosyl chain by 1,6-linkages (Ashour et al., 2022).

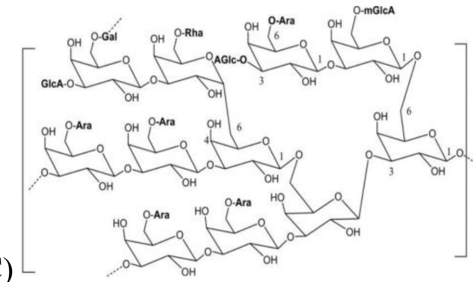
A)  B)  C) 

Figure 7. (A) *Acacia seyal* tree (B) Gum arabic, (C) Chemical structure of Arabic acid present in gum arabic (Al-Jubori et al., 2023)

Gum arabic comprises three distinct fractions: approximately 85-90 % consist of arabinogalactan, with minimal protein content (only 0.35 %); 10 % constitutes an arabinogalactan-protein complex, containing about 11.8 % protein; and the remaining 2 % consist of one or two glycoproteins, with a higher protein content of approximately 47.3 % (Lopez-Torrez et al., 2015). The chemical composition of gum arabic can vary based on factors such as its source, the age of the trees from which it was harvested, and prevailing climatic and soil conditions (Verbeken et al., 2003).

Gum arabic is widely used due to its high solubility and low viscosity at high concentrations (Sakloestsakun et al., 2015) and acts as an oil-water emulsifier (Vuillemin et al., 2019). It dissolves quickly in cold or warm water, and this property makes it the most soluble hydrocolloid (Chranioti and Tzia, 2014). In the food industry, GA is used as a stabilizer and is assigned the E number 414. It is used in the production of juices for dilution, gummy sweets such as gummy candies, and marshmallow cookies, chocolate sweets and edible glitter (Mariod, 2018). Furthermore, GA is used as an emulsifier and thickener for glazes, fillers, chewing gums and other confectionery products (Rinsky and Rinsky, 2009).

Research has shown that gum arabic-based coatings have the potential to effectively delay the ripening process and to maintain the postharvest quality of various fruits, including banana, tomato, papaya, and mango (Tiamiyu et al., 2023). Gum Arabic composite edible film has been reported to significantly control the respiration rate in chili pepper (Muthmainnah, 2019) and fresh guava (Murmu and Mishra, 2017), as well as to maintain the firmness of fruits and vegetables (Tahir et al., 2018). It also delays color change in products like dehydrated tomato slices and fresh strawberry fruit (Eltoum and Babiker, 2014, Tahir et al., 2018).

Application of a herbal formulation containing gum arabic (GA) directly to the teeth and gums significantly reduces gingival and plaque indices (Pradeep et al., 2012). In mice, GA supplementation in drinking water or diet reduces obesity by altering lipid metabolic gene expression and age-dependent fat deposition in visceral adipose tissue (Ahmed et al., 2016). Combining GA with atorvastatin decreases total cholesterol, LDL, and triglyceride levels in hyperlipidemia patients, lowering heart disease risk (Mohamed, 2015). Subjects consuming GA reported decreased caloric intake and increased satiety (Calame et al., 2011). Additionally, GA administration improves symptoms of acute non-bloody diarrhea, high body weight, and prevents severe dehydration in children (Salah et al., 2012).

7. Consumer attitude towards sustainability

Consumers have increasingly shifted their attention towards broader ethical issues and sustainable food products. This trend includes preferences for locally sourced items, products promoting animal welfare, fair-trade goods, seasonal produce, and those with low carbon footprints (Codron et al., 2006). In today's world, individuals face mounting challenges, such as environmental pollution, stress, and health issues linked to lifestyle choices, highlighting the need for sustainability (Topolska et al., 2021, Buerke et al., 2017). As a result, there is a rising demand for products meeting high sustainability standards.

Consumer choices are shaped by their perceptions of sustainability, influencing their attitude and behavior. Ethical concerns are increasingly prioritized, leading to a preference for sustainable food options (Sánchez-Bravo et al., 2020). Studies show that demographics like gender, marital status, and parenthood influence the willingness to pay more for environmentally friendly products (Laroche et al., 2001; Vecchio and Annunziata, 2015). Sustainable packaging also garners attention, with consumers willing to pay extra for eco-friendly options (Nguyen et al., 2020).

Interestingly, consumers may perceive products with sustainability claims as having superior quality, even if the quality of a product is not directly related to these claims (Ganglmair-Wooliscroft and Wooliscroft, 2022). Despite the growing awareness, there is still a gap between the attitude towards sustainability and the actual behavior, often due to price perceptions and skepticism (Kautish et al., 2022). Education and age are significant factors influencing consumers concern for food sustainability.

Voluntary labeling can drive changes in consumption patterns and enhance consumer awareness. However, effective utilization of sustainability information depends on the consumers motivation and understanding (Vecchio and Annunziata, 2015). Therefore, grasping the drivers of consumer acceptance is essential for launching sustainable products successfully (Baker et al., 2022).

8. Hypothesis, research objectives, and expected scientific contributions

This research hypothesizes the following:

1) Fruit by-products can successfully replace cocoa powder and extend the shelf-life of whole grain cookies without compromising sensory and physical properties.

2) Cookies with an edible film based on chitosan and gum arabic, along with fruit by-products, provide improved nutritional value, antioxidant activity, starch digestibility, and consumer acceptance.

3) Consumption of whole grain cookies with an edible film and fruit by-products does not lead to the formation of oxLDL receptors in healthy women.

The primary objective of this dissertation is to examine the feasibility of substituting cocoa powder in whole grain cookies with fruit by-products and to assess the effect of integrating an edible film on the cookies sensory and physical characteristics.
Secondly, to investigate consumer attitude, determine the nutritional profile, antioxidant activity, and starch digestibility, and evaluate the impact of consuming cookies containing both the edible film and fruit by-products on oxLDL receptor modulation in healthy women.

The research plan was divided into two parts:
The preliminary stage involved the development of whole grain cookies incorporating cocoa powder alongside grape and/or aronia pomace as elucidated in the *Supplementary* material. Additionally, efforts were made to produce edible films possessing favourable physical and sensory attributes, as documented in *Publication No. 1.* This section also focuses on determining the nutritional value, bioactive profile, and shelf-life of cookies with grape- and aronia pomace, both with and without an edible film enriched with grape seed extract as documented in *Publication No. 1* and *Publication No. 2*.

In the subsequent phase, this investigation delves into the effects of the consumption of cookies containing edible film and fruit by-products on oxLDL receptor modulation in healthy female subjects, as described in *Publication No. 2*. Furthermore, the investigation examines consumers' understanding of nutritional principles, their awareness of sustainability concerns, and their purchasing intentions regarding environmentally friendly cookies in Croatia, France, and North Macedonia, as detailed in *Publication No. 3*.

Throughout this dissertation the following research inquiries were investigated:

1) Can grape- and aronia pomace effectively substitute a portion of cocoa powder in cookies while preserving their sensory attributes? (*Supplementary*)

2) What are the physicochemical properties of edible films based on chitosan and gum arabic, enriched with grape seed extract? (*Publication No. 1*)

3) Does the application of edible films composed of chitosan and gum arabic, enhanced with grape seed extract, impact the quality and shelf-life of cookies? (*Publication No. 1*)

4) How does the substitution of cocoa powder with fruit by-products, coupled with the application of edible films, affect the nutritional composition, antioxidant capacity, and starch digestibility of cookies? (*Publication No. 2)*

5) Can the regular consumption of cookies—where 24 % of cocoa is replaced by grape- and aronia pomace and further coated with edible films enriched with grape seed extract—modulate oxLDL receptor levels in healthy women? (*Publication No. 2*)

6) What is the market potential for eco-friendly cookies in Croatia, France, and North Macedonia? (*Publication No. 3*)

This dissertation achieved the following:

(i) Formulation of cookies where 24 % of cocoa was replaced with grape- and aronia pomace, and subsequently coated with edible films enriched with grape seed extract, without compromising sensory characteristics.

(ii) Comprehensive investigation of the physical and chemical attributes of edible films derived from chitosan and gum arabic, supplemented with grape seed extract.

(iii) Evaluation of the effects of incorporating edible films enriched with grape seed extract on the nutritional composition, antioxidant capacity, starch digestibility, and shelf-life extension of the final cookie products.

(iv) Examination of the influence of consuming cookies containing grape- and aronia pomace-combined with edible films enriched with grape seed extract, on oxLDL levels in healthy female participants.

(v) Analysis of market potential and consumer perceptions regarding whole grain cookies featuring food by-products and edible films across Croatian, French, and North Macedonian population.

(vi) Contribution to further explore the potential uses of by-products in advancing functional food applications.

Chapter 2

Publications

Publication No. 1: Characteristics of edible films enriched with fruit by-products and their application on cookies

Food Hydrocolloids

Molnar, D., Novotni, D., Kurek, M., Galić, K., Iveković, D., Bionda, H., Ščetar, M. (2023) Characteristics of edible films enriched with fruit by-products and their application on cookies. *Food Hydrocoll.* **135**, 108191. https://doi.org/10.1016/j.foodhyd.2022.108191

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Dunja Molnar: Investigation, conceptualization, methodology, formal analysis, writing – original draft.

Dubravka Novotni: Investigation, supervision, conceptualization, methodology, resources, formal analysis, writing – review and editing.

Mia Kurek: Investigation, methodology, interpretation**,** writing – review and editing.

Kata Galić: Writing – review and editing.

Damir Iveković: Investigation, methodology.

Helena Bionda: Investigation.

Mario Ščetar: Investigation, supervision, conceptualization, methodology, resources, formal analysis, writing – review and editing.

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Characteristics of edible films enriched with fruit by-products and their application on cookies

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ABSTRACT

Edible coatings and films provide an environmentally-conscious alternative to plastic food packaging options. This study investigated physico-chemical, morphological and thermal properties of edible coatings and films based on chitosan and gum arabic (GA) enriched with grape seed extract (GSE). Their influence on the quality and shelf-life of whole grain cookies with grape and aronia (chokeberry) pomace (GAP) was also investigated. The addition of 0.1% GSE to the film formulations significantly increased thickness and resistance to water vapor, while reducing water solubility, visible light transmission, and rotational force. Moreover, the addition of GSE influenced the film color (turning it dark, reddish, and yellowish) and microstructure as shown by thermal, scanning electron microscopy and Fourier transform-infrared analysis. The addition of GSE led to an increase in the total phenolic content. Thus, edible coating led to lower formation of peroxides in cookies during storage. Cookies with edible coatings were harder and tougher but with much better sensory properties than control during six months of storage. Both, an accelerated shelf-life test and a direct shelf-life method confirmed that the GAP cookies with edible film were safe and of acceptable quality after six months of storage. The results of this study showed that GA and chitosan edible film enhanced with GSE had a positive impact on cookie shelf–life.

1. Introduction

Cookies are the most popular bakery product, widely consumed, with a wide range of flavors, long shelf-life and relatively low cost (Nagy et al., 2012). Due to the environmental factors during storage, such as light and oxygen, the fat composition of fat-rich cookies is changed, reducing the quality and nutritional value of cookies (Lu & Xu, 2010). It is therefore of great importance that packaging material provides protection from oxygen and water vapor (Galić et al., 2009). Oriented polypropylene (OPP) and metalized polymers are mostly used commercial materials for cookie packaging (Galić et al., 2009). Growing awareness of sustainability of products and processes have increased interest in novel packaging materials (such as edible films and coatings) that have a lower environmental impact (Siracusa et al., 2008) and could increase the quality and shelf-life of baked products (Díaz-Montes &Castro-Muñoz, 2021).

In the last few decades, chitosan has been one of the most frequently used biopolymer for the production of edible films and coatings. Chitosan is the most important derivative of chitin with a unique cationic structure comprised of β-1.4-linked 2–amino–2–deoxy–β–D-glucose (deacetylated D-glucosamine) and N–acetyl–D-glucosamine units (Fan & et al., 2018). It is often mentioned as a biodegradable, biocompatible, and naturally active antimicrobial agent that poses great film-forming properties as well as antioxidant activity (Vuillemin et al., 2019). Gum arabic (GA) is a mixture of polysaccharides, oligosaccharides, and glycoproteins collected from exudates of *Acacia senegal* or *Acacia seyal* trees (Goodrum et al., 2000). GA has antioxidant and antimicrobial properties against a variety of pathogenic bacteria and fungi (Bnuyan et al., 2015). In the scientific literature, it has been reported the use of GA alone or in combination with chitosan for preserving fruits (Khaliq et al., 2015). Although there is great interest in using edible films for prolongation of shelf-life, a survey of the scientific literature revealed that data on the shelf-life of cookies with edible coatings are limited.

Due to its low mechanical properties and water sensitivity, chitosan is often used in combination with other polymers. Combination of chitosan (CS) and gum arabic (GA), a powerful biomaterial complex might be obtained due to the unique properties of CS and the low viscosity and good emulsifying properties of GA. Combination of chitosan with other

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polymers is a key factor for application of edible films in food industry but also pharmaceutical industry (Xu, L., 2019; Xu, L., 2020, Xu, L., 2020). CS cannot effectively control drug release due to its rapid dissolution and degradation in the stomach and to overcome these obstacles, modifications of CS, such as blending oppositely charged polycations and negatively charged polyanions in a solution, would offer an advantage over native CS.

Although it is often considered as a waste, grape pomace, a by-product obtained by processing fruit into juices or wine, has been reported as a functional compound that increase the nutritional value and stability of cookies (Zbikowska & et al., 2018). Grape seed extract (GSE) has a high amount of various polyphenolic substances (anthocyanins, proanthocyanidins, flavan-3-ols) and dietary fiber (Sochorova & et al., 2020). The enrichment of chitosan with GSE was reported to prevent oxidation, improve quality, prevent microbiological spoilage, and extend the shelf-life of different foods (Sogut & Seydim, 2018). The influence of GSE on the shelf-life of packaged products, was reported for fish and meat products (Sogut & Seydim, 2018). Still, studies on the combination of GA and chitosan with GSE in the form of edible coatings remain scarce.

The objective of this study is to characterize GSE enriched films based on chitosan and GA and to determine how their application on cookies could extend the shelf-life of cookies and increase their quality. To understand the potential impact of film formulations on cookies, chitosan-GA blends prepared as self-standing films with or without active ingredients were characterized for their physicochemical, thermal and structural properties. Chitosan-GA formulations were applied as coatings on cookies. Also, the present study aimed to monitor the quality characteristics of coated cookies stored at different temperatures, to analyze the association between quality parameters (peroxide value) and sensory attributes. Q10-values and activation energy (Ea) were also determined. Shelf-life of coated cookies was predicted using Arrhenius equation.

2. Materials and methods

2.1. Materials and reagents for edible films and coatings preparation

Chitosan (France Chitin, Orange, FR, type 652, Mw 165 kDa, DA > 85%) and GA (Enologica vason s.p.a., San Pietro in Carino, Italy) were used for film preparation. MegaNatural Gold GSE was donated from Polyphenolics (USA; total phenolic content is 90% expressed as gallic acid equivalent (mg 100 g^{-1})). GSE was stored in original packaging at −18 °C. Acetic acid (glacial 100%, Merck, Darmstadt, Germany), distilled water, and glycerol (Dekorativna točka d.o.o., 99.5% purity, Croatia) were used for film-forming solutions (FFS) preparation.

2.2. Raw materials for cookie preparation

Wholemeal spelt flour (Siladi, Croatia) containing 12% protein and fine oat flakes (Crownfield, Germany) containing 13.5% proteins were bought at a local market. Cocoa powder (with 20% fat) and margarine (with butter) (70% fat) were purchased from Kraš and Zvijezda, Croatia, respectively. Dried red grape (*Vitis vinifera* L. variety Frankovka and Syrah, with seeds) pomace and aronia (*Aronia melanocarpa* L., without seeds) pomace, obtained as by-products from juice production, were provided from local producers (Davorka Šipek, Natkrižovljan, Cestica Community and Tomislav Jurendić, Koprivnica, Croatia). Dried grape and aronia pomace were ground in a laboratory ball mill (Cryomill, Retsch, Austria). Batches of 7 g of pomace were ground for 3 min in a 50 mL stainless steel container containing 12 steel balls (10 mm diameter) at a vibration frequency of 30 Hz to resemble the particle size of the cocoa powder (Molnar et al., 2020).

2.3. Film preparation

Two types of FFS were prepared; the first consisted of chitosan, GA and glycerol, and the second consisted of chitosan, GA, glycerol and GSE. Chitosan and GA solutions were prepared separately. Two grams of chitosan powder was dissolved in a 1% (v/v) aqueous acetic solution. GA powder was dissolved in distilled water to obtain a 5% (w/v) solution. Both solutions were continuously stirred on a magnetic stirrer (GA at 425 rpm and chitosan at 545 rpm), for 24 h at room temperature (23 ± 2 °C). GSE (0.1 g) was firstly dissolved in GA solution (40 min on a magnetic stirrer). When the whole extract was dissolved (as remarkable by the human eye), GA (with extract) and chitosan solutions were mixed to obtain an active film-forming solution (KGAE). Chitosan and GA without GSE served as control (KGA). Glycerol (20% of the dry weight of the polymer) was added to each FFS; KGAE and KGA, and 20 g of each FFS were poured into a glass Petri dish (118 mm diameter) and dried in a ventilated climate chamber (Memmert HPP110, Memmert, Buechenbach, Germany) for 46 h at 25 °C and relative humidity of 50%. Dry films were peeled off with tweezers and conditioned in a desiccator at 50% RH and 25 °C until analyzed.

2.4. Physical and chemical characterization of films

2.4.1. Total phenolic content of extract solution and coated cookies

Modified Folin–Ciocalteu (FC) method was used to determine the total phenolic content (TPC) in extract solution and coated cookies (Shortle, 2014). First, 100 μL of appropriately diluted extract solution was mixed with 200 μL of FC reagent and 2 mL of distilled water. After 3 min, 1 mL of 20% Na_2CO_3 (w/v) was added, stirred, and maintained at 50 °C for 25 min in a thermostatic bath.

For determination of TPC in cookies, approximately 2 g of ground cookies were placed in a tube together with 25 mL of 50% methanol/water solution (50:50; v/v). The tube was vortexed for 3 min, immersed in a water bath for 1 h and centrifuged for 10 min at 2500 rpm. Supernatant I was transferred to another tube and 25 mL acetone/water (70:30, v/v) solution was added to the tube with sediment after which the tube was vortexed again for 3 min, immersed in a water bath for 1 h, and centrifuged (2500 rpm, 10 min). Supernatant was combined with supernatant I and centrifuged for 15 min at 3500 rpm. The resulting supernatant was transferred into a tube, 500 μL of the extract was mixed with 1100 μL of distilled water and 100 μL of FC reagent. After 3 min, 300 μL 20% Na_2CO_3 (w/v) was added to each tube, stirred and maintained in the dark at room temperature for 2 h.

A spectrophotometer (model UV 1600 PC; VWR International, Leuven, Belgium) was used to measure the absorbance at 765 nm. For the blanks, the extraction solvent was used instead of GSE, and the analytical curve was made with gallic acid. TPC was expressed as mg of gallic acid equivalent (GAE) × g^{-1} of powdered extract. All measurements were performed in duplicate for FFS and in triplicate for cookies.

2.4.2. Film thickness

A digital micrometer with an accuracy of 1 μm was used to measure the thickness of film samples (Digimet, HP, Helios Preisser, Gammertingen, Germany). An average of 5 replicates was calculated.

2.4.3. Water vapor permeability measurements

Water vapor permeability (WVP) of films was measured gravimetrically using a modified ASTM E96-80 standard method (ASTM, 1980). The permeation cup was filled with 20 mL of distilled water (100% RH) and tightly closed with a film sample. Samples were placed in a ventilated climatic chamber (Memmert HPP110, Memmert, Germany) maintained at 30% RH and 25 ± 1 °C for 10 days. Samples were periodically weighted until the end of the experiment. Five replicates were performed for all samples.

WVP (g m^{-1} s^{-1} Pa^{-1}), was calculated at the steady-state of the permeation process with a constant weight change of the cell (Heising et al., 2015) as following:

$$WVP = \frac{\Delta m}{\Delta t \bullet A \bullet \Delta p} \bullet x \quad (1)$$

where $\Delta m/\Delta t$ represents the weight of moisture loss per unit of time (g s $^{-1}$), A represents the film area exposed to moisture transfer (9.08 10^{-4} m^2), x represents the film thickness (m) and Δp represents the difference in water vapor pressure between the two sides of the film (Pa).

2.4.4. Water solubility

Film solubility in water (WS) was done according to Shingel (2004). The initial dry weight of film samples (cut into squares 1 cm × 2 cm) was determined (W_i). Film samples were immersed in distilled water for 24 h. The remaining film segments were oven-dried to a constant weight (the ultimate weight of dry matter not dissolved in water) at 105 °C (W_f). WS (%) was calculated as follows:

$$WS\ (\%) = \frac{W_i - W_f}{W_i} \bullet 100 \quad (2)$$

Three replicates were done for all film types.

2.4.5. Transparency

Transparency is the ability of a material to transmit light. Transparency of edible films was measured at 600 nm using a spectrophotometer (PerkinElmer Lambda 25, USA) according to Peng et al. (2013). Transparency was calculated using the following equation:

$$T = Abs_{(600)} / x \quad (3)$$

where T is transparency, Abs_{600} is absorbance at 600 nm, and x is thickness of the sample (mm).

2.4.6. Color measurements

The color of dry films and fresh cookies were measured using the CIE-Lab color scale with a colorimeter (Konica Minolta Spectrophotometer CM3500d, Tokyo, Japan). Measurements were done on at least six different places on the film surface and ten different places on the cookies surface. The CIE-Lab color L^*, a^*, and b^* components were recorded. The overall color difference (ΔE) was calculated according to Eq. (4). (Pathare et al., 2013):

$$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2} \quad (4)$$

2.4.7. pH value

pH value of FFS without and with GSE was measured in a triplicate with a pH meter (3510 pH meter, Cole Parmer Ltd., United Kingdom) by immersing the electrode directly in a homogenized solution.

2.4.8. Viscosity

The apparent viscosity of FFS was determined using a viscometer (Lamy Rheology RM 100 Plus, France). Viscosity (m Pa s) was calculated from viscosity data and rotation speed was measured in triplicate.

2.4.9. Differential scanning calorimetry (DSC)

Thermal film properties were analyzed using a differential scanning calorimetry (DSC 214 Polyma Differential Scanning Calorimeter, NETZSCH-Gerätebau GmbH, Germany). An empty capsule was used as an inert reference and the calibration was performed using the indium standard. Nitrogen flow was used as a carrier gas for heating (40 mL min^{-1}) and cooling cycles (60 mL min^{-1}). The temperature program ranged between 25 °C and 340 °C for all samples, and was set as follows: a) equilibrating at 25 °C and heating to 340 °C at a rate of 10 °C min^{-1}, b) cooling down to 25 °C, c) reheating to 340 °C and d) final cooling down to 25 °C. All samples were preconditioned at 25 °C and <10% RH (silica gel, dry) for at least 5 days prior to experiments. Two repetitions were carried out for each sample and the average sample mass was around 8 mg.

2.4.10. Fourier Transform-Infrared (FTIR)

Fourier Transform-Infrared (FTIR) spectrometry (PerkinElmer Frontier, Beaconsfield, UK) was applied to analyze the structures of prepared films. Spectra of FTIR were measured in the 4000 to 400 cm^{-1} frequency range, using ZnSe crystal as attenuated total reflectance (ATR). For each measurement, 32 scans with a 4 cm^{-1} resolution were done. This analysis aimed to determine the molecular level modifications induced by the incorporation of GSE into the polymer chains.

2.4.11. Scanning electron microscopy (SEM)

Surface topography of the films based on chitosan and GA enriched with GSE was examined by scanning electron microscope Vega 3 LMH (Tescan, The Czech Republic). Film samples in the form of 5 × 5 mm squares were side fixed on specimen stubs with a conductive copper tape and coated with a thin layer of a gold-palladium alloy prior to imaging. Due to the high sensitivity of samples to electron beam irradiation, the samples were examined at the low beam energy of 2.0 keV, with the beam current and dwell time carefully optimized to avoid sample damage.

2.5. Cookies preparation

Three types of cookies were prepared: 1) the control cookies without pomace in recipe (CC); 2) cookies enriched with grape and aronia pomace (GAP), and 3) cookies with grape and aronia pomace coated with edible film (GAP with KGAE) (according to Molnar et al. (2020)). The control recipe contained (weight on total dough weight, in percentage): fine oat flakes 30%; whole meal spelt flour 25.2%, margarine 20%; brown sugar 13.5%; cocoa powder 4.8%; vanilla sugar 3.5%; tap water 2%; salt 0.4%, baking powder 0.3%; and sodium bicarbonate 0.3%. In GAP, 23.6% of cocoa powder amount (1.1% of total dough weight) was replaced with a mixture of cocoa powder (76.4%), grape pomace (17.5%) and aronia pomace (6.1%).

The cookies were prepared according to AACC (2000) method 10–50.05. The dough was prepared by mixing all the ingredients together. Then, it was rolled out to a thickness of 5 mm and shaped with a 5 cm diameter round cookie cutter. The pieces of dough were baked in the oven at 180 °C for 12 min in three batches.

2.6. Application of edible coating on cookies

The spraying method was used for the application of chitosan and GA based FFS with bioactive component (GSE) on cookies (GAP with KGAE). A spraying bottle with FFS was mixed well and each side of the cookie was spritzed twice followed by drying in the oven for 30 min at 80 °C.

2.7. Cookies storage and shelf-life test

Cookies were packed in a composite 32 μm thick BOPPAcPVDC film made of biaxially oriented polypropylene (BOPP) covered with acrylic/polyvinylidene chloride (AcPVDC) (ExxonMobil, Belgium). Pouches with cookies were hermetically double-sealed using a manual welding machine (UNIVAC S.r.l div Lavezzini, model Medium, Italy). Storage stability was determined using a direct method (DM) and an accelerated shelf-life test (ASLT).

For ASLT, 90 cookies of each type (CC, GAP, GAP with KGAE) were prepared, and each cookie was packed separately in a pouch. Cookies were stored at the following temperatures: 22 °C ± 1 °C, 30 °C ± 1 °C and 35 °C ± 1 °C in a thermostatic chamber (Desing, Serbia) for three months. Peroxide value and moisture content were analyzed at the beginning and after three months of ASLT. The Arrhenius equation was used to express the temperature dependence of the reaction rate:

$$\ln k = \ln A - E_a / RT \quad (5)$$

where k is the reaction rate constant; R is the molar gas constant (8.314 J K^{-1} mol^{-1}), T is absolute reaction temperature (K), E_a is activation energy (J mol^{-1}) and A is a preexponential factor. Reaction rate k was calculated according to equation (6) where B_0 is the peroxide value of fresh cookies, B is the peroxide value of cookies after three months of storage and T is time.

$$k = B - B_0 / T \quad (6)$$

The temperature dependence of deterioration of foods is often indicated in terms of Q10-value, which was calculated according to the following equation:

$$Q10 = R2 / R1^{e(10/T2-T!)} \quad (7)$$

Where $R2$ and $R1$ are defined as the ratio between the shelf-life value at a given temperature and $T1$ and $T2$ are temperatures. Q10-values, indicating deterioration of coated cookies were 1.7 and 1.4 for temperatures of 30 and 35 °C, respectively.

For direct testing of shelf-life, eight pouches containing 10 cookies of each type were stored at room temperature (22 °C ± 1 °C) and dark place for six months. A complete cookie was subjected to descriptive sensory analysis after 0, 3, 4, 5, 5.5, and 6 months of storage, according to ISO standards 16779:2015 (International Organization for Standardization, 2015).

2.8. Sensory analysis

Experienced assessors (12 females and 2 males), staff of the Faculty of Food Technology and Biotechnology, University of Zagreb made a sensory panel. Samples were presented in a random order, coded and served with water and coffee beans. Fresh cookies were evaluated for appearance, smell, flavor, texture and overall acceptability using a nine-point hedonic scale; with 1 dislike extremely and 9 like extremely (Svensson, 2012). Fresh and stored cookies for 3, 4, 5, 5.5, and 6 months of ambient storage were evaluated using descriptive sensory analysis carried out in accordance with ISO standards 16779:2015 (International Organization for Standardization, 2015). Cookies were evaluated on a scale of 0–10 for intensity (0–not detectable, 5–medium intensity, 10–high intensity): appearance (brown color, surface brightness), odor (whole grain, cocoa, forest fruit, rancid), flavor (sweet, bitter, sour, salty, rancid, pungent, bitter aftertaste) and mouth sensation (hardness, crunchiness, solubility, stickiness). Each panelist received a control cookie with defined intensities prior to analysis.

2.9. Moisture content

Moisture content **was determined gravimetrically** by using a moisture analyzer (PMB53 Adam Equipment, USA). The crushed cookie sample (3 g) was placed on a chamber tray (104 °C) and closed. Moisture content (%) determination was performed in triplicate, for each type of cookie.

2.10. Peroxide value

The peroxide value (PV, mmol kg^{-1}) of cookies was determined in duplicate following ISO 3960:2017 method (International Organization for Standardization, 2017). Three consecutive runs were performed for each sample.

2.11. Statistical analyzes

Xlstat–Pro (win) 7.5.3 was used for statistical analysis (Addinsoft, New York, NY, USA). The statistical differences between the ranks were assessed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. The changes in texture, sensory features, and acceptance of the cookies during storage were analyzed using principal component analysis (PCA) and Statistica 14 (Tibco Software, Palo Alto, USA). A confidence level of $p < 0.05$ was considered significant in all circumstances.

3. Results and discussion

3.1. Physical, barrier, and mechanical film properties

The physical properties of the investigated films are given in Table 1. The thickness of films with incorporated GSE was twice as thick (104.4 μm) as those without GSE (61.9 μm). An increase of thickness in chitosan-based films with the incorporation of 5, 10, and 15% of GSE had also been previously reported (Sogut & Seydim, 2018). Oliveira Filho et al. (2020) also showed that the incorporation of citrus limonia essential oil (>0.75%) in chitosan-based film increased its thickness. Thickness value is an important property since it influences the films appearance and even more important, its water vapor and gas barrier properties (Qiao et al., 2019).

The knowledge of the WVP helps to estimate if a certain type of film is adequate for a specific food product since it influences the moisture interaction with the surrounding environment (Xu et al., 2019). WVP was slightly higher in KGAE than in films without extract (Table 1). When two biopolymers containing opposite charges interact associatively; chitosan (polycation) and GA (polyanion) these result in complex structure based on the formation of networks and the location of trapping water within the network. Although GA possesses a hydrophilic nature, chitosan-GA mixture lead to a dense matrix structure and thereby, water could not easily permeate through the pores of the films. Still, adding hydrophilic grape seed extract (GSE) to chitosan-GA based film led to a higher permeability to water vapor (Wu & et al., 2014). A possible explanation is that extract alters the structure of the polymer matrix in such a way that the cohesion bonds between the polymer chains were weakened and the adhesion forces were strengthened, resulting in an increased permeability and lower antimicrobial effect. Greater atimicrobial potential might be in connection with film thickess; thicker coatings means that a higher amount of chitosan-GA would have been applied to cookies and therefore coatings might have greater antimicrobial potential. When applying chitosan–GA based coatings with GSE to cookies, thicker layer should be applied but also, longer drying time of cookies is needed. Water vapor is generally transferred through the hydrophilic portion of the film and is dependent on the ratio of hydrophobic and hydrophilic elements.

When moisture content becomes a limiting factor for shelf-life, water solubility (WS) provides information on the affinity of polymers for water. This is also a key aspect in determining the degradation properties when films are used as packaging materials. Films enriched with GSE had lower solubility in water compared to the film without the extract (Table 1). Due to the hydrophilic character of extract, a possible

Table 1
Thickness, water vapor permeability (WVP), water solubility (WS), transparency (T) at 600 nm, color properties (L^*, a^*, b^*, ΔE) of chitosan and gum arabic (GA) based films without (KGA) or with grape seed extract GSE (KGAE).

Property	KGA	KGAE
Thickness (μm)	61.9 ± 1.3^b	104.4 ± 7.8^a
WVP ($\times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1})	2.38 ± 0.15^b	3.31 ± 0.15^a
WS (%)	18.67 ± 3.28^b	12.44 ± 0.56^b
T (600 nm)	0.045 ± 0.001^b	0.618 ± 0.038^a
L^*	91.05 ± 0.23^a	49.32 ± 0.38^b
a^*	1.98 ± 0.11^b	14.58 ± 1.14^a
b^*	-5.24 ± 0.47^b	8.25 ± 1.04^a
ΔE	0	45.65 ± 0.56^a

Values are given as average ± standard error. Significant differences between samples ($p < 0.05$) are shown by different superscripts ($^{a-b}$) within a raw.

reason for lower solubility of film with GSE is uneven and incomplete bonding of extract in the film matrix. Similar findings were given by other authors who reported a reduction in WS with the addition of apple pomace to chitosan film (Riaz & et al., 2018). Opposite behavior was reported for chitosan films with tea polyphenols and essential oils (Wang et al., 2013).

When light-sensitive food is exposed to visible and ultraviolet light, the light barrier qualities of packaging materials play a key role in determining the packaging's capacity to preserve food from deterioration and loss of nutrients and flavors (De Moraes Crizel et al., 2018). According to obtained results, in the visible region (600 nm), films with GSE proved to be more effective barrier to light transmission than films without GSE (Table 1). Findings are consistent with those reported by Sogut and Seydim (2018) and OliveiraFilho et al. (2020). A decrease in film transparency could be due to the incorporation of colored phenolic compounds from GSE (Sogut & Seydim, 2018) and changes in matrix structure due to the electrostatic interactions between GA and chitosan (Xu et al., 2021).

The human eye could identify a unique coloration and the presence of agglomerated particles in GSE films. The composition of GSE and the powder particle size could explain this appearance (Kurek et al., 2021). The presence of antioxidants and anthocyanin pigments in the film increased the darkness ($<L^*$), redness ($>a^*$), and yellowness ($>b^*$) of the film samples as the concentration of GSE in the film increased. When the films are used to pack (wrap) items that are sensitive to photooxidation, the decrease in lightness may have a favorable effect (Kurek et al., 2021). Due to the presence of antioxidant compounds, but also acidic pH solution which affects the red color of anthocyanins, integration of GSE influenced the color change of film. In agreement with obtained results, Sogut and Seydim (2018) found lower values of parameter L^* and higher values of parameters a^* and b^* in samples with GSE. Similar findings were also reported by Moghadam et al. (2020). The total color difference ΔE for films with GSE indicates that these differences are extremely visible by human eye.

3.2. Viscosity and pH value of film formulations

pH values of all tested FFS (Table 2) were in the acidic region and therefore in the optimum pH interval (3.5 and 5) for the development of a polyelectrolyte complex between GA and chitosan (Espinosa-Andrews et al., 2007). GA carboxyl groups are protonated at pH below 3.5 and the contraction of the molecular backbone prevents the formation of complexes. The degree of ionization and solubility of chitosan decreases when the pH value exceeds 6 (Espinosa-Andrews et al., 2007) which was not the case with our samples. The results indicate that the addition of GSE had an almost negligible effect on the pH value (Table 2). Chitosan solution had the lowest pH (4.7) while GA solution with GSE (GAE) had the highest pH value (4.96). Unlike GA, chitosan is not soluble in distilled water but is soluble in a 1% aqueous acetic acid solution so this could be one of reasons for the lower pH of chitosan solution. When chitosan and GA solutions were combined (KGA), the pH of the resulting solution was somewhere between the values of the individual solutions.

The FFS's viscosity is a crucial characteristic since it impacts sprayability on food, particularly on cookies in the present study. In another context, it can also serve to estimate the polymer's stability in solution. Chitosan solutions had higher viscosity values than GA (Table 2). By mixing both solutions viscosity significantly decreased, probably due to the formation of stable polysaccharide-protein complexes upon mixing a positively charged chitosan (-NH_3^+) and negatively charged GA (-COO-). The addition of GSE led to an increase in the viscosity in simple formulations, while no significant difference was measured for chitosan-GA complex. Still, viscosity results were a key parameter for choosing the application method of FFS on cookies.

Chitosan solution with GSE (KE) or without GSE (K) and GA solution with GSE (GAE) or without GSE (GA) were used only for these two analyzes.

Table 2
Results on pH value, viscosity (V) and rotational force (T) of tested film forming solutions.

Sample	pH value	V (m Pa s)	T (m N m^{-1})
K	4.70 ± 0.04^{d}	68 ± 0^{c}	2.622 ± 0.064^{a}
KE	4.70 ± 0.04^{cd}	74 ± 0^{b}	2.861 ± 0.014^{a}
GA	4.93 ± 0.09^{ab}	40 ± 0^{de}	0.145 ± 0.004^{b}
GAE	4.96 ± 0.05^{a}	50 ± 0^{d}	0.209 ± 0.003^{b}
KGA	$4.86 \pm 0{,}01^{ab}$	20 ± 0^{e}	0.093 ± 0.002^{b}
KGAE	4.83 ± 0.02^{bc}	20 ± 0^{e}	0.067 ± 0.001^{b}

Values are given as average ± standard error. K–chitosan; KE–chitosan + GSE; GA–gum arabic; GAE–GA + GSE; KGA–chitosan + GA, KGAE–chitosan + GA + GSE. Significant differences between samples ($p < 0.05$) are shown by different superscripts (a–b) within a row.

3.3. Thermal film properties

Results of DSC measurements are given in Table 3. Changes in the inclination of the baseline were very weak in all measuring cycles, so no glass transition temperature for both chitosan and GA could be measured. Similar behavior was previously well reported in the scientific literature (Dong et al., 2004). Only chitosan powder showed an endothermic peak around 80.4 °C, assigned as the dehydration temperature, T_d and it was attributed to the small amounts of water absorbed during handling prior to measurement (Kurek et al., 2012). For other samples, no peaks could be seen below 100 °C, probably due to the low RH sample conditioning prior to measurements. The second endothermic peak for chitosan powder was measured at 186.8 °C, and at 167.7 °C for GA powder. Transition temperatures changed after film formation and in chitosan–based films they were lower than for chitosan powder (175.5 °C for simple chitosan film and 179.2 °C for KGAE film). In the scientific literature, these endothermic transitions are known as the dissociation temperature (T_{DS}). It is described as the dissociation process of the interchain hydrogen bonding of the chitosan, phase change from solid to a rubbery state, and loss of water of crystallization in GA (SaniMamman et al., 2020). As expected, these peaks disappeared in the second heating cycle (Chuang et al., 1999). Slightly higher values (3–4 °C) were measured in KGA blends. This might indicate that there was a weak molecular interaction between the chitosan and GA. In KGA film two endothermic peaks were observed at 178.8 and 191.6 °C, while in films enriched with GSE there was only one, but very broad, indicating some structural changes. According to the results, there was no degradation in KGA nor KGAE film at higher temperatures and therefore 80 °C was chosen as acceptable temperature for drying biscuits after FFS application.

3.4. Molecular interactions of film (FTIR)

FTIR spectral data are given in Fig. 1. Only slight changes were seen regarding to pure chitosan film, with no significant differences between KGA and KGAE. Both spectral data showed a broad band in the range of

Table 3
Thermal transition temperatures of tested samples.

Sample	T_d (°C)	T_{DS} (°C)
CS powder	80.4	186.8
GA powder	nd	167.7
GA	nd	nd
CS	nd	175.5
KGA	nd	178.8
		191.6
KGAE	nd	179.2

T_d-dehydration temperature; T_{DS}-dissociation temperature; CS–chitosan; GA–gum arabic; KGA–film made from chitosan and GA; KGAE film–film made from chitosan and GA and GSE; nd–not detected.

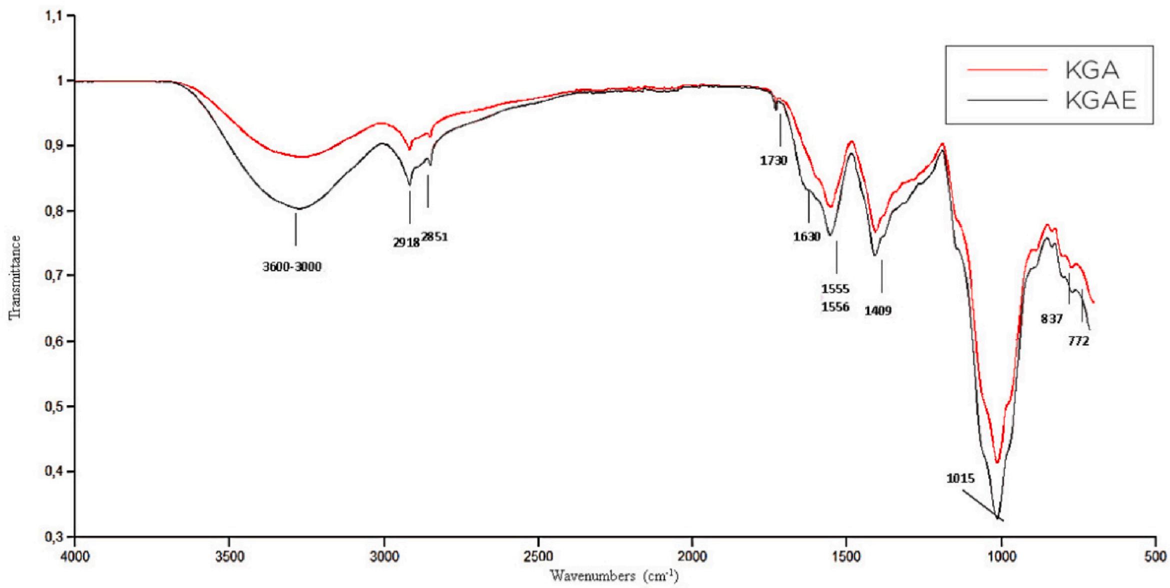

Fig. 1. Fourier transform-infrared (FTIR) for chitosan-GA film with grape seed extract GSE (KGAE) or without GSE (KGA).

3600–2900 cm^{-1} that were assigned to OH- and NH- stretching bands. Band at 2918 cm^{-1} was assigned to the asymmetric stretching of the aliphatic –CH_2 groups and at 2851 cm^{-1} to symmetric stretching CH_3/CH_2. The carbonyl stretching of the amide I peak characteristic for chitosan (band at 1643 cm^{-1}, spectra not given) was shifted to 1630 cm^{-1}, perhaps because the carboxylic groups in GA formed intermolecular hydrogen bonds with chitosan (Sakloetsakun et al., 2015). Similar data were given by others for NH_2 bending peak around 1550 cm^{-1} but this shift did not occur in the present study (1552 cm^{-1} for chitosan, spectra not given, 1556 cm^{-1} and 1555 cm^{-1} for KGA and KGAE, respectively). Peak at 1409 cm^{-1} corresponded to the asymmetric stretching vibration of –COO^-. Peaks below 1150 cm^{-1} were assigned to the skeletal C–O–C vibration and the vibration of amino group at the C2 position of a pyranose ring in chitosan.

3.5. Film morphology

Surface topography of the films was analyzed by scanning electron microscopy (SEM). Surface of the chitosan–GA films was found to be smooth, homogeneous and topographically featureless, with no sign of defects such as bubbles or cracks (not shown). In contrast to the unmodified chitosan–GA films, the films enriched with GSE (KGAE) were found to contain granular agglomerates 0.2–10 μm in size incorporated into the film matrix (Fig. 2). These agglomerates, most probably originating from the low solubility of GSE in the film forming solution, do not affect significantly the film homogeneity as they only sparsely appear in the film, as evident from the low-magnification SEM micrograph shown in Fig. 2a. In general, the surface of the chitosan–GA film containing GSE was smooth, with no cracks or pores (Fig. 2a), indicating a good structural integrity of the film and compactness of its structure.

3.6. Influence of edible coatings on color properties and acceptability of fresh cookies

The obtained results indicate that L^* was higher, while redness (a^*), and yellowness (b^*) were lower in cookies with KGAE than in cookies with simple chitosan and GA films (Table 4). The total color difference was the smallest for GAP with KGAE when compared to control without coating (Molnar et al., 2020) indicating that GSE, grape and aronia pomace might be good replacemenst for cocoa powder in cookies in terms of color acceptability.

Appearance, odor, texture and overall acceptability were similar

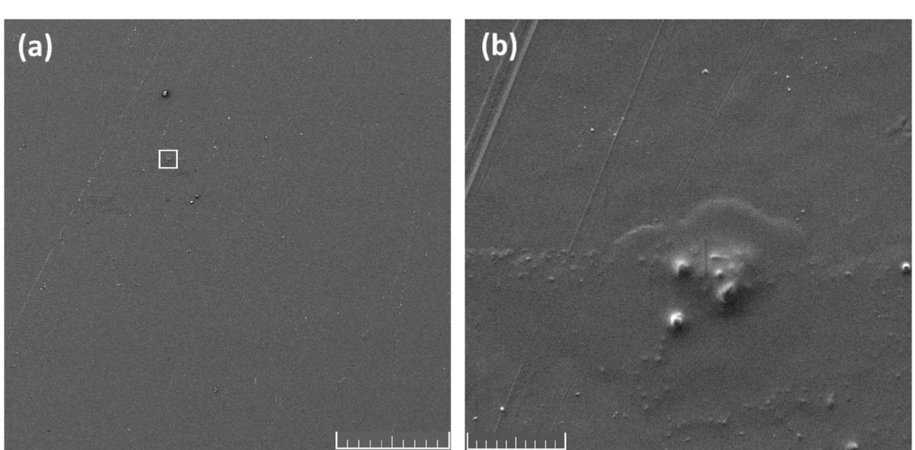

Fig. 2. SEM micrographs of the chitosan–GA film containing GSE. Fig. b shows a higher-magnification image of the squared region in Fig. a. Scale bars in Figs. a and b represent 200 and 10 μm, respectively.

Table 4
Color properties and consumer acceptability of fresh cookies with pomace depending on the applied film formulation.

Sample	Chitosan	GA	KGAE
L*	32.98 ± 0.80[b]	31.86 ± 0.70[c]	45.57 ± 0.70[a]
a*	7.93 ± 0.30[a]	8.00 ± 0.30[a]	3.52 ± 0.30[b]
b*	10.18 ± 0.70[a]	10.28 ± 0.50[a]	9.34 ± 0.70[b]
ΔE[a]	12.33	13.43	1.03
Appearance	6.7 ± 1.8[a]	8.0 ± 0.7[b]	7.6 ± 1.1[ab]
Odor	7.8 ± 1.4[a]	7.9 ± 0.7[a]	8.2 ± 0.9[a]
Flavor	6.8 ± 2.0[a]	7.7 ± 1.2[b]	7.9 ± 0.9[c]
Texture	5.5 ± 2.3[a]	6.8 ± 1.9[ab]	7.6 ± 1.0[b]
Overall acceptability	6.8 ± 2.5[a]	8.1 ± 0.6[b]	7.9 ± 0.9[b]

Values are given as average ± standard deviation. Cookies with grape and aronia pomace (GAP) + chitosan; GAP + GA; GAP + KGAE (chitosan + GA + GSE). Significant differences between samples ($p < 0.05$) are shown by different superscripts ([a–b]) within a column.
[a] Calculated relatively to the control cookie (CC) without pomaces in recipe and edible film.

between GAP cookies with GA or KGAE (Table 4) belonging to categories from “like slightly” (6.8) to “like very much” (8.2). Since the flavor is the most important sensory characteristic to consumers, the flavor of GAP with KGAE was the most acceptable and scored as “like very much”. The overall acceptability with chitosan was the lowest among all tested samples. Due to the best results on lightness, flavor, odor and texture, or in other words characterized as acceptable, chitosan and GA-based films with GSE was used for further analyzes of cookies shelf-life.

3.7. *Total phenolic content (TPC) of films with grape seed extract and fresh cookies*

The TPC results were the highest for GA solution 794.3 mg L^{-1}, followed by chitosan solution 717.1 mg $L^{-1}$and KGAE 712.8 mg L^{-1}. Similar findings were observed by Rodrigues et al. (2020) who found that the addition of GSE and Jabuticaba bark extract (*Brazilian grape tree*) showed a lower proportion of TPC in chitosan solution with added extract compared to the results when the extract was added to already formed polymer (chitosan and gelatin). Similar results were found when blackcurrant fruit waste was added to chitosan solution in comparison with pectin solution (Kurek et al., 2021). This might be related to changes in polyphenol structure and binding; when the extract was introduced to the polymer matrix (chitosan and gelatin) encapsulation of the extract was correlated to already established linkages and therefore the remaining free phenolic hydroxyls caused higher values of total phenolics in the sample (Rodrigues et al., 2020).

Following the above-mentioned results, the TPC was significantly higher in cookies with GAP and KGAE than in CC (Table 5), although CC contained cocoa that is also rich in polyphenols. Theagarajan et al. (2019) found an increase in TPC concentration in cookies containing grape pomace, that is consistent with findings in the present study. A small increase of TPC in GAP with KGAE compared to GAP could be due to the presence of KGAE. However, since the film weight was rather low (0.0005 g) it was insignificant considering the cookies average weight (4.0062 g).

3.8. *Cookies shelf-life*

3.8.1. *Moisture content and peroxide value of cookies during the accelerated storage*

The moisture content of cookies should be below 5% since an increasing amount of water can significantly affect their textural characteristics and result in a shorter shelf–life (Romani et al., 2016). According to the results (Table 5), although there were some changes in moisture content, it remained below 5% in all three types of cookies during 3 months of storage. The moisture content depended ($p < 0.001$)

Table 5
Total phenolic content (TPC), moisture content, and peroxide value (PV) of packaged control cookies (CC), cookies with grape and aronia pomace (GAP), and GAP with KGAE of fresh cookies and during the accelerated storage for 3 months at different temperatures.

Parameters	CC	GAP	GAP with KGAE
TPC of fresh cookies (μg GA g d. w $^{-1}$)	1.39 ± 0.01[a]	1.58 ± 0.02[b]	1.63 ± 0.03[b]
Moisture of fresh cookies (%)	3.55 ± 0.06[aC]	3.67 ± 0.06[aB]	2.72 ± 0.05[bB]
PV of fresh cookies (mmol/kg)	3.09 ± 0.09[aA]	1.59 ± 0.10[cD]	2.57 ± 0.13[bD]
	After 3 months of storage		
Moisture T22 °C (%)	3.82 ± 0.03[bB]	4.09 ± 0.12[aA]	3.16 ± 0.04[cA]
Moisture T30 °C (%)	4.51 ± 0.09[aA]	3.09 ± 0.08[bC]	2.98 ± 0.04[bA]
Moisture T35 °C (%)	2.52 ± 0.07[aD]	2.89 ± 0.03[bC]	2.96 ± 0.09[bA]
PV (mmol/kg) T22 °C	4.66 ± 0.04[aB]	2.67 ± 0.09[bC]	4.46 ± 0.07[aC]
PV (mmol/kg) T30 °C	4.53 ± 0.07[bB]	3.94 ± 0.04[cB]	4.81 ± 0.09[aB]
PV (mmol/kg) T35 °C	4.97 ± 0.04[cA]	8.3 ± 0.13[aA]	6.99 ± 0.10[bA]

Values are given as average with standard deviation. CC–control cookies; Cookies with grape and aronia pomace (GAP); GAP + KGAE (chitosan + GA + GSE). Significant differences between samples ($p < 0.05$) are shown by different superscripts within a row ([a–c]) or during storage within a column (A-D).

on sample and storage conditions. Still, the moisture content least changed in GAP with KGAE during storage. This might be related to the additional drying step after KGAE application and the obtained low moisture content in the fresh cookies. Thus, it is necessary to monitor the moisture content of cookies after applying edible coatings.

The PV in food products is a measure of fat rancidity or the degree of primary lipid oxidation. The PV was statistically dependent ($p < 0.001$) on the interaction between sample and storage conditions. Among fresh cookies, CC had the highest PV. In all cookies, the PV was progressively increased over 3 months as the storage temperature was higher (Table 5) but remained below 10 mmol O_2 kg^{-1} which is considered critical (Niki, 2009). Nevertheless, after storage at 35 °C the PV was significantly higher in both pomace-enriched and coated samples compared to CC. This might be due to the higher antioxidant activity of CC with cocoa powder compared to cookies with GAP or that peroxides turned into secondary oxidation products. Nevertheless, Zaky et al. (2020) found that the PV of cookies without cocoa can be reduced up to 20% with the addition of higher concentrations of grape pomace extract (3%) when stored in low-density polyethylene (LDPE) bags at room temperature for 6 months.

The activation energy (Ea) was calculated using the Arrhenius equation and is given in Table 6. Although, the lowest activation energy was found for CC, according to ASLT, the highest shelf–life was expected for CC due to the lowest increase in PV at 35 °C. The activation energy of a chemical reaction is closely related to its rate; the higher the activation energy, the slower the chemical reaction will be. Although, the

Table 6
Activation energy, reaction rate, and predicted shelf-life of packaged control cookies (CC), cookies with grape and aronia pomace (GAP) and GAP with KGAE according to Arrhenius equation.

Parameters	CC	GAP	GAP with KGAE
Activation energy (J mol^{-1})	44642.3	162870.7	106725.4
Slope	−5369.53	−19589.9	−12836.8
Reaction rate (*k*) at 30 °C	0.012	0.020	0.019
Reaction rate (*k*) at 35 °C	0.016	0.056	0.037
Shelf-life (days) at 30 °C	576	429	398
Shelf-life (days) at 35 °C	441	151	202

activation energy of cookies with grape and aronia pomace and KGAE is higher resulting in slower chemical reaction and slower decrease rate of cookies quality, according to ASLT the highest increase in PV at 30 °C, was detected for cookies with grape and aronia pomace and KGAE. Highest increase in PV might have a great impact to shorter shelf life of the cookie due to the higher permeability to water vapor after application of grape seed extract (GSE).

3.8.2. Textural and sensory properties of cookies during real-time storage

Storage properties of cookies can be changed by varying in product formulation, structural characteristics or moisture content and water activity (Romani et al., 2016). After 6 months of storage at ambient conditions, a rancid odor was detected only in CC (Fig. 3a) which indicates that the secondary oxidation products were developed. This marked the end of the CC shelf-life because the sensory properties of the product were unacceptable at this point. This was unexpected according to ASLT estimated shelf-life but was in accordance with results of activation energy indicating that the oxidation reaction will run faster, decreasing the quality of cookies (Table 6). The lowest TPC content in CC might be the reason for the development of rancid odor and the shortest shelf-life of CC cookies. Thus, those samples were not further sensory evaluated. The first two components of PCA (Fig. 3b) with eigen values of 4.98 and 2.05 explained 70% of the variance. Hardness (0.97), toughness (0.85), brittleness (0.83), fruity flavor (0.83), surface gloss (0.87) and rancidity were all highly linked to the first component (0.84). The second component was mostly related to cocoa butter (−0.87) and overall perception (−0.80). Fig. 3(b) shows that cookies grouped according to the formulation. GAP samples were related to the cocoa odor, overall impression and teeth adhesiveness. GAP with KGAE was associated with surface gloss, brittleness, hardness, fruity flavor and acid taste suggesting that the coating might have affected these parameters. GAP with KGAE was related to rancid odor, but the least differentiated with storage time indicating the possibility of even longer shelf–life as indicated by sensory evaluation.

4. Conclusion

In the present study novel hydrocolloid and pomace extract formulations were designed for use as edible coatings for cookies. Formulations in form of films, made from chitosan and gum arabic, had desirable characteristics for the intended product (cookie). The addition of grape seed extract led to an increase in the total phenolic content and film thickness (41%) giving the good structural integrity with no negative effects on the film's appearance. The addition of the grape seed extract reduced transparency (92%), and increased water vapor barrier (30%) without structural changes at elevated temperatures, indicating that chitosan and gum arabic films with grape seed extract had desirable properties that could improve quality and extend the target product shelf-life. Designed formulations were successfully applied to the laboratory scale-made cookies. Although coatings changed the texture properties of cookies, more importantly, their application resulted in the extension of cookies shelf-life (for at least 30 days compared to the control cookies) which was confirmed with the direct method. Since slightly different results were obtained in direct method study compared with the accelerated shelf–life method (lowest peroxide value of control cookies at 35 °C), in future studies it is envisable to make additional analysis like measuring anisidine value and sensory evaluation. Also, future studies should focus on novel edible film formulations that would enable new cookie formulations with prolonged shelf-life differing from existing ones on the market.

Ethical statement

Ethical approval for the involvement of human subjects in this study was granted by Faculty of Food Technology and Biotechnology, University of Zagreb Research Ethics Committee, Reference number 251-69-11-20-37, dtd 12/14/2020.

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CRediT authorship contribution statement

Dunja Molnar: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft. **Dubravka Novotni:** Investigation, Supervision, Conceptualization, Methodology, Resources, Formal analysis, Writing – review & editing. **Mia Kurek:** Investigation, Methodology, interpretation, Writing – review & editing. **Kata Galić:** Writing – review & editing. **Damir Iveković:** Investigation, Methodology. **Helena Bionda:** Investigation. **Mario Ščetar:** Investigation, Supervision, Conceptualization, Methodology, Resources, Formal analysis, Writing – review & editing.

Declaration of competing interest

For paper entitled Characteristics of edible films enriched with fruit by-products and their application on cookies, submitted to Food Hydrocolloids

by Dunja Molnar, Dubravka Novotni, Mia Kurek, Kata Galić, Damir Iveković, Helena Bionda, Mario Ščetar, the authors declare that they

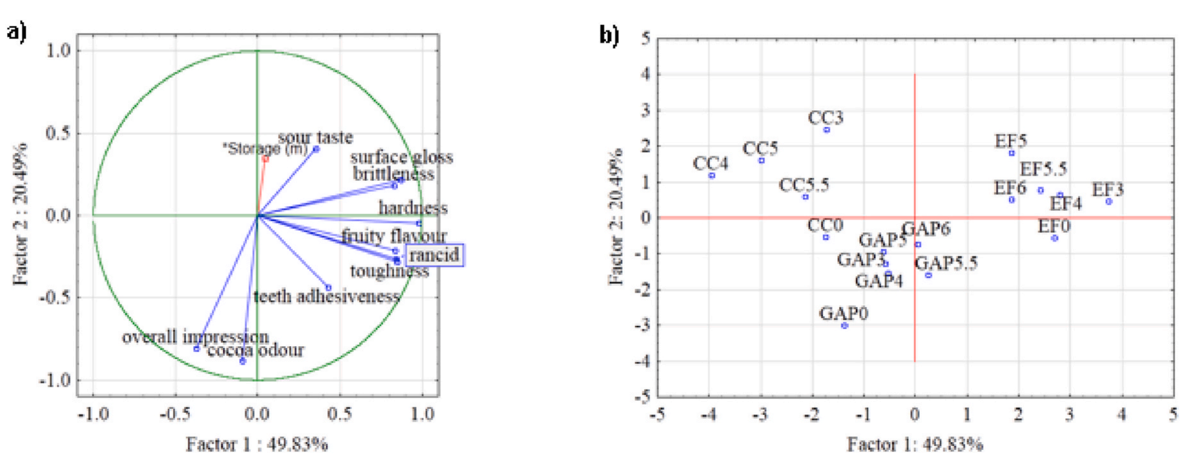

Fig. 3. Principal component loadings (a) and score (b) plots of instrumental (brittleness, breaking strength) and sensory attributes of control cookies (CC), GAP and GAP with KGAE (EF) during 0, 1, 3, 4, 5, 5.5 and 6 months of ambient storage.

have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

AACC. (2000). *Approved methods of the American association of cereal chemists International.11. Izd*. St. Paul, MN, SAD: AACC - American Association of Cereal Chemists.

ASTM. (1980). *-80 standard test method for water vapor transmission of materials* (p. E96). ASTM International.

Bnuyan, I., Hindi, N. K. K., & Jebur, M. H. (2015). In vitro antimicrobial activity of gum Arabic (Al MannaandTayebat) prebiotics against infectious *pathogens. International Journal of Pharmacy and Pharmaceutical Research, 3*(3), 77–85.

Chuang, W. Y., Young, T. H., Yao, C. H., & Chiu, W. Y. (1999). Properties of the poly (vinyl alcohol)/chitosan blend and its effect on the culture of fibroblast in vitro. *Biomaterials, 20*(16), 1479–1487. https://doi.org/10.1016/s0142-9612(99)00054-x

De MoraesCrizel, et al. (2018). Biodegradable films based on gelatin and papaya peel microparticles with antioxidant properties. *Food and Bioprocess Technology, 11*, 536–550. https://doi.org/10.1007/s11947-017-2030-0

Díaz – Montes, E., & Castro – Muñoz, R. (2021). Edible films and coatings as food-quality preservers: An overview. *Foods, 10*, 249. https://doi.org/10.3390/foods10020249

Dong, Y., Ruan, Y., Wang, H., Zhao, Y., & Bi, D. (2004). Studies on glass transition temperature of chitosan with four techniques. *Journal of Applied Polymer Science, 93*, 1553–1558. https://doi.org/10.1002/APP.20630

Espinosa - Andrews, H., Baez - Gonzalez, J. G., Cruz - Sosa, F., & Vernon - Carter, E. J. (2007). Gum Arabic-chitosan complex coacervation. *Biomacromolecules, 8*(4), 1313–1318. https://doi.org/10.1021/bm0611634

Fan, et al. (2018). Synthesis, characterization, and antifungal evaluation of diethoxyphosphorylpolyaminoethyl chitosan derivatives. *Carbohydrate Polymers, 190*, 1–11. https://doi.org/10.1016/j.carbpol.2018.02.056

Galić, K., Ćurić, D., & Gabrić, D. (2009). Shelf-life of packaged bakery goods - a review. *Critical Reviews in Food Science and Nutrition, 49*(5), 405–426. https://doi.org/10.1080/10408390802067878

Goodrum, L. J., Patel, A., Leykam, J. F., & Kieliszewski, M. J. (2000). Gum Arabic glycoprotein contains glycomodules of both extensin and arabinogalactan-glycoproteins. *Phytochemistry, 54*(1), 99–106. https://doi.org/10.1016/s0031-9422(00)00043-1

Heising, J. K., van Boekel, M. A. J. S., & Dekker, M. (2015). Simulations on the prediction of cod (Gadus morhua) freshness from an intelligent packaging sensor concept. *Food Packaging and Shelf Life, 3*, 47–55. https://doi.org/10.1016/J.FPSL.2014.10.002

International Organization for Standardisation. (2017). *Animal and vegetable fats and oils – determination of peroxide value – iodometric (visual) endpoint determination*. ISO Standard No. 3960:2017.

International Organization for Standardization. (2015). *Sensory analysis —assessment (determination and verification) of the shelf-life of foodstuffs*. ISO Standard No. 16779:2015.

Khaliq, G., Mohamed, M. T. M., Ali, A., Ding, P., & Ghazali, H. M. (2015). Effect of gum Arabic coating combined with calcium chloride on physico- chemical and qualitative properties of mango (Mangifera indica L.) fruit during low temperature storage. *Scientia Horticulturae, 190*, 187–194. https://doi.org/10.1016/J.SCIENTA.2015.04.020

Kurek, M., Benbettaieb, N., Ščetar, M., Chaudy, E., Repajić, M., Klepac, D., , … Galić, K., et al. (2021). Characterization of food packaging films with blackcurrant fruit waste as a source of antioxidant and color sensing intelligent material. *Molecules, 26*(9), 2569. https://doi.org/10.3390/molecules26092569

Kurek, M., Brachais, C-H., Nguimjeu, C. M., Bonnotte, A., Voilley, A., Galić, K., Debeaufort, F., et al. (2012). Structure and thermal properties of a chitosan coated polyethylene bilayer film. *Polymer Degradation and Stability, 97*(8), 1232–1240. https://doi.org/10.1016/j.polymdegradstab.2012.05.039

Lu, L.-X., & Xu, F. (2010). Effects of initial oxygen concentration and oxygen-barrier property of film on fat oxidation rate of packed cookies. *European Food Research and Technology, 231*, 347–351. https://doi.org/10.1007/s00217-010-1282-y

Moghadam, M., Salami, M., Mohammadian, M., Khodadadi, M., & Emam – Djomeh, Z. (2020). Development of antioxidant edible films based on mung bean protein enriched with pomegranate peel. *Food Hydrocolloids, 104*, Article 105735. https://doi.org/10.1016/j.foodhyd.2020.105735

Molnar, D., Novotni, D., Krisch, J., Bosiljkov, T., & Ščetar, M. (2020). The optimisation of cookie formulation with grape and aronia pomace powders as cocoa substitutes. *Croatian Journal of Food Technology, Biotechnology and Nutrition, 15*, 38–44. https://doi.org/10.31895/hcptbn.15.1-2.5

Nagy, H. P. S., Kaur, J., Dar, B. N., & Sharma, S. (2012). Effect of storage period and packaging on the shelf-life of cereal bran incorporated cookies. *American Journal of Food Technolology, 7*, 301–310. https://doi.org/10.3923/ajft.2012.301.310

Niki, E. (2009). Lipid peroxidation: Physiological levels and dual biological effects. *Free Radical Biology andMedicine47*, 469–484. https://doi.org/10.1016/j.freeradbiomed.2009.05.032

Oliveira Filho, et al. (2020). Chitosan film with citrus LimoniaEssentil oil: Physical and morphological properties and antibacterial activity. *Colloids and Interfaces, 4*(2), 18. https://doi.org/10.3390/colloids4020018

Pathare, P. B., Opara, U. L., & Al-Said, F. A. J. (2013). Color measurement and analysis in fresh and processed foods: A review. *Food and Bioprocess Technology, 6*, 36–60. https://doi.org/10.1007/s11947-012-0867-9

Peng, Y., Wu, Y., & Li, Y. (2013). Development of tea extracts and chitosan composite films for active packaging materials. *International Journal of Biological Macromolecules, 59*, 282–289. https://doi.org/10.1016/j.ijbiomac.2013.04.019

Qiao, G., Xiao, Z., Ding, W., & Rok, A. (2019). Effect of chitosan/nano-titanium dioxide/thymol and tween films on ready-to-eat cantaloupe fruit quality. *Coatings, 9*, 828. https://doi.org/10.3390/coatings9120828

Riaz, et al. (2018). Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols. *International Journal of Biological Macromolecules, 114*, 547–555. https://doi.org/10.1016/j.ijbiomac.2018.03.126

Rodrigues, M. A. V., Vicente Bertolo, M. R., Aparecida Marangon, C., da Conceição Amaro Martins, V., & de GuzziPlepis, A. M. (2020). Chitosan and gelatin materials incorporated with phenolic extracts of grape seed and jabuticaba peel: Rheological, physicochemical, antioxidant, antimicrobial and barrier properties. *International Journal of Biological Macromolecules, 160*, 769–779. https://doi.org/10.1016/j.ijbiomac.2020.05.240

Romani, S., Rocculi, P., Tappi, S., & Dalla Rosa, M. (2016). Moisture adsorption behaviour of cookie during storage investigated by using a new Dynamic Dewpoint method. *Food Chemistry, 195*, 97–103. https://doi.org/10.1016/j.foodchem.2015.06.114

Sakloetsakun, D., Preechagoon, D., Bernkop–Schnürch, A., & Pongjanyakul, T. (2015). Chitosan–gum Arabic polyelectrolyte complex films: Physicochemical, mechanical and mucoadhesive properties. *Pharmaceutical Development and Technology*, 1–10. https://doi.org/10.3109/10837450.2015.1035727

Sani Mamman, I., Teo, Y. Y., & Misran, M. (2020). Synthesis, characterization and rheological study of Arabic gum-grafted-poly (methacrylic acid) hydrogels. *Polymer Bulletin*. https://doi.org/10.1007/s00289-020-03267-4

Shingel, K. I. (2004). Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. *Carbohydrate Research, 339*, 447–460. https://doi.org/10.1016/j.carres.2003.10.034

Shortle, et al. (2014). Influence of extraction technique on the anti-oxidative potential of hawthorn (Crataegus monogyna) extracts in bovine muscle homogenates. *Meat Science, 98*, 828–883. https://doi.org/10.1016/j.meatsci.2014.07.001

Siracusa, V., Rocculi, P., Romani, S., & Dalla Rosa, M. (2008). Biodegradable polymers for) food packaging: A review. *Trends in Food Science & Technology, 19*(12), 634–643. https://doi.org/10.1016/j.tifs.2008.07.003

Sochorova, et al. (2020). Health effects of grape seed and skin extracts and their influence on biochemical markers. *Molecules, 25*(22), 5311. https://doi.org/10.3390/molecules25225311

Sogut, E., & Seydim, A. C. (2018). The effects of Chitosan and grape seed extract-based edible films on the quality of vacuum packaged chicken breast fillets. *Food Packaging and Shelf Life, 18*, 13–20. https://doi.org/10.1016/J.FPSL.2018.07.006

Svensson, L. (2012). *Design and performance of small scale sensory consumer tests*. Retrived from Uppsala website http://stud.epsilon.slu.se/50 45/1. (Accessed 7 October 2020).

Theagarajan, et al. (2019). Valorisation of grape pomace (cv. Muscat) for development of functional cookies. *International Journal of Food Science and Technology, 54*, 1299–1305. https://doi.org/10.1111/ijfs/14119

Vuillemin, M. E., Michaux, F., Muniglia, L., Linder, M., & Jasniewski, J. (2019). GumArabic and chitosan self-assembly: Thermodynamic and mechanism aspects. *Food Hydrocolloids, 96*, 463–474. https://doi.org/10.1016/J.FOODHYD.2019.05.048

Wang, L., Dong, Y., Men, H., Tong, J., & Zhou, J. (2013). Preparation and characterization of active films based on chitosan incorporated tea polyphenols. *Food Hydrocolloids, 32*(1), 35–41. https://doi.org/10.1016/J.FOODHYD.2012.11.034

Wu, et al. (2014). Propertiesand antimicrobial activity of silver carp (*Hypophthalmichthys molitrix*) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. *Food Packaging and Shelf Life, 2*, 7–16. https://doi.org/10.1016/j.fpsl.2014.04.004

Xu, L., Chu, Z., Wang, H., Cai, L., Tu, Z., Liu, H., Zhu, C., Shi, H., Pan, D., Pan, J., & Fei, X. (2019). Electrostatically assembled multilayered films of biopolymer enhanced nanocapsules for on-demand drug release. *ACS Applied Polymer Material, 2*, 3429–3438.

Xu, et al. (2019). Structure, physical and antioxidant properties of chitosan-gum Arabic edible films incorporated with cinnamon essential oil. *International Journal of Biological Macromolecules, 134*, 230–236. https://doi.org/10.1016/j.ijbiomac.2019.04.189

Xu, L., Wang, H., Chu, Z., Cai, L., Shi, H., Zhu, C., Pan, D., Pan, J., Fei, X., & Lei, Y. (2020). Temperature-responsive multilayer films of micelle-based composites for controlled release of a third-generation EGFR inhibitor. *ACS Applied Polymer Material, 2*, 741–750.

Xu, L., Zhang, X., Chu, Z., Wang, H., Li, Y., Shen, X., Cai, L., Shi, H., Zhu, C., Pan, J., & Pan, D. (2021). Temperature-responsive multilayer films based on block copolymer-coated silica nanoparticles for long-term release of favipiravir. *ACS Applied Polymer Material, 4*, 14014–14025.

Zaky, A. A., Mohsen, E. F. S. Y., Ashour, M. S., & Asiamah, E. (2020). Utilization of grape pomace extract as a source of natural antioxidant in cookies. *Food Science, 8*(1), 108–126.

Zbikowska, et al. (2018). Effect of addition of plant extracts on the durability and sensory properties of oat flake cookies. *Journal of Thermal Analysis and Calorimetry, 134*(2), 1101–1111. https://doi.org/10.1007/s10973-018-7301-0

Publication No. 2: Application of fruit by-products and edible film to cookies: Antioxidant activity and concentration of oxidized LDL receptor in women—A first approach

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applied sciences

Article

Application of Fruit By-Products and Edible Film to Cookies: Antioxidant Activity and Concentration of Oxidized LDL Receptor in Women—A First Approach

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Abstract: Cookie consumption can change the serum level of oxidized low-density lipoprotein (oxLDL) and oxLDL receptors, both playing important roles in the pathogenesis of atherosclerosis and cardiovascular diseases. This study investigated the nutritional value and the antioxidant activity of whole grain cookies in which 24% of the cocoa powder was substituted with grape and aronia pomace and were further coated with edible films enriched with grape seed extract (GAP with KGAE) as well as the effects of their consumption on the serum level of oxLDL receptors in women. The proximate composition, mineral content, antioxidant activity, and starch digestibility in vitro of experimental and control cookies were determined. A group of 12–13 healthy women (median age 36) consumed 45 g of GAP with KGAE or commercial cookies for 10 days. The results showed that GAP and KGAE cookies had increased flavonoid content (22%) and antioxidant potential (27–73%) compared to the control. The content of slowly digestible starch prevailed over rapidly digestible starch. The serum concentrations of the oxLDL receptors between the test and control groups were similar. We can conclude that the moderate consumption of whole grain cookies with fruit by-products does not lead to the formation of oxLDL receptors in healthy women.

Keywords: oxidized LDL receptor; starch digestibility; biscuits; chitosan; gum arabic; grape pomace; aronia; chokeberry pomace; cocoa substitute; grape seed extract

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1. Introduction

The excessive consumption of cookies that are high in sugar and saturated fat can lead to chronic non-communicable diseases such as cardiovascular disease (CVD) and diabetes type II [1]. CVD is a leading cause of premature death and disability in Europe, according to the World Health Organization [2]. Cookie consumption changes the blood concentration of glucose, but also triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein [1]. This alone does not cause CVD because LDL must first be oxidized. If there is an imbalance between radical formation (production of reactive oxygen species) and radical removal (action of antioxidants), oxidized low-density lipoprotein (oxLDL) can form [3]. The newly formed oxLDL triggers the onset of atherosclerosis in the following steps: increased expression of adhesion molecules on vascular endothelial cells, the attraction of leukocytes to endothelial cells, the sequestration of leukocytes into the intimal layer, macrophage activation, the release of cytokines and reactive oxygen species (ROS), and plaque formation [4]. In addition, the lectin-like receptor-1 for oxLDL plays an important role in triggering atherosclerosis since different actions commence when oxLDL binds to the oxLDL receptor in different cell types. This binding increases the absorption of oxLDL in vascular smooth muscle cells (VSMCs) and macrophages and promotes the production of foam cells. Moreover, it induces apoptosis in VSMCs and contributes to endothelial activation [5]. However, to our knowledge, no study investigated the effect of cookie consumption on the formation of oxLDL receptors in humans.

Polyphenols and other antioxidants in human nutrition represent a valuable nutritional tool in preventing CVD, diabetes, osteoporosis, and cancer [6]. Polyphenols counteract the oxidation of LDL and the subsequent deposition of atherosclerotic material in tissues [7]. Epidemiological studies have shown that polyphenols present in berries [8], cocoa [9] and red wine [10] are strong antioxidants that can slower CVD progression.

Recently, fruit-by products have become interesting for the recovery of their antioxidants. Aronia or chokeberry (*Aronia melanocarpa*) is the richest source of polyphenols among berry fruits [11], with a very high content of procyanidins, anthocyanidins, and phenolic acids, while flavonols are present in lower amounts [12]. Aronia is mostly used for juice production, in which aronia pomace remains a by-product. In addition to their high antioxidant capacity, the main polyphenolic constituents of aronia also possess anti-inflammatory, anticancer, antimicrobial, antiviral, antidiabetic, antiatherosclerotic, antihypertensive, antiplatelet, and anti-inflammatory properties [13]. Grapes (*Vitis vinifera*), are one of the most widely grown crops worldwide [14] and are used for wine or juice production. The remaining grape pomace and seeds contain significant amounts of dietary fiber with high antioxidant activity due to the natural presence of polyphenols and other bioactive compounds, indicating the potential use of this sustainable resource as a food or beverage ingredient [15–18]. In our previous studies, we found that up to 24% of cocoa powder can be successfully replaced in cookie recipes with a mixture of grape and aronia pomace (GAP) without affecting their sensory acceptability [18].

Polysaccharide-based edible films are a form of biodegradable food packaging [19] that not only fulfill the role of protection but also reinforce the mechanical strength and enhance the phenolic content of a food [20]. Edible films are used for various food categories, including baked goods [20,21]. Previously, we found that the application of an edible film based on chitosan and gum arabic enriched with grape seed extract (KGAE) positively affects cookies' shelf life [20]. Although there is great interest in the health benefits of edible films enriched with bioactive compounds, the detailed nutritional profile of coated cookies is missing.

The aim of the present study was to investigate the effects of replacing cocoa powder with fruit by-products and the application of edible film on the nutritional value, antioxidant activity, and starch digestibility of cookies. The second goal of this study was to investigate the effects of cookies consumption on oxLDL receptors in healthy women.

2. Materials and Methods

2.1. Materials for Preparation of Cookies and Edible Films

Fine rolled oats (Crownfield, Germany) with a protein content of 13.5% and whole spelt flour (Siladi, Croatia) with a protein content of 12% were purchased at a local market. Margarine (containing 30% butter) (70% fat) and cocoa powder (20% fat) were purchased from Zvijezda and Kraš (Zagreb, Croatia), respectively. Dried red grape pomace (*Vitis vinifera* L., Frankovka and Syrah varieties, with seeds, containing 9.2% fat) and aronia pomace (*Aronia melanocarpa* L., without seeds, containing 4.0% fat) were obtained as by-products from local juice producers (Davorka Šipek, Natkrižovljan, Cestica municipality, and Tomislav Jurendić, Koprivnica, Croatia). Dried grape and aronia pomaces (7 g) were ground in a laboratory ball mill (Cryomill, Retsch, Golling an der Salzach, Austria) with 12 steel balls (10 mm diameter) for 3 min in a 50-mL stainless steel container at a vibration frequency of 30 Hz to achieve the median diameter of the 50th percentile (36 ± 5 μm) similar as cocoa powder (41 ± 3 μm) [18].

The edible film was prepared using chitosan (France Chitin, Orange, France, type 652, Mw 165 kDa, DA > 85%), gum arabic (GA) (Enologica vasons. p.a., San Pietro in Carino, Italy) and MegaNatural Gold grape seed extract (GSE) donated by Polyphenolics (Madera, CA, USA; the total phenolic content was 90%, expressed as mg of gallic acid equivalent per 100 g). GSE was stored in its original packaging at −18 °C. A film-forming solution (FFS) was prepared using acetic acid (Merck, Darmstadt, Germany) and purified water.

2.2. Cookies and Edible Film Preparation

Three types of cookies were prepared according to Molnar et al. [20]: control cocoa cookies without fruit pomace in the recipe (CC), cookies enriched with grape and aronia pomace (GAP), and cookies with grape and aronia pomace covered with edible film (GAP with KGAE). The recipe for CC contained (with a percentage of spelt flour and oat flakes combined weight in brackets): fine rolled oats 300 g, whole spelt flour 252 g, margarine 200 g (36.2%), brown sugar 135 g (24.5%), cocoa powder 48 g (8.7%), vanilla sugar 35 g (6.3%), tap water 20 g (3.6%), salt 4 g (0.7%), baking powder 3 g (0.5%), and sodium bicarbonate 3 g (0.5%). In GAP and GAP with KGAE, 11 g (23.6%) of cocoa powder was replaced with a mixture of grape pomace (8.2 g which is 17.5% of cocoa) and aronia pomace (2.8 g which is 6.1% of cocoa) [18].

The FFS, consisting of chitosan, GA and GSE, was prepared as described previously by Molnar et al. [21]. First, chitosan powder was dissolved in 1% (v/v) aqueous acetic solution while GA powder was dissolved in distilled water to obtain 5% (w/v) solutions. Then, GSE (1 g/L) was added to the GA solution and mixed for 40 min on a magnetic stirrer. Finally, the GA containing GSE and the chitosan solutions were mixed in a 50:50 ratio to obtain an active FFS (KGAE). After spraying KGAE over the GAP cookies, they were dried for 30 min at 80 °C in an oven [20].

CC, GAP, and GAP with KGAE were used for nutritional and biochemical analyses as well as for the determination of starch digestibility, while GAP with KGAE was used in the intervention study along with commercial cookies.

Commercial cookies (Cioccograno, Mulino Bianco, Barilla) purchased at the local market contained 22 g of fat of which 10 g were saturated fatty acids, 60.4 g of carbohydrates of which 23.4 g were sugars, 7.6 g of protein, 6.3 g of dietary fiber and 0.43 g of salt per 100 g.

2.3. Determination of Proximate Composition of Cookies

Ash content was determined after incineration at 550 °C according to the AACC 08-01 method [22]. Moisture content was determined gravimetrically using a moisture analyzer (PMB53 Adam Equipment, Oxford, CT, USA). Protein content was analyzed according to the standard Kjeldahl method [23] and the conversion factor 6.25 was used for the calculation. Fat content was analyzed according to ISO 6492:1999 [24] and using the Foss Soxtec TM 8000 extraction system and the Foss Hydrotec TM 8000 Hydrolasys system (Foss, Hilleroed, Denmark), while the methyl esters of fatty acids [saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA)] were determined in accordance with ISO 12966-4:2015 [25] using an Agilent 7890B gas chromatograph (Santa Clara, CA, USA). Crude fiber content was measured using the FIBERTEC 2010 and M6 (Foss Analytical AB, Höganäs, Sweden). The method is based on successive steps of chemical treatments to dissolve the non-fiber components and the final determination of the residue obtained. The determination of mineral content was performed according to the European standards EN 14084:2003 [26] and EN 15763:2010 [27]. The determination of ash, protein and fat content was performed in duplicate, while the determination of crude fiber and minerals was performed in triplicate for each cookie sample.

2.4. In Vitro Starch Digestibility

Starch digestibility was studied in vitro using a slightly modified method of Englyst [28], which directly correlates with the glycemic response [29]. The method is based on the measurement of glucose released from a test food during a time-limited incubation with digestive enzymes under controlled conditions and allows the determination of free glucose (FG), rapidly available glucose (RAG), rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and total starch (TS). Heat-stable amylase (A3306) used to determine total starch, as well as pancreatin (P7547), amyloglucosidase (A7095), and invertase (I4504) used for in vitro hydrolysis of starch, were purchased from Sigma Aldrich Co. (St. Luis, MO, USA). The absorbance of the standards and samples was measured at 490 nm using a microplate reader (VICTOR X3, PerkinElmer, Shelton, CT, USA). The following formulas were used to calculate TS, RDS, SDS, RS, and RAG:

$$\mathrm{TS} = (\mathrm{TG} - \mathrm{FG}) \times 0.9 \tag{1}$$

$$\mathrm{RDS} = (\mathrm{G}_{20} - \mathrm{FG}) \times 0.9 \tag{2}$$

$$\mathrm{SDS} = (\mathrm{G}_{120} - \mathrm{G}_{20}) \times 0.9 \tag{3}$$

$$\mathrm{RS} = (\mathrm{TG} - \mathrm{G}_{120}) \times 0.9 \tag{4}$$

$$\mathrm{RAG} = \mathrm{At} \times \mathrm{Vt} \times \mathrm{C} \times \mathrm{D}/\mathrm{As} \times \mathrm{Wt} \times 100 \tag{5}$$

where G_{20} represents glucose released after 20 min, G_{120} represents glucose released after 120 min, At is the absorbance of the test solution, Vt is the total volume of the test solution, C is the concentration of standard (mg glucose/mL), D is the dilution factor, As is the absorbance of standard and Wt is the weight (in mg) of the sample taken for analysis.

2.5. Determination of Flavonoid Content and Antioxidant Activities

The extract was prepared as described by Molnar et al. [20]. It was used for the measurement of flavonoid content and antioxidant activities in triplicate using a UV 1600PC spectrophotometer (VWR International, Leuven, Belgium).

For the determination of flavonoid content, 2 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 100 µL of distilled water were pipetted into a cuvette. A blank sample was prepared in the same manner, using the extraction solvent instead of the extract and distilled water instead of 10% aluminum chloride. After 30 min, the absorbance was measured at 415 nm. The six-point calibration curve (0.02–0.10 µmol/mL) was prepared using a rutin standard solution.

For the ferric reducing antioxidant power (FRAP) assay, 300 µL of the extract was mixed with 200 µL of methanol and acetone solution (50:50, *v*/*v*) and 2 mL of the FRAP reagent (temperate at 37 °C) was immediately added. The absorbance was measured at 593 nm. Radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH). In a microcuvette, 150 µL of the extract was mixed with 0.95 mL of a 0.06 mM DPPH solution. After standing in the dark for 30 min, absorbance was measured at 517 nm. For the 2,2′-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) assay, 150 µL of the extract was mixed well with 2 mL of $ABTS^{+}$ and shaken. The extraction solvent was used for the blank samples. The absorbance was measured after 6 min with a blank sample (extraction solvent instead of extract) at 734 nm. The five-point standard calibration curves (0.02–0.10 µmol/mL) were prepared for FRAP, DPPH, and ABTS antioxidant tests using a Trolox solution (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

2.6. Determination of oxLDL

The study was designed as a randomized controlled trial. It was carried out in May 2021, at the University Hospital Center Sestre Milosrdnice, the Department of Clinical Chemistry (Zagreb, Croatia).

2.6.1. Subjects

A cohort of 25 women (aged 23–60) was randomly divided into two groups. The control group included 12 women who consumed 45 g of commercial cookies (Cioccograno, Mulino Bianco, and Barilla). The test group included 13 women who consumed 45 g of GAP with KGAE. Participants were advised to eat 30 g of cookies at breakfast and 15 g of cookies between meals daily for 10 days. Study participants were nonsmokers, did not suffer from chronic diseases, were not taking medications to treat cardiovascular diseases, and had not taken dietary supplements for at least three months prior to the start of the study. Subjects who were pregnant, had a body mass index (BMI) of >30 kg/m^2, or were vegetarians were excluded because these factors affect oxidative stress, which would affect the results of the study. In addition, subjects who consumed more than 500 mL of flavonoid-rich foods (tea, coffee, cocoa, fruit juices) were excluded, as determined by a food intake frequency questionnaire. Subjects suffering from allergies to any of the ingredients were also excluded. All subjects were asked to maintain their usual lifestyle and to report any illness if it occurred during the study. All participants signed an informed consent form to participate in this study.

2.6.2. Anthropometric and Biochemical Parameters

Assessment of participants included anthropometric measurements (body height (BH), bodyweight (BW), and waist and hip circumference), dietary methods (Food Frequency Questionnaire (FFQ)), and biochemical methods (determination of glucose, triglycerides, total cholesterol, HDL and LDL cholesterol, oxLDL receptor, and iron in blood/serum). Anthropometric measurements were performed at baseline to calculate body mass index (BMI = BW (kg)/BH^2 (m)), and waist-to-hip ratio (WHR). All necessary information about the study participants (age, level of education, number of people in the household, alcohol, medications, dietary supplements, allergy status, frequency of consumption of food prepared outside the home, and level of physical activity) was provided in the first section of the FFQ. The reference period for data collection was the month preceding the participation.

Dietary intake was followed using the modified validated FFQ [30]. Foods were divided into nine categories (cereals, fruits, vegetables, dairy products, legumes, fats, nuts and seeds, sweets, and beverages). A Likert scale was used to determine frequencies and the following responses were possible: always (every day—5), often (three to five times a week—4), sometimes (once a week—3), rarely (once or twice a month—2), and never (1).

All biochemical parameters were measured at the beginning of the study in the fasting state, while the oxLDL receptor was also measured 2 h after consumption of cookies, and after 10 days of the intervention. Glucose, iron, and lipid profile concentrations were measured with the Abbott Architect c8000 (Abbott Laboratories, Chicago, IL, USA) using the manufacturer's original reagents. The oxidized LDL receptor was determined using an enzyme-linked immunosorbent assay (ELISA) kit for lectin-like oxidized LDL receptor 1 (LOX1) (SEB859Hu 96) (CLOUD-CLONE CORP., Houston, TX, USA) according to the manufacturer's instructions. Briefly, the method is a double antibody sandwich ELISA on wells precoated with a LOX1-specific antibody. After incubation of the standards and samples with the pre-coated wells, the next step was to add avidin conjugated to horseradish peroxidase followed by biotin-conjugated antibodies to each well. When the 3,3′5,5′-Tetramethylbenzidine substrate is added, the color of the wells changes only in the presence of LOX1. Sulfuric acid stops the reaction. The concentration of LOX1 in the samples is proportional to the color intensity measured at 450 nm. The detection range of the method is 12.5–800 pg/mL. The ELISA was performed manually, with automated washing steps on the Hydroflex Microplate Washer, and absorbance was measured on Infinite F50 Readers (Tecan Ltd., Männedorf, Switzerland).

The data collected from the subjects were kept confidential. Moreover, the names of the subjects were encrypted during the processing of the sample and the analysis of the results and were known only to the principal investigator. At the end of the study, all personal data and biological materials were disposed of appropriately. Approval for this research was granted by the Ethics Committee of the University Hospital Center Sestre Milosrdnice (251-29-11-21-01-9) and the Ethics Committee of the Faculty of Medicine, University of Zagreb (380-59-10106-21-111/122).

2.7. Data Analysis

Statistical differences between chemical composition, antioxidant level, and starch digestibility were assessed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. Results of the FFQ are expressed as a median with 25th and 75th percentile using a box plot obtained in Statistica 10 (StatSoft, Tulsa, OK, USA). Biochemical data were analyzed using the Mann-Whitney test or Fisher's exact test and the Spearman correlation coefficient. The nonparametric paired test (Wilcoxon's signed-rank test) was used to compare differences before and after the intervention within the same group. Xlstat-Pro (win) 7.5.3 GraphPad Prism 8.4.3 was used for statistical analysis (Addinsoft, New York, NY, USA). A significance level of $p < 0.05$ was considered in all statistical analyses.

3. Results and Discussion

3.1. Proximate Composition of Cookies

The nutritional profile of CC, GAP, and GAP with KGAE is shown in Table 1. The cookies differed slightly in fat, saturated fat, protein, crude fiber, TS, glucose, and mineral content, suggesting that fruit by-products (grape and aronia pomace) can be successfully used as a partial substitute for cocoa powder.

In all types of cookies, SDS prevailed over RDS. Lower RDS content and higher SDS content directly correlate with a lower glycemic response [29,31]. The amount of SDS was 1.3- to 2-fold higher in our cookies compared with the results of Garsetti et al. [31], suggesting that their consumption might lead to longer satiety and that the cookies might have a medium to low glycemic index.

A significantly higher RDS (19%) was observed in GAP with KGAE than in CC. This could be due to the spraying of water-based edible film over cookies and the subsequent drying process. Starch in cookies is only partly gelatinized, but heating in the presence of water leads to a disruption of the inter- and intramolecular hydrogen bonds between starch chains, causing the chains to separate and become more available to digestive enzymes [32]. In contrast to our results, Diao et al. [33] found that the addition of 3% and 6% chitosan lowered the digestion rate of waxy maize starch by reducing amylase-driven hydrolysis reactions. Here, the chitosan was applied on the cookie surface in the form of an edible film. Similarly, Bae et al. [34] found that the addition of up to 2% of GA effectively reduces in vitro starch digestibility by lowering RDS content while increasing the RS content of Segoami rice noodles. However, since our results showed that cookies containing GAP with KGAE had the highest RDS, it may be that a thicker layer of edible film must be used to represent an appropriate choice for lowering the glycemic index of the final product. In addition to fiber, RS has a positive effect on the natural flora of the gastrointestinal tract [35] and was present in all three cookie varieties. In both of our modified cookies, GAP and GAP with KGAE, the RS content increased, although the difference was insignificant from CC.

Table 1. Proximate composition, starch digestibility in vitro, minerals, and antioxidant activity (FRAP, ABTS and DPPH) of different types of cookies.

Parameter	CC	GAP	GAP with KGAE
Total Fat (g/100 g)	18.65 ± 0.17 [a]	18.35 ± 0.16 [a]	19.16 ± 0.08 [b]
SFA (g/100 g)	8.2 ± 0.08 [a]	8.1 ± 0.08 [a]	8.4 ± 0.04 [b]
MUFA (g/100 g)	6.6 ± 0.06 [a]	6.5 ± 0.09 [a]	6.8 ± 0.03 [b]
PUFA (g/100 g)	3.4 ± 0.03 [a]	3.3 ± 0.05 [b]	3.4 ± 0.01 [a,b]
TS (% dry weight)	39.99 ± 0.54 [a]	42.69 ± 1.49 [a]	41.96 ± 0.94 [a]
RDS (% dry weight)	17.63 ± 14.41 [a]	18.63 ± 0.47 [a,b]	21.06 ± 1.45 [b]
SDS (% dry weight)	21.46 ± 2.03 [a]	21.40 ± 2.62 [a]	19.02 ± 3.40 [a]
RS (% dry weight)	0.88 ± 0.43 [a]	2.65 ± 1.45 [a]	1.88 ± 1.87 [a]
RAG (% dry weight)	29.49 ± 1.23 [a]	30.71 ± 0.47 [a]	32.70 ± 1.82 [a]
Free Glucose (% dry weight)	9.89 ± 0.33 [a]	10.01 ± 0.21 [a]	9.30 ± 0.32 [a]
Protein (g/100 g)	10.1 ± 0.01 [a]	8.9 ± 0.16 [a]	9.3 ± 0.01 [b]
Crude fiber (g/100 g)	2.29 ± 0.06 [a]	2.20 ± 0.01 [a]	2.15 ± 0.05 [a]
Minerals as ash (g/100 g dry weight)	2.04 ± 0.18 [a]	1.99 ± 0.11 [a]	1.99 ± 0.17 [a]
Iron (mg/kg)	33 ± 2 [b]	35 ± 1 [a]	33 ± 2 [b]
Calcium (mg/kg)	60 ± 3 [a]	59 ± 3 [a]	52 ± 2 [b]
Magnesium (mg/kg)	326 ± 12 [a]	292 ± 24 [b]	239 ± 19 [c]
Sodium (mg/kg)	4206 ± 25 [a]	3649 ± 31 [b]	3376 ± 29 [c]
Flavonoid (mmol rutin/100 g dry weight)	0.145 ± 0.02 [a]	0.139 ± 0.02 [a]	0.177 ± 0.02 [b]
FRAP (mmol Trolox/100 g dry weight)	3.022 ± 0.11 [c]	3.963 ± 0.03 [b]	4.316 ± 0.21 [a]
ABTS (mmol Trolox/100 g dry weight)	11.47 ± 0.58 [a]	13.88 ± 0.46 [b]	14.58 ± 0.90 [c]
DPPH (mmol Trolox/100 g dry weight)	0.074 ± 0.01 [a]	0.110 ± 0.01 [b]	0.128 ± 0.01 [c]

Different letters (a–c) in the same row indicate significant results ($p < 0.05$, Tukey's test). CC—cocoa cookies; GAP—cookies with grape and aronia pomace; GAP with KGAE—cookies with grape and aronia pomace and edible film; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; TS—total starch: RDS—readily digested starches; SDS—slowly digested starches; RS—resistant starches; RAG—rapidly available glucose, FRAP—ferric reducing antioxidant power; ABTS—2,2′-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid); DPPH—2,2-diphenyl-1-picrylhydrazyl.

The mineral composition differed between the cookies. The iron content was slightly higher in GAP than in the other two samples, probably due to the addition of red grape pomace. According to the literature, iron content in cocoa powder is about 25 mg/100 g [36]; in dry aronia pomace, it ranges from 7.5 to 8.6 mg/100 g [12]; whereas in red grape pomace, it ranges from 117 to 398 mg/100 g of dry weight, depending on the grape variety [37]. The concentrations of calcium and magnesium were higher in CC (13% and 27% respectively) than in GAP with KGAE, whereas GAP content was in between. This could be due to the fact that cocoa is naturally abundant in calcium and magnesium, which could reduce the risk of hypertension and atherosclerosis [36,38]. On the other hand, CC had also the highest sodium content, which is disadvantageous in the risk of CVD [2,38]. It is interesting to note that GAP with KGAE was lower in each mineral analyzed than the GAP sample. It could be due to the presence of GA in edible film, which has been reported to be effective in the removal of boron [39]. Therefore, future studies should investigate the effect of edible films on the bioaccessibility of essential minerals in foods.

The flavonoid content was the highest in GAP with KGAE, which was 21% higher compared with CC. This agrees with the total phenolic content measured in our previous study [20]. The CC showed the lowest antioxidant potential, which indicated the relevance of using fruit by-products in cookies. The FRAP antioxidant capacity of aronia pomace is 52.2 ± 0.2 mmol TE/100 g on a dry weight basis [40]. In grape pomace, it ranges from 110 to 530 µmol TE/g [41]. In comparison, cocoa powders have FRAP antioxidant activity from 110–454 µmol TE/g [42], of which 54% is lost in baking [43]. Foods with higher amounts of polyphenols (such as anthocyanins, flavonols, and phenolic acids) have a greater ability to reduce Fe and scavenge both DPPH and ABTS [42]. In this study, GAP with KGAE showed the highest antioxidant potential in every antioxidant assay, which depending on the test was 27–73% higher compared to CC, and even 5–9% higher than GAP. Similarly, Pawłowska et al. [44] found an increased antioxidant activity (36% in ABTS and 83% in DPPH) in muffins in which cocoa powder was completely replaced with carob powder (5% at flour basis). We showed an additional benefit of applying an edible film with GSE as a source of antioxidants since the FFS contained phenolics at a concentration of 0.71 g GAE/L [20]. Our results agree with the study of Cádiz-Gurrea et al. [45] who showed that GSE shows a stronger antioxidant capacity and a higher phenolic and flavan-3-ol content than cocoa.

Due to the highest antioxidant potential and flavonoid content, GAP and KGAE were further used for the intervention study in comparison with the commercial cookies of similar proximate composition.

3.2. Baseline Characteristics

Characteristics and concentrations of biochemical parameters at baseline for the control and test groups are shown in Table 2 and the results of the FFQ are shown in Figure 1.

The participants in the study had normal BMI. Most of the women, particularly in the test group, were physically active and consumed dietary supplements and alcohol ($p = 0.041$). This can be explained by the fact that many women probably exercise and take supplements to achieve the socially prescribed ideal body image [46]. Most of the biochemical parameters did not differ at baseline. The only statistically significant difference was observed in the higher concentration of HDL cholesterol in the test group ($p = 0.029$), which could be due to their higher consumption of fish and marine products compared to the control group (Figure 1). However, the concentrations for both groups were within the reference interval, so this difference was not considered clinically relevant.

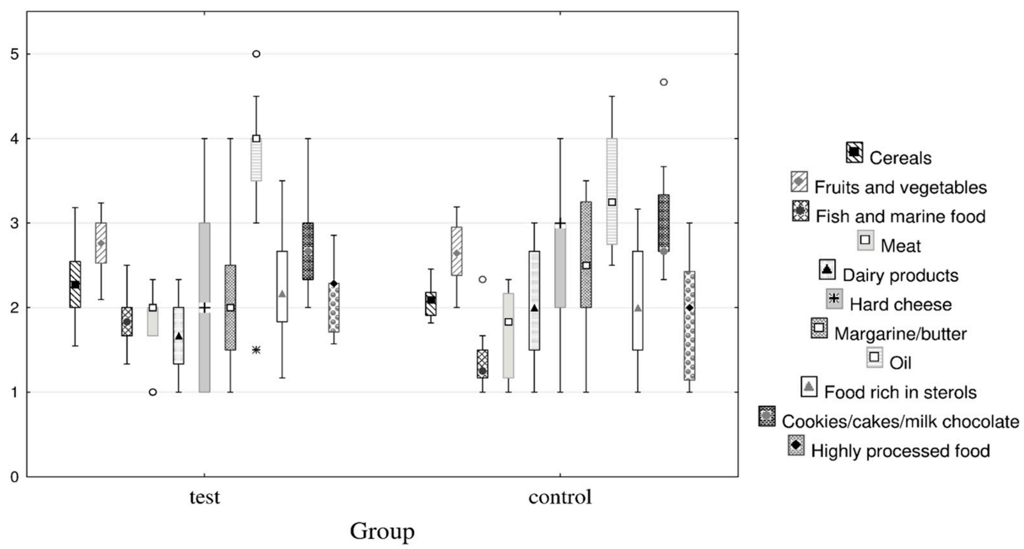

Figure 1. Box-Whisker plot of food frequency questionnaire by the control and test group (median with 25th and 75th percentile (Box value) and non-outlier range (Whisker value); markers ° denote outliers and * extreme).

Table 2. Baseline characteristics of the subjects at the beginning of the study (with *p* value between the control and test group).

Parameter	Total	Control Group	Test Group	*p*
Age (years) median (range) (span)	36 (23–60)	36 (23–60)	36 (25–46)	0.200 *
Chronic disease (N/total)	6/25	2/12	4/13	0.645 **
Medicaments (N/total)	7/25	2/12	5/13	0.378 **
Allergies (N/total)	1/25	1/12	0/13	0.480 **
Alcohol consumption (N/total)	13/25	4/12	9/13	0.115 **
Physical activity (N/total)	16/25	5/12	11/13	0.041 **
Food supplements (N/total)	10/25	2/12	8/13	0.041 **
BMI (kg/m^2) (span)	22.3 (19.6–24.5)	22.1 (19.6–24.9)	22.2 (19.1–24.5)	0.957 *
Waist circumference (cm)	76 (68–86)	77 (74–80)	72 (67–87)	0.869
Hip circumference (cm)	98 (90–105)	101 (87–105)	96 (91–107)	0.717
Waist-to-hip ratio	0.77 (0.74–0.81)	0.77 (0.75–0.80)	0.78 (0.71–0.83)	0.716
Blood glucose (mmol/L)	5.0 (4.8–5.3)	5.2 (5.0–5.3)	5.0 (4.7–5.2)	0.218 *
Cholesterol (mmol/L)	4.7 (4.2–5.2)	4.6 (4.0–5.3)	4.7 (4.4–5.1)	0.604 *
Cholesterol HDL (mmol/L)	1.6 (1.5–1.7)	1.5 (1.4–1.6)	1.7 (1.6–1.7)	0.029 *
Cholesterol LDL (mmol/L)	2.5 (2.3–3.1)	2.5 (2.1–3.2)	2.5 (2.3–2.9)	0.849 *
Triglycerides (mmol/L)	0.8 (0.7–1.1)	0.8 (0.7–1.3)	0.8 (0.7–1.0)	0.509 *
Iron (Fe) (µmol/L)	16 (11–20)	13 (10–17)	18 (11–24)	0.210 *
UIBC (µmol/L)	41 (32–52)	40 (34–50)	41 (32–53)	0.849 *
TIBC (µmol/L)	57 (53–63)	55 (51–61)	63 (53–65)	0.126 *
Fe saturation (%)	27 (19–38)	26 (18–32)	35 (19–39)	0.369 *

* Mann-Whitney test, ** Fisher exact test. BMI—body mass index; UIBC—unsaturated iron-binding capacity; TIBC—total iron binding capacity.

Food-item consumption frequencies are on a 1 to 5 Likert scale (5—every day; 4—three to five times a week; 3—once a week; 2—one to two times a month; 1—never).

The diet of participants contained items from each food group. The consumption of biscuits/cake/milk chocolate (including some extremes) was higher while the consumption of fish and marine products was lower in the control group than in the test group.

3.3. Oxidized LDL Receptor

The concentration of oxLDL receptors did not statistically differ between the test or control group at the beginning or the end of the study (mean 0.41 ng/mL and 0.39 ng/mL, respectively; $p = 0.462$) (Table 3).

There was no statistically significant predictor of change in concentration of the oxLDL receptors at day 10 in either group (Table 4), which was confirmed by the study results.

Similar to our results, Pokimica et al. [47] found 100 mL/day of aronia juice (chokeberry) at a high or low polyphenol dose during the 8-week intervention had no positive effect on the change in oxLDL levels. A similar randomized, double-blind trial of forty-four patients who had suffered myocardial infarction found that oxLDL levels in the intervention group decreased significantly by 29% after 6 weeks of oral intake of aronia flavonoid extract [48]. It should be noted that this study was performed on patients after myocardial infarction who received concomitant statin therapy. In addition, several studies reported the potential of hydroxytyrosol-enriched biscuits for lowering oxidized LDL levels in humans [49,50]. Nevertheless, it must be considered that the above studies observed the effect of chokeberry extract or grape pomace only on oxLDL and not on the oxLDL receptor. Moreover, the putative benefits of increased consumption of polyphenols have limited bioavailability and the mechanisms by which these compounds may modulate lipid metabolism should be further investigated. Here, we can conclude that whole grain cookies can be safely consumed in moderate amounts without the risk of increasing oxLDL in healthy women.

Table 3. Concentration of oxidized low-density lipoprotein (oxLDL) receptors in control and test group at the beginning of the study and after intervention.

Parameter	Total	Control Group	Test Group	*p* (Control vs. Test Group)
oxLDL receptor (0 h) (ng/mL)	0.29 (0.18–0.47)	0.29 (0.19–0.52)	0.29 (0.18–0.47)	0.765 *
oxLDL receptor (after 2 h) (ng/mL)	0.42 (0.25–0.48)	0.37 (0.24–0.45)	0.45 (0.30–0.60)	0.276 *
p (0 vs. 2 h)		0.753 **	0.917 **	
oxLDL receptor (after 10 days) (ng/mL)	0.42 (0.27–0.46)	0.43 (0.24–0.45)	0.37 (0.27–0.50)	0.744 *
p (0 vs. 10 days)		0.583 **	1.000 **	
p (2 h vs. 10 days)		0.480 **	0.600 **	

* Mann-Whitney test; ** Wilcoxonov test.

Table 4. Logistic regression analysis of the prediction of the fall in oxidized low-density lipoprotein (oxLDL) receptor concentration on the 10th day of study.

	Control Group			Test Group		
	OR	95% CI	*p*	OR	95% CI	*p*
Age	1.02	0.92–1.14	0.684	0.85	0.63–1.14	0.276
Chronic disease	2.33	0.11–50.99	0.590	0.42	0.03–5.71	0.512
Medications	-	-	0.997	0.25	0.02–3.34	0.295
Alcohol	2.27	0.55–9.35	0.255	0.94	0.27–3.28	0.922
Physical activity	1.67	0.15–18.88	0.680	0.57	0.03–11.85	0.718
Food supplementation	-	-	0.998	0.22	0.02–2.45	0.219
BMI (kg/m^2)	1.11	0.73–1.69	0.613	0.76	0.50–1.17	0.212
Waist circumferences (cm)	0.94	0.80–1.11	0.464	0.94	0.82–1.07	0.316
Hip circumferences(cm)	0.97	0.84–1.10	0.607	0.91	0.77–1.06	0.23
WHR	-	-	0.549	-	-	0.620
Glucose (mmol/L)	0.13	0.00–5.32	0.280	21.35	0.36–1252.45	0.141
Cholesterol (mmol/L)	2.16	0.59–7.94	0.246	2.44	0.21–28.26	0.476
Cholesterol HDL (mmol/L)	11.22	0.01–12,329.25	0.499	0.50	0.00–95.95	0.795
Cholesterol LDL (mmol/L)	2.27	0.50–10.33	0.287	3.17	0.26–38.37	0.365
Triglycerides (mmol/L)	5.32	0.12–229.97	0.384	0.88	0.03–22.34	0.940
Iron (μmol/L)	1.10	0.87–1.39	0.401	1.05	0.89–1.24	0.593
UIBC (μmol/L)	0.86	0.70–1.05	0.139	0.98	0.88–1.09	0.711
TIBC (μmol/L)	0.81	0.61–1.08	0.145	0.99	0.86–1.15	0.964
Fe saturation (%)	1.08	0.94–1.24	0.264	1.03	0.94–1.13	0.553

OR—odds ratio; 95% CI—95% confidence interval; BMI—body mass index; WHR—waist-to-hip ratio; UIBC—unsaturated iron-binding capacity; TIBC—total iron binding capacity.

3.4. Oxidized LDL Receptor and Waist Circumference

In our study, we observed an inverse correlation between waist circumference and oxLDL receptor concentration in the control group on the 10th day of the study ($r = -0.67$; $p = 0.034$) (Table 5), but not in the test group. A possible reason for this could be a wider range of waist circumference in the test group.

Sikora et al. [51] conducted a study to investigate the effects of two-month supplementation with aronia extract on angiotensin-converting enzyme activity in patients with metabolic syndrome. They showed a slight decrease in BMI (30.9 to 30.4 kg/m^2; $p < 0.001$) and a moderate decrease in waist circumference (95–93.7 cm; $p = 0.001$) in the obese population compared to the control group, which showed a sharp increase in both parameters. Several other studies investigating the effects of 300–500 mg of aronia extract per day on overweight/obese patients with metabolic syndrome found no association between oxLDL and waist circumference or change in waist circumference alone [52,53]. In our study, we did not observe a correlation between BMI and oxLDL receptors, which may be because our participants had normal BMI.

Table 5. Correlation of concentration of oxidized low-density lipoprotein (oxLDL) receptor with anthropometric parameters in control and test group.

Parameter	oxLDL Receptor (2 h)				oxLDL Receptor (10 d)			
	Control Group		Test Group		Control Group		Test Group	
	r *	*p*	r *	*p*	r *	*p*	r *	*p*
Age	−0.25	0.443	−0.43	0.141	0.05	0.880	0.29	0.336
Body mass index (kg/m^2)	−0.14	0.665	−0.11	0.734	−0.42	0.176	0.48	0.094
Waist circumference (cm)	−0.18	0.614	−0.13	0.680	−0.67	0.034	0.31	0.336
Hip circumference (cm)	−0.34	0.334	−0.25	0.429	−0.31	0.390	0.25	0.436
Waist-to-hip ratio	−0.12	0.748	0	0.983	−0.60	0.068	0.39	0.216

* Spearman's rank correlation coefficient. Values in bold indicate statistically significant results.

3.5. Oxidized LDL Receptor and Serum Iron

We observed quite contradictory results regarding the relationship between serum iron and oxLDL receptor levels (Table 6).

Table 6. Correlation of oxidized low-density lipoprotein (oxLDL) receptor concentration with biochemical parameters in the control and test groups.

Parameters	oxLDL Receptor (2 h)				oxLDL Receptor (10 Days)			
	Control Group		Test Group		Control Group		Test Group	
	r *	*p*	r *	*p*	r *	*p*	r *	*p*
Glucose (mmol/L)	−0.15	0.637	0.52	0.067	−0.33	0.294	−0.23	0.443
Cholesterol (mmol/L)	0.33	0.290	−0.22	0.472	0.13	0.678	−0.03	0.921
Cholesterol HDL (mmol/L)	0.40	0.197	0.08	0.784	0.30	0.352	0.22	0.473
Cholesterol LDL (mmol/L)	0.33	0.296	−0.17	0.579	0.19	0.554	0.04	0.886
Triglycerides (mmol/L)	−0.25	0.429	0.15	0.618	0.21	0.518	−0.01	0.964
Iron (Fe) (µmol/L)	0.25	0.434	0.08	0.788	0.69	0.012	−0.62	0.022
UIBC (µmol/L)	−0.23	0.476	−0.19	0.530	−0.47	0.121	0.23	0.452
TIBC (µmol/L)	−0.31	0.331	−0.39	0.193	−0.02	0.948	−0.28	0.356
Fe saturation (%)	0.29	0.358	0.04	0.897	0.61	0.037	−0.47	0.105

* Spearman's rank correlation coefficient. Values in bold indicate statistically significant results. HDL—high-density lipoprotein; LDL—low-density lipoprotein, UIBC—unsaturated iron-binding capacity; TIBC—total iron binding capacity.

The oxLDL receptor had a significant direct relationship with serum iron levels ($r = 0.69$; $p = 0.012$) and iron saturation ($r = 0.61$; $p = 0.037$) in the control group and a significant inverse relationship with serum iron levels ($r = -0.62$; $p = 0.022$) and iron saturation ($r = -0.47$; $p = 0.105$) in the tested group at day 10. This could be due to the fact that the control group had a lower serum iron concentration at baseline. Whether this was a coincidence remains to be determined, but it is known that aronia pomace has an indirect effect on increasing blood iron levels [54]. Jakovljevic et al. [53] demonstrated that oral supplementation with aronia extract (0.45 mL/kg/day) for four weeks in combination with different diets (high fat vs. standard) resulted in a significant increase in serum iron levels in rats regardless of diet. In contrast to our results, Brouwers et al. [55] found in their study that serum ferritin concentration and haptoglobin phenotype (Hp) were independently associated with circulating oxLDL levels in males. On the other hand, D'Amelio et al. [56] showed in their study that the association depends on iron-related genes, especially haptoglobin phenotypes. The Hp of the individual has antioxidant properties and determines the availability of iron to the cells. It could be that the Hp was not uniform in our subjects and thus contributed to the different results. A recent study that examined the relationship between iron stress and oxidative damage in patients with metabolic syndrome found a strong association between serum ferritin and oxLDL levels. In fact, people with elevated ferritin and oxLDL levels had a higher risk of metabolic syndrome [57]. In addition, there is evidence that some polyphenols may affect the bioavailability of iron, which in turn may increase the risk of developing cardiovascular disease, especially in higher-risk populations [58].

Our study has some possible limitations. The size of the groups studied was small, and they were only composed of healthy women. Furthermore, we were not able to monitor the dietary intake of our participants during the intervention, although participants were instructed not to change their dietary habits and lifestyle significantly. In addition, a longer intervention period is required.

4. Conclusions

In this study, we investigated the nutritional profile, starch digestibility, and antioxidant potential of whole grain cookies with fruit by-products and edible film enriched with grape seed extract as well as the concentration of oxidized LDL receptors after their consumption by women. We found that the partial replacement of cocoa powder with grape and aronia pomace and the use of an edible film did not negatively affect the nutritional profile or starch digestibility of the cookies but contributed to their flavonoid content and antioxidant potential. Our results showed that moderate cookie consumption does not increase the concentrations of oxidized LDL receptors in healthy women. Nevertheless, our study provides information about a possible relationship between oxidized LDL receptor concentration and waist circumference. In addition, our results provide evidence for the potential preventive effect of whole grain cookies on cardiovascular disease prevention. Further studies with more participants and a longer intervention period need to be conducted to confirm the efficacy of this potential prevention. The improved antioxidant properties of the cookies suggest that fruit by-products and edible films should be further investigated for potential applications in the development of functional foods.

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References

1. DiNicolantonio, J.J.; Lucan, S.C.; O'Keefe, J.H. The evidence for saturated fat and for sugar related to coronary heart disease. *Prog. Cardiovasc. Dis.* **2016**, *58*, 464–472. [CrossRef] [PubMed]
2. Benjamin, E.J.; Muntner, P.; Alonso, A.; Bittencourt, M.S.; Callaway, C.W.; Carson, A.P.; Chamberlain, A.M.; Chang, A.R.; Cheng, S.; Das, S.R.; et al. Heart disease and stroke statistics-2019 update: A report from the American Heart Association. *Circ. J.* **2019**, *139*, e56–e528. [CrossRef]
3. Leopold, J.A.; Loscalzo, J. Oxidative mechanisms and atherothrombotic cardiovascular disease. *Drug Discov.* **2008**, *5*, 5–13. [CrossRef] [PubMed]
4. Obermayer, G.; Afonyushkin, T.; Binder, C.J. Oxidized low-density lipoprotein in inflammation-driven thrombosis. *J. Thromb.* **2018**, *16*, 418–428. [CrossRef]
5. Poznyak, V.A.; Nikiforov, G.N.; Markin, M.A.; Kashirskikh, A.D.; Myasoedova, A.V.; Gerasimova, V.E.; Orekhov, N.V. Overview of oxLDL and its impact on cardiovascular health: Focus on atherosclerosis. *Front. Pharmacol.* **2021**, *11*, 613780. [CrossRef]
6. Scalbert, A.; Johnson, I.T.; Saltmarsh, M. Polyphenols: Antioxidants and beyond. *Am. J. Clin. Nutr.* **2005**, *81*, 215S–217S. [CrossRef] [PubMed]
7. Marrugat, J.; Covas, M.I.; Fitó, M.; Schröder, H.; Miró-Casas, E.; Gimeno, E.; López-Sabater, M.C.; de la Torre, R.; Farré, M. Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation. *Eur. J. Nutr.* **2004**, *43*, 140–147. [CrossRef]
8. Li, S.H.; Zhao, P.; Tian, H.B.; Chen, L.H.; Cui, L.Q. Effect of grape polyphenols on blood pressure: A meta-analysis of randomized controlled trials. *PLoS ONE* **2015**, *10*, e0137665. [CrossRef]
9. Jumar, A.; Schmieder, R.E. Cocoa flavanol cardiovascular effects beyond blood pressure reduction. *J. Clin. Hypertens.* **2016**, *18*, 352–358. [CrossRef]
10. Cosmi, F.; Di Giulio, P.; Masson, S.; Finzi, A.; Marfisi, R.M.; Cosmi, D.; Scarano, M.; Tognoni, G.; Maggioni, A.P.; Porcu, M.; et al. Regular wine consumption in chronic heart failure impact on outcomes, quality of life, and circulating biomarkers. *Circ. Heart Fail.* **2015**, *8*, 428–437. [CrossRef]
11. Jakobek, L.; Šeruga, M.; Medvidović-Kosanović, M.; Novak, I. Antioxidant activity and polyphenols of Aronia in comparison to other berry species. *Agric. Conspec. Sci.* **2007**, *72*, 301–306.
12. Jurendić, T.; Ščetar, M. *Aronia melanocarpa* products and by-products for health and nutrition: A review. *Antioxidants* **2021**, *10*, 1052. [CrossRef]
13. Jurikova, T.; Mlcek, J.; Skrovankova, S.; Sumczynski, D.; Sochor, J.; Hlavacova, I.; Snopek, L.; Orsavová, J. Fruits of black chokeberry *aronia melanocarpa* in the prevention of chronic diseases. *Molecules* **2017**, *22*, 944. [CrossRef] [PubMed]
14. Food and Agriculture Organization of the United Nations (FAO). 2018. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 14 October 2022).
15. Acan, B.G.; Kilicli, M.; Bursa, K.; Toker, O.S.; Palabiyik, I.; Gulcu, M.; Yaman, M.; Gunes, R.; Konar, N. Effect of grape pomace usage in chocolate spread formulation on textural, rheological and digestibility properties. *LWT* **2021**, *138*, 110451. [CrossRef]
16. Vilela, A.; Cruz, I.; Oliveira, I.; Pinto, A.; Pinto, T. Sensory and nutraceutical properties of infusions prepared with grape pomace and edible-coated dried–minced grapes. *Coatings* **2022**, *12*, 443. [CrossRef]
17. Lončarević, I.; Petrović, J.; Teslić, N.; Nikolić, I.; Maravić, N.; Pajin, B.; Pavlić, B. Cocoa spread with grape seed oil and encapsulated grape seed extract: Impact on physical properties, sensory characteristics and polyphenol content. *Foods* **2022**, *11*, 2730. [CrossRef]
18. Molnar, D.; Novotni, D.; Krisch, J.; Bosiljkov, T.; Ščetar, M. The optimisation of biscuit formulation with grape and aronia pomace powders as cocoa substitutes. *Croat. J. Food Technol. Biotechnol. Nutr.* **2020**, *15*, 38–44. [CrossRef]
19. Kumar, N. Polysaccharide-based component and their relevance in edible film/coating: A review. *Nutr. Food Sci.* **2019**, *49*, 793–823. [CrossRef]
20. Molnar, D.; Novotni, D.; Kurek, M.; Galić, K.; Iveković, D.; Bionda, H.; Ščetar, M. Characteristics of edible films enriched with fruit by-products and their application on cookies. *Food Hydrocoll.* **2023**, *135*, 108191. [CrossRef]
21. Mouzakitis, C.K.; Sereti, V.; Matsakidou, A.; Kotsiou, K.; Biliaderis, C.G.; Lazaridou, A. Physicochemical properties of zein-based edible films and coatings for extending wheat bread shelf life. *Food Hydrocoll.* **2022**, *132*, 107856. [CrossRef]
22. American Association of Cereal Chemists (AACC). *Approved Methods of the American Association of Cereal Chemists International*, 11th ed.; American Association of Cereal Chemists: St. Paul, MN, USA, 2000. Available online: https://www.cerealsgrains.org/resources/Methods/Pages/default.aspx (accessed on 26 September 2022).
23. *ISO 1871:2009*; Food and Feed Products—General Guidelines for the Determination of Nitrogen by the Kjeldahl Method. International Organization for Standardization: Geneva, Switzerland, 2009. Available online: https://www.iso.org/standard/41320.html (accessed on 15 September 2022).

24. *ISO 6492:1999*; Animal Feeding Stuffs—Determination of Fat Content. International Organization for Standardization: Geneva, Switzerland, 1999. Available online: https://www.iso.org/standard/12865.html (accessed on 15 September 2022).
25. *ISO 12966-4:2015*; Animal and Vegetable Fats and Oils—Gas Chromatography of Fatty Acid Methyl Esters—Part 4: Determination by Capillary Gas Chromatography Method. International Organization for Standardization: Geneva, Switzerland, 2015. Available online: https://www.iso.org/standard/63503.html (accessed on 15 September 2022).
26. *EN 14084:2003*; Foodstuffs. Determination of Trace Elements. Determination of Lead, Cadmium, Zinc, Copper and Iron by Atomic Absorption Spectrometry (AAS) after Microwave Digestion. European Standard: Plzen, Czech Republic, 2003. Available online: https://standards.iteh.ai/catalog/standards/cen/5a1ec234-434f-42a0-8447-b5c00aee9bae/en-14082-2003 (accessed on 4 October 2022).
27. *EN 15763:2010*; Foodstuffs. Determination of Trace Elements. Determination of Arsenic, Cadmium, Mercury and Lead in Foodstuffs by Inductively Coupled Plasma Mass Spectrometry (ICP–MS) after Pressure Digestion. European Standard: Plzen, Czech Republic, 2010. Available online: https://standards.iteh.ai/catalog/standards/sist/44d9ee33-afaf-49df-b541-f6b4b28e97e6/sist-en-15763-2010 (accessed on 4 October 2022).
28. Englyst, H.N.; Kingman, S.M.; Cummings, J.H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* **1992**, *46*, S33–S50. [PubMed]
29. Vujić, L.; Čepo, D.V.; Dragojević, I.V. Impact of dietetic tea biscuits formulation on starch digestibility and selected nutritional and sensory characteristics. *LWT–Food Sci. Technol.* **2014**, *62*, 647–653. [CrossRef]
30. Babić, D.; Sindik, J.; Missoni, S. Development and validation of a self-administered food frequency questionnaire to assess habitual dietary intake and quality of diet in healthy adults in the Republic of Croatia. *Coll. Antropol.* **2014**, *38*, 1017–1026. Available online: https://hrcak.srce.hr/128218 (accessed on 4 April 2021).
31. Garsetti, M.; Vinoy, S.; Lang, V.; Holt, S.; Loyer, S.; Brand-Miller, J.C. The glycemic and insulinemic index of plain sweet biscuits: Relationships to in vitro starch digestibility. *J. Am. Coll. Nutr.* **2005**, *24*, 441–447. [CrossRef] [PubMed]
32. Chung, H.J.; Lim, H.S.; Lim, S.T. Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch. *J. Cereal Sci.* **2006**, *43*, 353–359. [CrossRef]
33. Diao, Y.; Sia, X.; Shanga, W.; Zhoua, Z.; Wanga, Z.; Zhenga, P.; Strappeb, P.; Blanchard, C. Effect of interactions between starch and chitosan on waxy maize starch physicochemical and digestion properties. *CYTA J. Food.* **2017**, *15*, 327–335. [CrossRef]
34. Bae, I.Y.; Oh, I.K.; Jung, D.S.; Lee, H.G. Influence of arabic gum on in vitro starch digestibility and noodle-making quality of segoami. *Int. J. Biol. Macromol.* **2019**, *125*, 668–673. [CrossRef]
35. Liu, X.; Martin, D.A.; Valdez, J.C.; Sudakaran, S.; Rey, F.; Bolling, B.W. Aronia berry polyphenols have matrix-dependent effects on the gut microbiota. *Food Chem.* **2021**, *359*, 129831. [CrossRef]
36. Cinquanta, L.; Di Cesare, C.; Manoni, R.; Piano, A.; Roberti, P.; Salvatori, G. Mineral essential elements for nutrition in different chocolate products. *Int. J. Food Sci. Nutr.* **2016**, *67*, 773–778. [CrossRef]
37. Antonić, B.; Jančíková, S.; Dordević, D.; Tremlová, B. Grape pomace valorization: A systematic review and meta-analysis. *Foods* **2020**, *9*, 1627. [CrossRef]
38. Steinberg, F.M.; Bearden, M.M.; Keen, C.L. Cocoa and chocolate flavonoids: Implications for cardiovascular health. *J. Am. Diet. Assoc.* **2003**, *103*, 215–223. [CrossRef] [PubMed]
39. Duran, H.; Yavuz, E.; Sismanoglu, T.; Senkal, B.F. Functionalization of gum arabic including glycoprotein and polysaccharides for the removal of boron. *Carbohydr. Polym.* **2019**, *226*, 115139. [CrossRef]
40. Oszmiański, J.; Lachowicz, S. Effect of the production of dried fruits and juice from chokeberry (*Aronia melanocarpa* L.) on the content and antioxidative activity of bioactive compounds. *Molecules* **2016**, *21*, 1098. [CrossRef] [PubMed]
41. Ayuda-Durán, B.; González-Manzano, S.; Gil-Sánchez, I.; Moreno-Arribas, M.V.; Bartolomé, B.; Sanz-Buenhombre, M.; Guadarrama, A.; Santos-Buelga, C.; González-Paramás, A.M. Antioxidant characterization and biological effects of grape pomace extracts supplementation in *Caenorhabditis elegans*. *Foods* **2019**, *8*, 75. [CrossRef]
42. Todorovic, V.; Milenkovic, M.; Vidovic, B.; Todorovic, Z.; Sobajic, S. Correlation between antimicrobial, antioxidant activity, and polyphenols of alkalized/nonalkalized cocoa powders. *J. Food Sci.* **2017**, *82*, 1020–1027. [CrossRef] [PubMed]
43. Stahl, L.; Miller, K.B.; Apgar, J.; Sweigart, D.S.; Stuart, D.A.; McHale, N.; Ou, B.; Kondo, M.; Hurst, W.J. Preservation of cocoa antioxidant activity, total polyphenols, flavan-3-ols, and procyanidin content in foods prepared with cocoa powder. *J. Food Sci.* **2009**, *74*, C456–C461. [CrossRef] [PubMed]
44. Pawłowska, K.; Kuligowski, M.; Jasińska-Kuligowska, I.; Kidoń, M.; Siger, A.; Rudzińska, M.; Nowak, J. Effect of replacing cocoa powder by carob powder in the muffins on sensory and physicochemical properties. *Plant Foods Hum. Nutr.* **2018**, *73*, 196–202. [CrossRef] [PubMed]
45. Cádiz-Gurrea, M.L.; Borrás-Linares, I.; Lozano-Sánchez, J.; Joven, J.; Fernández-Arroyo, S.; Segura-Carretero, A. Cocoa and grape seed byproducts as a source of antioxidant and anti-inflammatory proanthocyanidins. *Int. J. Mol. Sci.* **2017**, *18*, 376. [CrossRef]
46. Klein, K.M. Why Don't I Look Like Her? The Impact of Social Media on Female Body Image. Senior Thesis, Claremont Colleges, Claremont, CA, USA, 2013. Available online: http://scholarship.claremont.edu/cmc_theses/720 (accessed on 10 November 2022).
47. Pokimica, B.; García-Conesa, M.T.; Zec, M.; Debeljak-Martačić, J.; Ranković, S.; Vidović, N.; Petrović-Oggiano, G.; Konić-Ristić, A.; Glibetić, M. Chokeberry juice containing polyphenols does not affect cholesterol or blood pressure but modifies the composition of plasma phospholipids fatty acids in individuals at cardiovascular risk. *Nutrients* **2019**, *11*, 850. [CrossRef]

48. Naruszewicz, M.; Łaniewska, I.; Millo, B.; Dłużniewski, M. Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infraction (MI). *Atherosclerosis* **2007**, *194*, e179–e184. [CrossRef]
49. Mateos, R.; Martínez-López, S.; Arévalo, G.B.; Amigo-Benavent, M.; Sarriá, B.; Bravo-Clemente, L. Hydroxytyrosol in functional hydroxytyrosol-enriched biscuits is highly bioavailable and decreases oxidised low density lipoprotein levels in humans. *Food Chem.* **2016**, *205*, 248–256. [CrossRef]
50. Conterno, L.; Martinelli, F.; Tamburini, M.; Fava, F.; Mancini, A.; Sordo, M.; Conterno, L.; Martinelli, F.; Tamburini, M.; Fava, F.; et al. Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. *Eur. J. Nutr.* **2019**, *58*, 63–81. [CrossRef] [PubMed]
51. Sikora, J.; Broncel, M.; Mikiciuk-Olasik, E. Aronia melanocarpa Elliot reduces the activity of angiotensin I-converting enzyme—In vitro and ex vivo studies. *Oxid. Med. Cell. Longev.* **2014**, *2014*, 739721. [CrossRef] [PubMed]
52. Xie, L.; Vance, T.; Kim, B.; Lee, S.G.; Caceres, C.; Wang, Y.; Hubert, P.A.; Li, J.-Y.; Chun, O.K.; Bolling, B.W. Aronia berry polyphenol consumption reduces plasma total and low-density lipoprotein cholesterol in former smokers without lowering biomarkers of inflammation and oxidative stress: A randomized controlled trial. *Nutr. Res.* **2017**, *37*, 67–77. [CrossRef] [PubMed]
53. Jakovljevic, V.; Milic, P.; Bradic, J.; Jeremic, J.; Zivkovic, V.; Srejovic, I.; Nikolic Turnic, T.; Milosavljevic, I.; Jeremic, N.; Bolevich, S.; et al. Standardized Aronia melanocarpa extract as novel supplement against metabolic syndrome: A rat model. *Int. J. Mol. Sci.* **2018**, *20*, 6. [CrossRef] [PubMed]
54. Broncel, M.; Kozirog, M.; Duchnowicz, P.; Koter-Michalak, M.; Sikora, J.; Chojnowska-Jezierska, J. Aronia melanocarpa extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome. *Med. Sci. Monit.* **2010**, *16*, CR28–CR34. [PubMed]
55. Brouwers, A.; Langlois, M.; Delanghe, J.; Billiet, J.; De Buyzere, M.; Vercaemst, R.; Rietzschel, E.; Bernard, D.; Blaton, V. Oxidized low-density lipoprotein, iron stores, and haptoglobin polymorphism. *Atherosclerosis* **2004**, *176*, 189–195. [CrossRef]
56. D'Amelio, P.; Cristofaro, M.A.; Tamone, C.; Morra, E.; Di Bella, S.; Isaia, G.; Grimaldi, A.; Gennero, L.; Gariboldi, A.; Ponzetto, A.; et al. Role of iron metabolism and oxidative damage in postmenopausal bone loss. *Bone* **2008**, *43*, 1010–1015. [CrossRef]
57. Leiva, E.; Mujica, V.; Sepúlveda, P.; Guzmán, L.; Núñez, S.; Orrego, R.; Palomo, I.; Andrews, M.; Arredondo, M.A. High levels of iron status and oxidative stress in patients with metabolic syndrome. *Biol. Trace Elem. Res.* **2013**, *151*, 1–8. [CrossRef]
58. Lesjak, M.; Hoque, R.; Balesaria, S.; Skinner, V.; Debnam, E.S.; Srai, S.K.S.; Sharp, P.A. Quercetin inhibits intestinal iron absorption and ferroprotein transporter expression in vivo and in vitro. *PLoS ONE* **2014**, *9*, e102900. [CrossRef]

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Article

Consumer Nutritional Awareness, Sustainability Knowledge, and Purchase Intention of Environmentally Friendly Cookies in Croatia, France, and North Macedonia

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Abstract: The increasing demand for greater utilization of byproducts in the food industry has been driven by growing interest in environmental sustainability. This paper examines the market potential and consumer attitudes toward whole-grain and sustainable cookies made with food byproducts and edible films. Additionally, particular attention was given to evaluating levels of sustainability knowledge and nutritional awareness, willingness to purchase environmentally friendly cookies with food byproducts, and to appraise differences in answers between countries and generations. An online questionnaire was used to collect data from Croatian (n = 472), French (n = 166), and North Macedonian consumers (n = 119) aged between 18 and 62, predominantly women (82%) with higher education degrees. Results showed that even if chocolate-coated cookies remain very popular, North Macedonians prefer whole-grain and plain cookies, while the French prefer chocolate-coated cookies and Croats prefer both types of cookie. The majority of consumers (96%) were interested in purchasing environmentally friendly cookies. However, consumers' interest in purchasing cookies with food byproducts was generally low, which may be related to their limited knowledge of byproducts. In conclusion, there is market potential for whole-grain cookies with food byproducts, but brand, price, and consumer education may be critical to their success.

Keywords: food byproducts; generational differences; nutritional awareness; sustainability knowledge

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1. Introduction

Consumers are showing an increasing inclination toward food that offer health benefits and are increasingly interested in the health benefits of food rich in nutrients and bioactive compounds such as antioxidants, polyphenols, fiber, minerals, and vitamins [1,2]. As a result, efforts have been made to develop food that can maintain and improve people's health [3,4]. Food based on whole grains, which are rich in nutrients, fibers, and phytochemicals, with proven health benefits, play significant role in maintaining human health and wellbeing [5]. Although the amount of whole-grain food on the market is constantly increasing [6], its consumption remains low.

Cookies are a very popular cereal-based food consumed by both children and adults worldwide [7], so they could be used as a suitable vehicle for nutrient incorporation [8]. The highest number of newly developed products can be found in the sweet cookie category, where claims such as high in/source of fiber and whole grain are among the top five used claims [9]. Cookie consumption does not have to be contrary to the healthy indulgence trend and can even be used as part of nutritional strategies to tackle several nutrient deficiencies as well as chronic and nutrition-related diseases [10,11], such as diabetes, celiac disease, obesity, and cardiovascular diseases [12–15]. On top of this, the sustainability in the supply chain of cocoa as a popular ingredient in cookies has been challenged.

Consumer interest in the environmental sustainability of food production has also increased in recent years [16]. One of the main problems facing the global food system is food waste, as more than one-third of the food produced for human consumption is either lost or wasted each year [17–20]. In this regard, byproducts from the fruit industry can be an optimal source of bioactive compounds and can serve as value-adding ingredients for cookies [21]. For instance, grape and aronia (chokeberry) pomace could serve as a partial substitute for cocoa powder in cookies [22,23]. Nevertheless, the market for cookies with byproducts as upcycling ingredients is still limited.

Packaging plays an important role in the marketing and shelf life of cookies. The use of edible and biodegradable films could reduce the amount of disposable packaging used and therefore reduce environmental pollution and CO_2 emissions. These materials should be employed in multilayer packaging, improve organoleptic properties, supplement nutritional value, serve as carriers for antioxidants and microbiological agents, and control the transfer of moisture, gasses, and other compounds in the food system [24]. Edible films based on chitosan and gum arabic enriched with grape seed extract can improve the bioactive profile [12] and extend the shelf life of cookies [24] without affecting product quality or consumer taste.

Based on the above, it could be suggested that the fortification of popular cookies with fruit byproducts and edible films could result in a product that easily delivers important nutrients such as antioxidants, minerals, and fiber to a wider population [12,24]. However, understanding how and why consumers purchase functional or sustainable (upcycled) foods is important for the sustainable development of this sector [20,25]. Previous studies investigated and explained consumer behavior related to purchase intention, acceptance, and consumption of cookies [19,26,27], which depends on the sensory profile of cookies and consumer habits, demographic background, personal relevance of functional benefits [28,29], marketing strategies, and on-pack labeling [30]. Various factors such as availability, quality, brand, novelty, packaging, and pricing have been identified to affect the behavior of cookies consumers. [15,31]. However, only a limited number of studies have investigated the market potential of functional and sustainable cookies.

The aim of this study was to investigate the market potential and consumers attitudes towards whole-grain and sustainable cookies in three Mediterranean countries (Croatia, France, and North Macedonia). Special attention was paid to differences between countries and generations in the preference of cookie type, consumption motivation, nutritional awareness, sustainability knowledge, and willingness to purchase cookies containing food byproducts. Relations between participants responses and their country of residence and generation group were evaluated through analysis of variance, principal component, and cluster analysis. Further on, the importance of sociodemographic factors (education, country of residence, generation, and gender) on willingness to purchase cookies, which are beneficial to health but also the environment or contain food byproducts, was assessed using classification and a regression tree. Our hypothesis was that a purchase intention is positively influenced by consumer education degree and differs between generations and countries.

2. Materials and Methods

2.1. Study Design and Study Population

This cross-sectional study was conducted using an anonymous online questionnaire created on the Google Forms online survey platform. The questionnaire was created in Croatian and then translated into French and Macedonian. Data collection was conducted in two intervals from April 2022 to June 2023. The research team distributed invitations to participate in this study to their professional networks and personal contacts via e-mail and social networks (Facebook® and LinkedIn®). To increase the number of study participants, research team members asked potential participants to forward the invitations to their contacts. Eligible participants were adults above 18 years of age, residents of Croatia, France, or North Macedonia with access to a computer, tablet, or phone with an internet connection. A total of 797 participants (Croatia (n = 500), France (n = 172), North Macedonia (n = 125)) completed the online questionnaire. Participants who responded that they never consume cereal-based foods (n = 40) were excluded from further analysis. Based on the study by Šedík et al. [32], participants were divided into four generations as follows: Generation Z (born between 2004 and 1997), Generation Y (born between 1996 and 1981), Generation X (born between 1980 and 1972), and Generation Silver (born between 1971 and 1952). Sociodemographic characteristics of participants who consumed cereals are shown in Table 1. Generation Y was the most strongly represented. Most study participants were female, employed and had a higher level of education (bachelor's or master's degree).

Table 1. Participants' characteristics for the total sample and according to gender (numbers of participants with percentages in brackets).

Sociodemographic Characteristic				
	Overall (n = 757)	Croatia (n = 472)	France (n = 166)	North Macedonia (n = 119)
Gender [1], n (%)				
Female	621 (82)	389 (82)	133 (80)	99 (83)
Male	135 (18)	83 (18)	32 (19.3)	20 (17)
Age cohorts, n (%)				
Generation Z	191 (25)	58 (12)	116 (70)	17 (14)
Generation Y	337 (45)	278 (59)	17 (10)	42 (35)
Generation X	153 (20)	88 (19)	20 (12)	45 (38)
Generation Silver	76 (10)	48 (10)	13 (8)	15 (13)
Highest education qualification, n (%)				
Primary school	4 (0.5)	3 (0.6)	1 (0.6)	/
Secondary school	87 (11.5)	74 (16)	6 (3.6)	7 (6)
Bachelor's or master's	497 (66)	276 (58.4)	109 (65.8)	112 (94)
PhD	169 (22)	119 (25)	50 (30)	/
Employment status, n (%)				
Employed, full- or parttime	503 (66.4)	381 (81)	20 (12)	102 (86)
Student	154 (20.3)	30 (6.4)	114 (69)	10 (8)
Entrepreneur	30 (4)	30 (6.4)	/	/
Retired	10 (1.3)	5 (1.1)	1 (0.6)	2 (2)
Other	60 (8)	24 (5.1)	31 (18.4)	5 (4)

[1] Participant (0.1%) did not want to report their gender.

All participants were voluntarily enrolled in the study and were assured of strict confidentiality. In the participant information sheet at the beginning of the online questionnaire, potential participants were provided with detailed information about the authors of the study, affiliations, the main research findings, the indication that the results will be used in a doctoral dissertation, and the estimated time required to complete the questionnaire (10–12 min). All participants had the right to withdraw from the study at any time without consequence. There were no adverse health effects that could be caused by participation in the present study. Participants did not receive any financial or other compensation for their participation in the study.

All participants signed an informed consent form to participate in this study. This study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Food Technology and Biotechnology (University of Zagreb Faculty, Croatia, 251-69-11-20-37; 14 December 2020), the Faculty Committee of the Faculty of Technology and Metallurgy (Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia, 09-1273/1; 10 November 2020), and the ONIRIS institution (Oniris VetAgroBio—Ecole Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique—French Republic Agricultural Ministry National HighSchool, France, 23-657; 12 February 2023).

The research data were collected using a structured online questionnaire adapted from Čukelj et al. (2016) [33], which consisted of a total of 43 multiple-choice questions divided into several sections: sociodemographics (5 questions), cookie consumption (9 questions), purchase intention (8 questions), brand (4 questions), nutrition and health (9 questions), and sustainability (3 questions).

Sociodemographic information included questions regarding age, gender, education, employment status, and country of residence. In the cereal product consumption section, data regarding the type of products consumed, frequency of consumption, and consumption habits were collected. The cookie consumption section included questions regarding preferred product type, consumption habits (reasons and key drivers for consumption), as well as the frequency of consumption. Data collected in the nutrition and health category focused on reading nutrition labels, and intake of dietary fiber and bioactive compounds. The section on sustainability focused on general knowledge and familiarity with sustainability/edible films and fruit byproducts. Within the purchase intention section, the brand preference for purchasing domestic or foreign producers, the willingness to pay a higher price for modified cookies or buy cookies of improved nutritional value/cookies with food industry byproducts/cookies with positive environmental impact was investigated.

Consumption of cereal products and cookies, as well as food rich in fiber and biologically active compounds, was assessed using a 5-point Likert scale ranging from 1 (never) to 5 (always). Consumer purchase behavior and nutrition awareness were measured using a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Finally, participants were asked to indicate their willingness to purchase nutritionally enhanced and sustainable products (cookies with byproducts of the food industry) on a 2-point scale from 1 (no) to 2 (yes). Zero was included in these questions as a "do not know" or "would not like to answer" option.

2.2. Nutritional Awareness Index and Sustainability Knowledge Score

The index of nutritional awareness (INA) indicates greater understanding or improved application of recent and accepted nutritional principles [33]. It was formed from five questions or statements with loadings of 0.6 or more, which explained 45% of the total variance after factor analysis (Table 2). Questions ("How often do you consume food rich in dietary fiber?"; "Do you introduce biologically active compounds into your body daily?"; "Are you reading food labels (nutritional data and ingredient list)?") and statements ("I watch what I eat to maintain my health" and "I watch what I eat to maintain a good appearance and prevent weight gain") were answered on 5-point Likert scales (ranging from "always" (5) to "never" (1) for questions or from "I completely agree" (5) to "I do not agree at all" (1) for statements). After adding up the individual grades, a total sum was divided with maximum possible score (25) and an INA between 0 and 1 was formed for each participant. Such an approach has already been described in the literature for similar purposes [33].

In similar manner, the sustainability knowledge score (SKS) was calculated from one question on sustainable development and two statements on specific knowledge of fruit byproducts and edible films, which accounted for 52% of the total variance after factor analysis (Table 2). The question "Are you familiar with the term Sustainable Development?" was answered with "yes" (score 2) or "no" (score 1) while statements regarding edible films and fruit byproducts were answered using the 5-point Likert scales ranging from "I completely agree" (5) to "I do not agree at all" (1). After adding up the individual grades, a total sum was divided with a maximum score (12) that could be achieved, forming an SKS between 0 and 1 for each participant.

Table 2. Contribution of questions to the index of nutrition awareness and sustainability knowledge score.

Index of Nutrition Awareness	**Component**
Do you introduce biologically active compounds into your body daily?	0.608
How often do you consume food rich in dietary fiber?	0.713
To what extent do you agree with the following statement: "I watch what I eat to maintain my health"?	0.765
How much do you agree with the following statement: "I watch what I eat to maintain a good appearance and prevent weight gain"?	0.644
Are you reading food labels; particularly nutritional values and ingredient list on food products?	0.615
Explanation of variance	45%
Eigenvalue	2.257
Sustainability knowledge score	
Are you familiar with the term Sustainable Development?	0.533
How much do you agree with the following statement: "Edible films are active packaging systems that extend product shelf life, improve product quality, and contribute to the nutritional quality of the final product"?	−0.798
To what extent do you agree with the following statement: "Grape and/or aronia pomace is a byproduct of the food industry rich in dietary fiber and polyphenols"?	−0.806
Explanation of variance	52%
Eigenvalue	1.57

2.3. Data Analysis

All qualitative data were coded and evaluated using descriptive statistics with Stata and Microsoft Excel and analyzed.

Descriptive statistic, frequency distribution, and correlation analyses were performed to assess consumer behavior and attitude. Statistical differences between countries and generations were assessed using Kruskal–Wallis analysis of variance (ANOVA) with multiple comparison (two-tailed). Only variables that differed significantly between generations or countries were included in the principal component analysis (PCA). Data mining included classification and regression tree (C&RT), and an importance plot was generated using standard classification and regression tree after pruning on variance. Boxplots show median with 25 and 75 percentile and nonoutlier range. The answers obtained from all participants were also analyzed by means of multivariate analysis employing hierarchical cluster analysis using Ward's method. The distance between samples was calculated using the square of the Euclidean distance with Minitab 17 statistical software. Statistica 14 (Tibco Software, Palo Alto, USA) was used for other data analysis, and a confidence level of $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Cookie Consumption and Preference Choice

It is a well-known fact that cookies are, in general, a good source of energy, which 39% of the participants agreed with. In France and Croatia, cookies were consumed more frequently (one to five times per week) than in North Macedonia (one to two times per month) ($p < 0.001$). In addition, North Macedonians prefer whole-grain and plain cookies, while the French prefer chocolate-coated cookies and the Croats prefer both whole-grain and chocolate-coated cookies (Figure 1a). In agreement, Čukelj et al. [33] found that chocolate and whole-grain cookies were the most popular cookies among Croats.

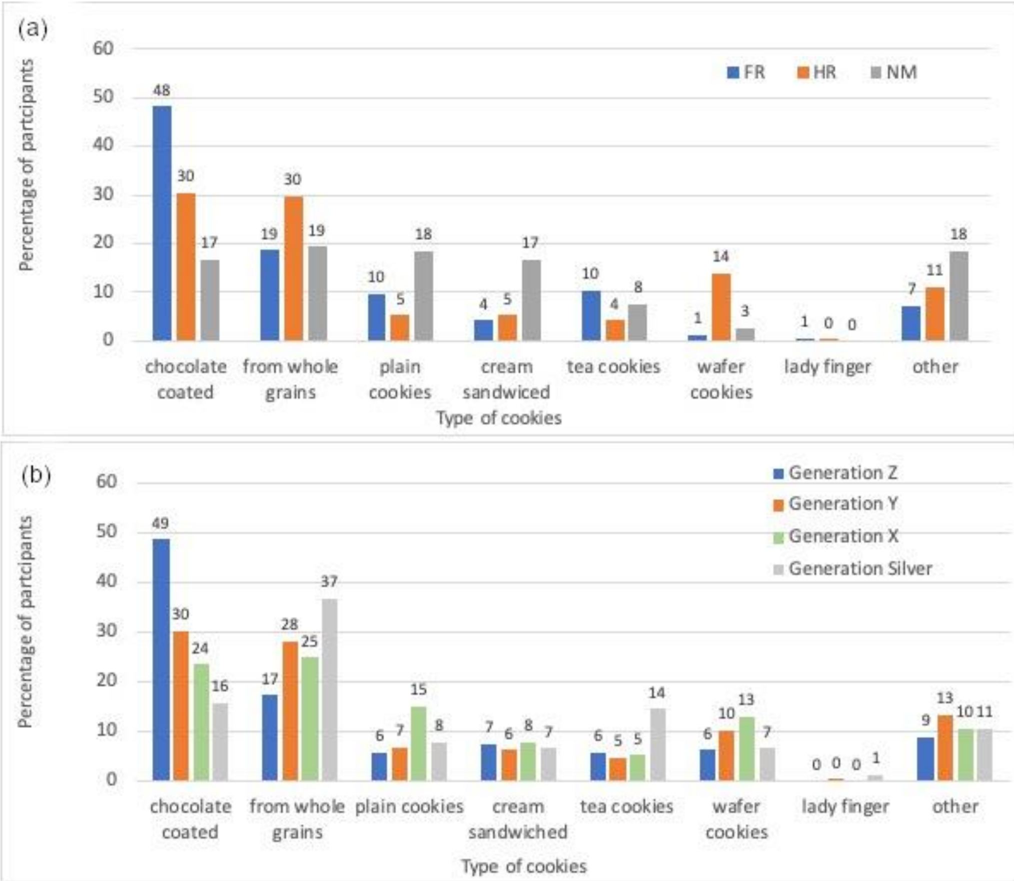

Figure 1. Preference (in percentage) of cookies type by (**a**) country of residence (FR = France; HR = Croatia; NM = North Macedonia) and (**b**) generation.

Regarding the type of cookie, Generation Silver prefers whole-grain cookies, while the younger generations, especially Generation Z, prefer chocolate-coated cookies (Figure 1b). The assumption is that the younger generations value taste over nutritional value and healthy eating. This was also confirmed by responses to the question of whether they consider food to be one of the greatest pleasures, which Generation Z strongly agreed with, compared to the other generations, who partially agreed (median). Consistent with a previous study [34], it was found that Generation Z does care less about healthy eating and that they place less value on health advice. Overall, chocolate-coated cookies remain the most popular option, but Generations X and Silver tend to prefer whole-grain products due to their more health-conscious lifestyles.

Further findings highlight Generation Z as unique consumers compared to other generations. Generation Z answered in the affirmative more often ($p = 0.021$) than Generation Silver when asked if they preferred cookies to other sweets. Consumption of cookies as a morning or afternoon snack was reported more frequently by Generation Z (daily or three to five times per week) than by other generations ($p < 0.001$). While this reflects the nonideal diet of today's generation, which is heavily influenced by the media, it is also possible that this generation lacks the cooking skills to prepare balanced meals or snacks themselves and relies on ready meals and available snacks from the store.

3.2. Nutritional Awareness and Interest in Health and Nutritional Value of Cookies

Compared to the other two nationalities, French participants reported eating cookies more often out of habit, boredom, or stress ($p < 0.001$) (Figure 2a). This may suggest that the French may be emotional consumers. Such findings are supported by the results of several studies; one of them examined the eating habits of elderly French people based on emotions and found that positive emotions were associated with higher food intake and vice versa [35]. Another reason could be that most of the French participants were students, for whom studies suggest that stress can play such a great role in their eating behavior that it can lead to eating disorders. [36]. When comparing generations, our result showed that Generation Z was more likely to consume cookies out of habit, boredom, or stress ($p = 0.039$) than the others (Figure 2b). Similar results were reported by Čukelj et al. [33], who showed a clear relation between habits and emotions. While it is not compelling that younger generations are more emotionally mature and resilient, our findings are in accordance with a study [37] that looked at stressful eating habits of Generation Z. Another study [38], which focused exclusively on the female Generation Z population, found mixed attitudes toward eating habits, with some females having eating disorders. Durukan and Gul [39] found that Generation Z's eating habit discipline was significantly lower than that of previous generations, which is consistent with our data. Again, it is important to keep in mind that Generation Z was born in a time characterized by a variety of global issues. More recently, the COVID-19 pandemic also had profound effects on each generation, but Generation Z experienced more stress-related changes [40]. It should be noted that more detailed studies are needed to accurately determine the reason chosen by participants (habit vs. boredom vs. stress).

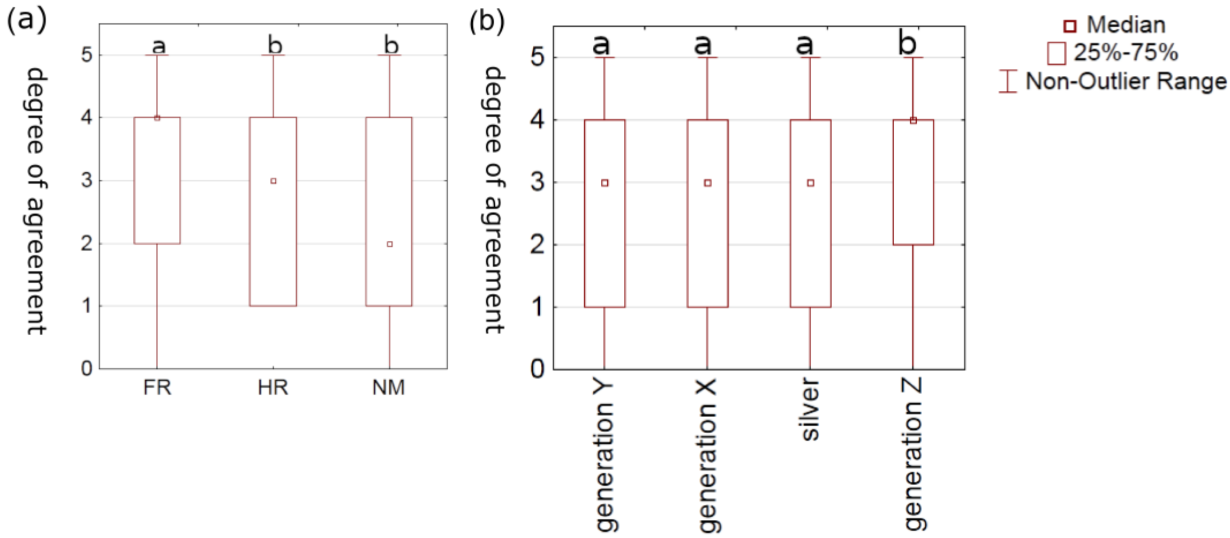

Figure 2. The agreement degree of consumers on the question whether they eat cookies out of habit, boredom, stress and/or other emotions depending on their (**a**) country of residence (FR = France; HR= Croatia; NM = North Macedonia) and (**b**) generation (1 = "I do not agree at all"; 5 = "I completely agree"). Boxplots with different letters represent statistical difference ($p < 0.05$) between countries or generations.

The majority of participants (86%) agreed that regular consumption of whole-grain products has a positive effect on their health. When asked if cookies are a good source of energy, opinions were divided. While some participants partially agreed (31%), others (32%) were undecided or disagreed (25%). Similarly, when asked if cookies are a good source of fiber, 28% of participants agreed, 30% could not decide, and 32.5% disagreed. In contrast to the Croats, the French did not rate the fiber content of cookies as very important ($p < 0.001$). This is in line with Lairon et al. [41], who reported that the overall intake of dietary fiber in the French population is insufficient. The reason could be that the French pay more attention to other aspects of cookies, such as sensory and hedonistic dimensions, or that they expect low sugar and calorie content.

Unlike Generation Z, the participants of Generation Y agreed more ($p = 0.0234$) on the question "How important is it that cookies have less sugar and/or calories?" (Figure 3). This is supported by data showing that the majority of Generation Y responded positively when asked if they were aware of weight gain and therefore health. Similarly, Generation Z ranked the importance of cookies with higher fiber content lower than other generations (Figure 3). In terms of fiber consumption, we found a significant difference ($p = 0.0002$) between Generation Z, who reported consuming fiber most frequently (43%), three to five times per week, and Generation Y, who reported consuming fiber more frequently (38.5% of participants reported consuming daily and 42% of participants reported consuming it three to five times per week). An even larger difference ($p < 0.001$) was found between Generation Z (median = seldom) and the other generations (median = often) in terms of regular consumption of biologically active substances. It could be that Generation Z does not know exactly what biologically active substances or the related health benefits are. This could indicate that Generation Z generally lacks health education and health awareness. In contrast, a previous report [42] has shown that Generation Z is more health conscious than Generation Y. In another study, the health state of Generation Z was related to the successful health education that is received at a younger age [43].

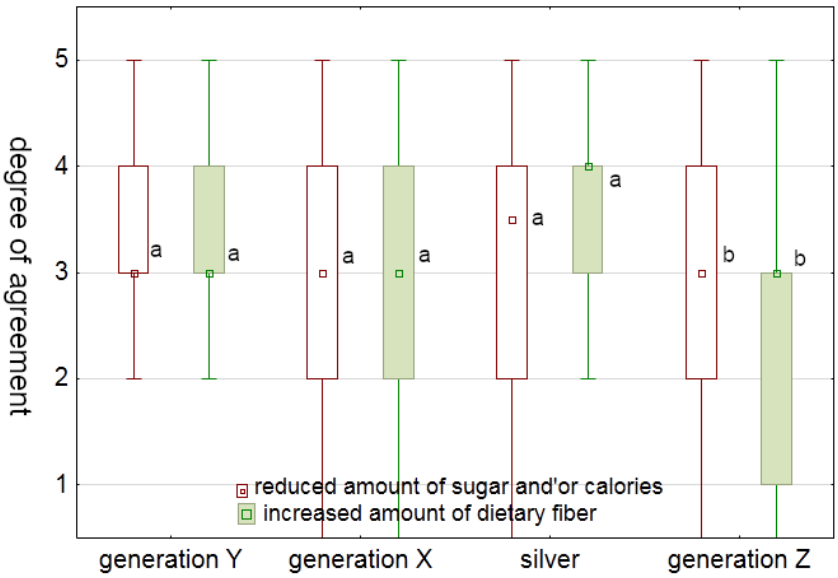

Figure 3. The importance that cookies have a reduced amount of sugar and/or calories or increased amount of dietary fiber to consumers of different generations (1 = "I do not agree at all"; 5 = "I completely agree"). Boxplots with different letters represent statistical difference ($p < 0.05$) within the same question between generations.

Results indicate that Croatian and French consumers generally pay more attention to the ingredient list and labeling of cookies than North Macedonian consumers ($p = 0.0007$). The reason for this could be that nutrition labeling was only recently introduced in Northern Macedonia and people still need to learn how to read the labels. In addition, Generation Y reported reading food labels more often (always 34% and often 40%) than Generation Z (always 20% and often 34%). This suggests that there might be a lack of awareness due to inefficient health education for this generation. The lack of interest in health and misconceptions about appropriate nutrition might be caused by the influence of social media and advertisements.

Overall, the index of dietary awareness was significantly lower in French participants (0.67) compared to Croats (0.80) and Northern Macedonians (0.79) (Figure 4a). As expected, Generation Z has the lowest nutritional awareness compared to others, making them the main target group for health education (Figure 4b). Therefore, additional education is needed in the different population groups to improve nutrition knowledge and awareness.

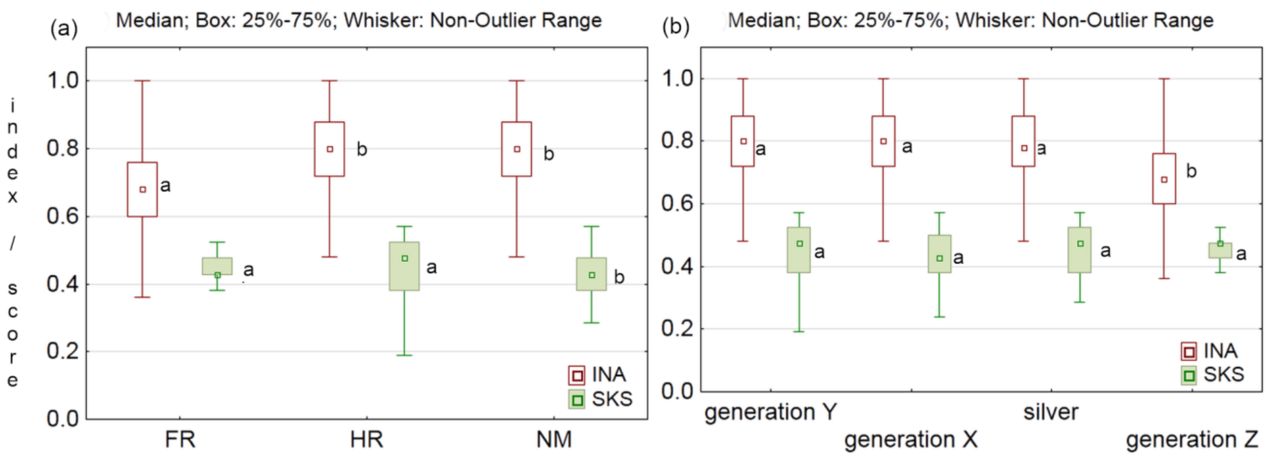

Figure 4. Nutritional awareness index (INA) and sustainability knowledge score (SKS) by (**a**) country of residence (FR = France; HR = Croatia; NM = North Macedonia) and (**b**) generation. Boxplots with different letters represent statistical difference ($p < 0.05$) within the INA or SKS between countries or generations.

3.3. Sustainability Knowledge Score (SKS)

Regardless of country or generation, the majority of participants (84%) were familiar with the importance of sustainable product development. In addition, some participants fully agreed (38%) or partially agreed (32%) that grape and aronia pomace are byproducts of the food industry rich in fiber and polyphenols, while some (26%) could not decide or disagreed (4%). Similarly, about half of the respondents agreed that edible films can extend shelf life (18% fully agreed, 32.5% partially agreed, while 41% neither agreed nor disagreed). This shows that knowledge about fruit byproducts and edible films is still insufficient. Differently from our study, Alonso and O'Neill [44] found that mature US consumers are more related to grape byproducts than the younger consumers group.

Our SKS depended significantly ($p < 0.001$) on the countries, with a median of 0.45 in France and 0.46 in Croatia, significantly different from 0.42 in Northern Macedonia (Figure 4a). Nevertheless, the consumer education level was the most important predictor of SKS. Based on the C&RT analysis, compared to the level of education, whose importance was 1, importance of the country of residence was 0.51, generation 0.38, and gender 0.27. Participants with a doctorate or master's degree had a significantly ($p < 0.001$) higher SKS score (median 0.48) than participants with a bachelor's degree, secondary or primary education (median 0.38). The lack of knowledge and understanding about food byproducts leads to a negative attitude towards their use [45]. Murillo et al. [46] showed that knowledge and previous consumption have a positive influence on the willingness to try food that contain seafood byproducts, whereas consumers' concern about sensory quality, safety, and nutrition are main reasons for their avoidance. Sustainability knowledge could benefit consumers' health and the economy of the food industry. Therefore, further education is needed on the potential use of fruit byproducts and edible films in the production of sustainable food.

3.4. Purchase Intention

Regardless of country of origin or generation, some participants (29%) showed interest in immediately purchasing new, attractive cookies from the market, but most were not interested. The majority of participants (79%) confirmed their interest in purchasing cookies with improved nutritional value that would positively impact their health, and most (67%)

would be eager to pay a higher price for such cookies. In addition, about half of the participants report that they prefer whole-grain cookies (1) because they are good for their health (36% fully agreed and 15% partially agreed) and (2) because they provide a longer feeling of satiety (33% fully agreed and 12% partially agreed). The intention to purchase whole-grain cookies because they are good for health and because consuming cookies provides a longer feeling of fullness were intercorrelated (r = 0.76), and both intentions were also correlated with attitudes that it is important for cookies to have reduced sugar and/or calorie content (r = 0.61; 0.52), increased fiber content (r = 0.63; 0.62), and detailed information about cookies composition and nutritional value (r = 0.54; 0.53). Similarly, Čukelj et al. [33] found that participants with higher nutrition awareness were more likely to purchase the functional cookies. These findings suggest that further public education and promotion of the benefits of whole-grain products with a focus on food-based targets and messaging may be important to increase the consumption of whole grain cookies and thus the intake of dietary fiber [47].

France differed significantly from the other countries on several questions. When asked if participants would purchase foreign brands, the French were least likely to do so (Figure 5). Furthermore, compared to the other two countries ($p = 0.0013$), the French do not care about the manufacturer or brand as long as it is a product from France. This suggests that the French consider their own brands or the brand they usually consume or already know to be of higher quality and would prefer to purchase them. According to a survey by Statista (2021), French consumers expect brands to focus more on products made in France and French manufacturers [48].

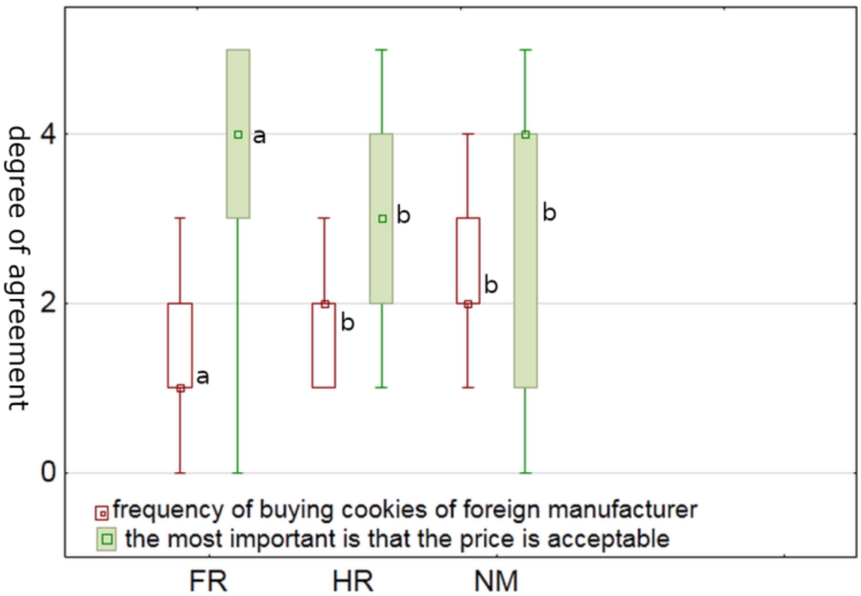

Figure 5. The influence of price and manufacturer on purchase intention by country of origin; FR = France, HR = Croatia, NM = North Macedonia (1 = never/I do not agree at all; 5 = always/I completely agree). Boxplots with different letters represent statistical difference ($p < 0.05$) within the same question between countries or generations.

In addition to brand choice, the price was also an important factor between countries ($p < 0.001$). In particular, the French agreed that an acceptable price was the most important factor (Figure 5). The results suggest that the most French population tends to be frugal when purchasing cookies. This makes sense given the turbulent changes that the French and global economies have undergone in recent years [49]. Recession, budget cuts, inflation, and stagnation in productivity all contribute to the struggling economy [50], leading consumers to purchase more affordable alternatives. However, compared to Croatia and Macedonia, France has a lower inflation rate and higher GDP, which indicate there might be other factors influencing this preference for more affordable cookies [51].

When looking at purchase intention by generation, Generation Y was more selective in choosing cookie brands and may have preferred imported cookies. Compared to the other generations, Generation Z seems to be more open-minded ($p = 0.0199$) when it comes to trying new types of cookies (Table S1 and Figure S1), but for them, a higher price was identified as a barrier to purchasing cookies. The fact that whole-grain cookies are good for health was not the key purchasing driver for Generation Z (Table S1), unlike the others ($p < 0.001$). This could be because Generation Z grew up in times of economic stress (i.e., high inflation) and is more concerned with pricing. The other generations are financially considerate, but not as much as Generation Z. This is also confirmed by evidence of greater frugality among the youngest generation [52]. A previous study of consumers in Kelantan [53] identified the key factors driving Generation Z to purchase snacks: packaging, price, available health information, availability, and taste.

French and Croatian people were most likely to purchase cookies that had a positive environmental impact in addition to health benefits (median test: chi-square = 7.291, $p = 0.026$) (Table S2, Figure S1). Similarly, according to a study by Coderoni et al. [54], most participants prefer purchasing food enriched with byproducts that have lower environmental impact and promote health. Yet, the influence of the participants' education level was a more important predictor of purchasing environmentally friendly cookies than the country of residence (Figure 6a).

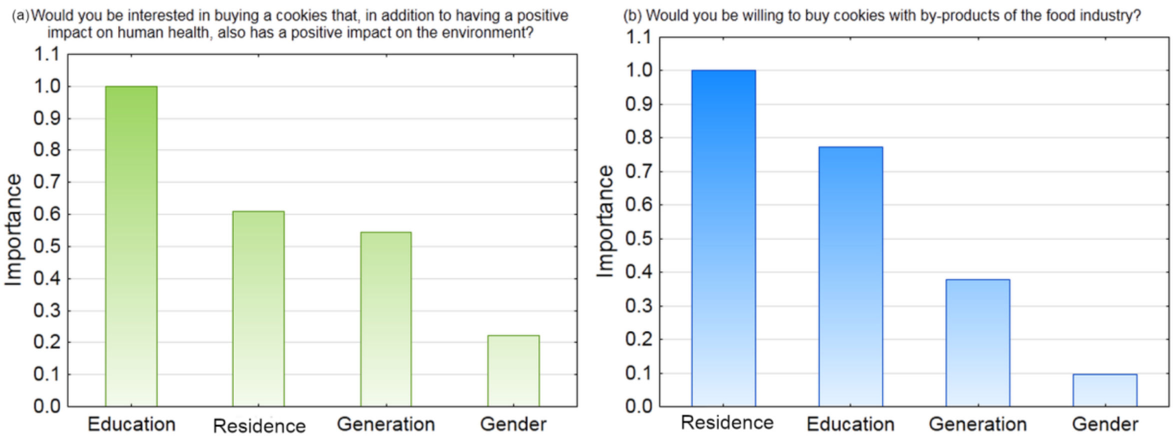

Figure 6. The importance plot of education level, country of residence, generation, and gender on consumer interest in purchasing cookies that (**a**) have a positive impact not only on human health but also on the environment and (**b**) contain byproducts of the food industry.

Most participants were not enthusiastic about the idea of purchasing cookies containing food byproducts (Tables S1 and S2). This could be due to their lack of knowledge, shown by the SKS, and not knowing the health benefits of byproducts. Country of residence and education level were identified as the most important factors influencing the willingness to purchase cookies with food byproducts (Figures 6b and S2). Among the three countries, French participants were the most interested in purchasing cookies with byproducts, while North Macedonians were the least interested (median test: chi-square = 74.208, $p < 0.001$). According to Yilmaz and Kahveci [20], generation, gender and commitment to recycling at home influences the willingness to purchase upcycled food. Consumers are more interested in purchasing products containing byproducts if they can expect a higher quality and better taste and if they believe that these products can contribute to solving the food waste problem [20]. Indeed, French consumers are more and more sensitive and have information about zero food waste and upcycling trends [55,56]. In contrast to our results, Grasso and Asioli [19] reported that although most British consumers had never heard of upcycled ingredients, they would be willing to purchase food products containing them.

Our results suggest that investments should be made to increase knowledge about the benefits that food byproducts can provide, such as upcycled ingredients for environmental protection. In particular, Mediterranean countries with large production of grapes and other berries could benefit from using their byproducts.

3.5. Principal Components and Clusters

Principal component loadings (PCA) and score plots for questions with significant differences by country and generation are shown in Figure 7. PCA of the presented data by country extracted two components with eigenvalues of 8.82 and 3.18 that explained the complete variance. In terms of countries, North Macedonian consumers were more associated with the nutritional aspects of cookie consumption, whereas French and Croatian participants were more characterized by their consumption and purchase patterns. The greatest difference was found between French and North Macedonian participants, specifically in the type of cookies preferred, reasons for consumption, preference for purchasing cookies from domestic or foreign producers, interest in and willingness to purchase cookies with byproducts and/or nutritionally improved cookies (Figure 7a). While the French consume cookies more frequently, often out of boredom or stress, North Macedonians prefer chocolate-coated cookies and consume them less frequently, as discussed in Section 3.1. In addition, the French prefer domestic producers and are most interested in purchasing cookies with byproducts that have a positive impact on the environment and people's health, while Macedonians are more open to foreign producers, as described in Section 3.4.

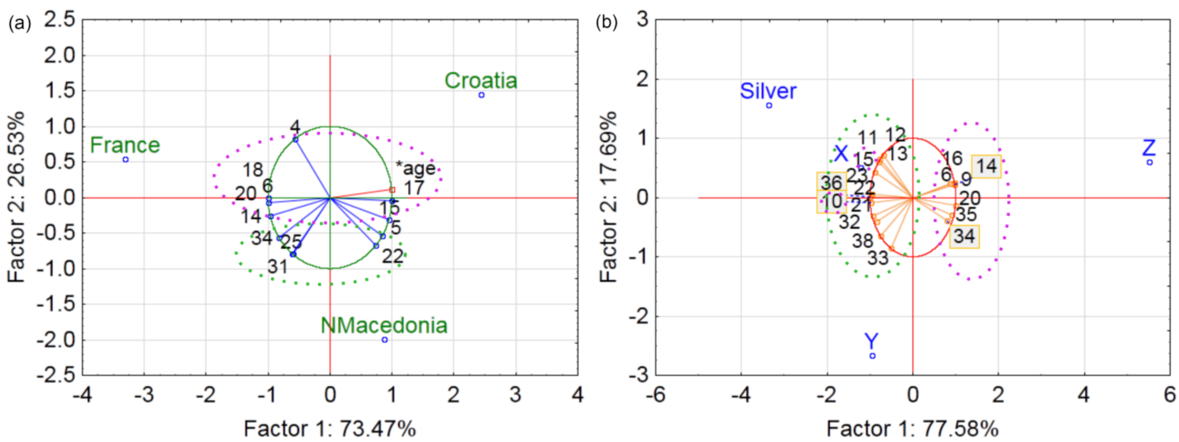

Figure 7. Principal component loadings and score plots of most significant questions by (**a**) country and (**b**) generation (Z, X, Y, and Silver). Legend: nutrition-related questions are circled with green dots (11—cookies are a good source of energy; 12—cookies can be a good source of dietary fiber; 13—eating cookies because they have fewer calories than some other sweets; 21—importance of reduced amount of sugar and/or calories when purchasing cookies; 22—importance of an increased amount of dietary fiber; 32—frequency of consumption of biologically active compounds; 33—frequency of consumption of dietary fiber; 36—eating to maintain my health; 37—reading nutritional values and ingredient list on food products; 38—index of nutrition awareness), consumption- and purchase-related questions are circled with pink dots (4—frequency of eating cereal-based products; 5—consumption of whole-grain products has a positive effect on health; 6—frequency of eating cookies; 9—eating cookies as a morning and/or afternoon snack; 10—eating cookies with coffee/tea; 14—eating cookies out of habit, boredom, stress, and/or other emotions; 15—favorite type of cookies; 16—purchasing cookies from a domestic producer; 17—purchasing cookies from a foreign producer; 18—manufacturer is not the key driver when purchasing cookies 20—importance of price when choosing cookies; 23—purchasing whole-grain cookies because they are good for health; 25—importance of having detailed nutritional information for cookies; 31—interest in purchasing cookies that have a positive effect not only on human health but also on the environment; 34—willingness to purchase cookies with byproducts from food industry; 35—eating is one of the greatest pleasures).

PCA of the presented data between generations (Figure 7b) explained that the first two components accounted for 95.27% of the total variance, with eigenvalues of 14.74 and 3.36, respectively. Whereas Generation Z was associated with the consumption and purchase habits, more mature generations were more concerned with the nutritional aspects. The largest difference found between generations Y and Z can be attributed to the type of cookies consumed (with coffee/tea or as an afternoon snack), the reasons for consumption, the consumption of bioactive compounds and dietary fiber, and reading labels.

Cluster analysis was also performed to determine the similarity between countries and generations (Figure 8). The data can be divided into two main groups consisting of three clusters: blue, red, and green. The first group, as well as the blue cluster, represents France and proves that most of the participants belong to Generation Z. This group differs from the second group, consisting of the red and green cluster representing Croatia and North Macedonia. The differences between France and North Macedonia were already noted in the PCA. It was also shown that the participants in France tend to be emotional eaters, while Croats and North Macedonians tend to have similar eating habits and cookie preferences, thus belonging to the same group. This is not surprising, since most of the French participants were part of the student population, unlike participants from the other two countries. Moreover, Croatia and Northern Macedonia are geographically closer (Balkan Peninsula) and were part of the same country in the past (former Yugoslavia). On the other hand, the participants from North Macedonia provided contrary answers to some of the questions, which is why they form a separate group. As for the second group, the number of participants was more evenly distributed among generations, especially in Northern Macedonia, while the Croatian population was slightly dominated by Generation Y.

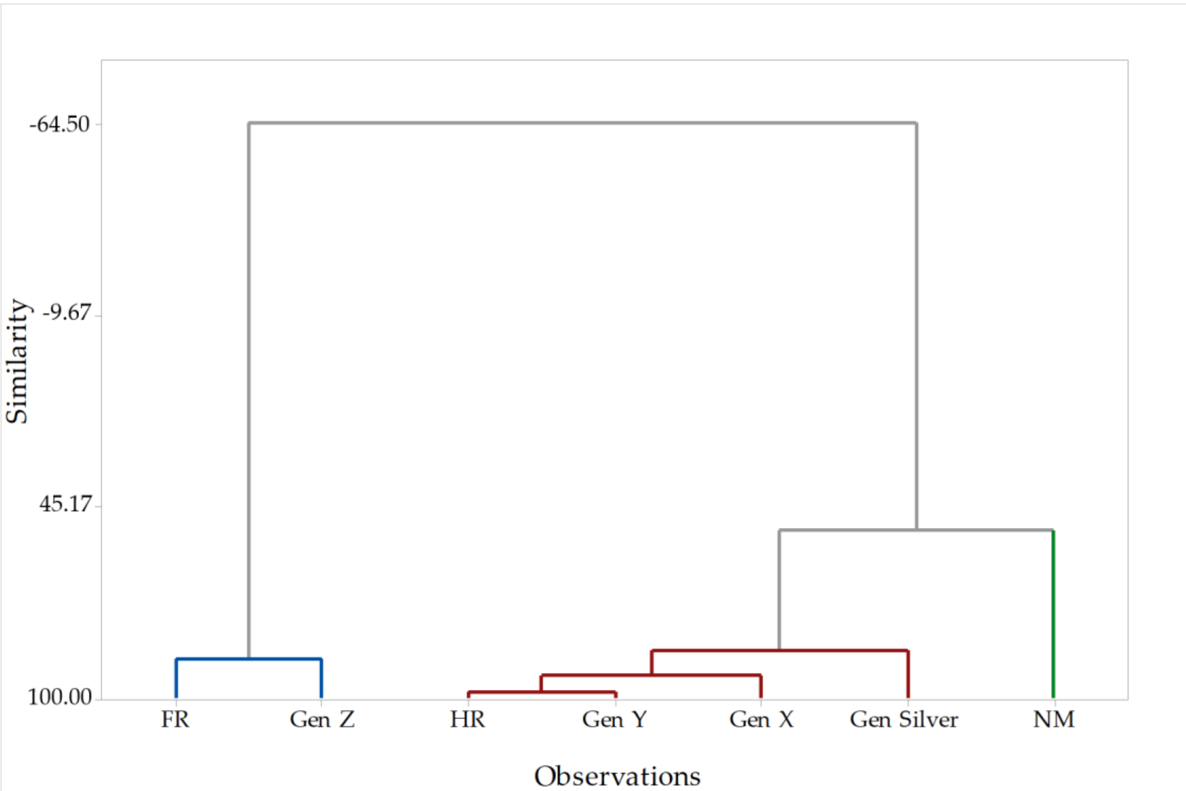

Figure 8. Cluster diagram of the answers (observations) of all participants depending on the country of origin (FR = Franc; HR = Croatia; NM = North Macedonia) and generation (GEN). Different colors are needed as they indicate different clusters in cluster analysis.

3.6. Limitations of Study

The main limitation of this study is that most of the study participants were female, which significantly affects its representativeness. On the other hand, women are responsible for purchasing in most households. Another limitation is the uneven number of participating populations from France, Northern Macedonia, and Croatia as well as the small number of participants and uneven distribution between generations. Nevertheless, our results could serve as a guide for the development of new products and marketing strategies for healthier and more sustainable cookies.

4. Conclusions

This study investigated the market potential and consumer attitudes towards whole-grain cookies with food byproducts and edible films in Croatian, French, and Northern Macedonian populations. Our study confirms that cookies are a popular food that many consumers eat regularly. Whole-grain cookies are among the most preferred types of cookies, which reflects consumer nutritional awareness. Consumer attitudes and consumption habits vary by country and generation, with Generation Z differing from other generations. The majority of consumers are familiar with sustainability and interested in purchasing cookies that are beneficial to the environment and their health. However, their knowledge of fruit byproducts or edible films and their willingness to purchase cookies with food byproducts is limited. Additional educational programs are needed for all generations to raise awareness of the opportunities that upcycled byproducts or edible films offer for environmental protection but also human health. Hence, the findings of this study could be used to develop a marketing and education strategies for communicating the benefits of using upcycled ingredients in producing food of enhanced nutrition value. Such strategies could result in consumers' better acceptability of food with upcycled ingredients and thus a rise in circular economy. In particular, Mediterranean countries with big production of grapes and related byproducts could benefit from such strategies. Future studies should explore the relevance of various educational programs to upcycling food byproducts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods12213932/s1. Table S1: Purchase intention of whole-grain, nutritionally improved and sustainable cookies by generations (number with percentage in brackets). Table S2: Purchase intention of whole-grain, nutritionally improved, and sustainable cookies by country (number with percentage in brackets); Figure S1: Classification and regression tree of willingness to buy environmentally friendly cookies; Figure S2: Classification and regression tree of willingness to buy cookies with food byproducts.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Zagreb Food Technology and Biotechnology (Croatia, 251-69-11-20-37; 14 December 2020), the Faculty Committee of the Faculty of Technology and Metallurgy (University Ss Cyril and Methodius in Skopje, Republic of North Macedonia, 09-1273/1; 10 November 2020), and the ONIRIS Institution (Oniris VetAgroBio– Ecole Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique—French Republic Agricultural Ministry National HighSchool, France, 23-657; 12 February 2023).

Data Availability Statement: The data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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References

1. Sajdakowska, M.; Jankowski, P.; Gutkowska, K.; Guzek, D.; Żakowska-Biemans, S.; Ozimek, I. Consumer acceptance of innovations in food: A survey among Polish consumers. *J. Consum. Behav.* **2018**, *17*, 253–267. [CrossRef]
2. Petrescu, D.C.; Vermeir, I.; Petrescu-Mag, R.M. Consumer understanding of food quality, healthiness, and environmental impact: A cross-national perspective. *Int. J. Environ. Res. Public Health* **2020**, *17*, 169. [CrossRef] [PubMed]
3. Bagchi, D. *Nutraceutical and Functional Food Regulations in the United States and around the World*, 3rd ed.; Academic Press: London, UK, 2019.
4. Galanakis, C.M. The food systems in the era of the coronavirus (COVID-19) pandemic crisis. *Foods* **2020**, *9*, 523. [CrossRef]
5. Gil, A.; Ortega, R.M.; Maldonado, J. Wholegrain cereals and bread: A duet of the Mediterranean diet for the prevention of chronic diseases. *Public Health Nutr.* **2011**, *14*, 2316–2322. [CrossRef] [PubMed]
6. Kantike, I.; Eglite, A. Consumer behaviour in the market of wholegrain products. In Proceedings of the International Scientific Conference, Jelgava, Latvia, 26–27 April 2012.
7. Florence, S.P.; Asna, U.; Asha, M.R.; Jyotona, R. Sensory, physical and nutritional qualities of cookies prepared from pearl millet (*Opennisetum typhoideum*). *Food Process. Technol.* **2014**, *5*, 377. [CrossRef]
8. Kadam, S.U.; Prabhasankar, P. Marine foods as functional ingredients in bakery and pasta products. *Food Res. Int.* **2010**, *43*, 1975–1980. [CrossRef]
9. Atlantic Area Healthy Food Eco-System (AHFES). Available online: https://www.ahfesproject.com/services/consumer-and-market-trends-reports/ (accessed on 18 August 2023).
10. Canalis, M.S.B.; Leon, A.E.; Ribotta, P.D. Effect of inulin on dough and biscuit quality produced from different flours. *Int. J. Food Stud.* **2017**, *6*, 13–23. [CrossRef]
11. Singh, A.; Kumar, P. Gluten free approach in fat and sugar amended biscuits: A healthy concern for obese and diabetic individuals. *J. Food Process. Preserv.* **2017**, *42*, e13546. [CrossRef]
12. Molnar, D.; Gabaj, N.N.; Vujić, L.; Ščetar, M.; Krisch, J.; Miler, M.; Štefanović, M.; Novotni, D. Application of fruit by-products and edible film to cookies: Antioxidant activity and concentration of oxidized LDL receptor in women—A first approach. *Appl. Sci.* **2023**, *13*, 5513. [CrossRef]
13. Kekalih, A.; Sagung, I.O.A.A.; Fahmida, U.; Ermayani, E.; Mansyur, M. A multicentre randomized controlled trial of food supplement intervention for wasting children in Indonesia-study protocol. *BMC Public Health* **2019**, *19*, 305. [CrossRef]
14. Buffière, C.; Hiolle, M.; Peyron, M.A.; Richard, R.; Meunier, N.; Batisse, C.; Rémond, D.; Dupont, D.; Nau, F.; Pereira, B.; et al. Food matrix structure (from biscuit to custard) has an impact on folate bioavailability in healthy volunteers. *Eur. J. Nutr.* **2020**, *60*, 411–423. [CrossRef] [PubMed]
15. Puerta, P.; Laguna, L.; Tárrega, A.; Carrillo, E. Relevant elements on biscuits purchasing decision for coeliac children and their parents in a supermarket context. *Food Qual. Prefer.* **2022**, *98*, 104496. [CrossRef]
16. Sörqvist, P.; Haga, A.; Holmgren, M.; Hansla, A. An eco-label effect in the built environment: Performance And comfort effects of labeling a light source environmentally friendly. *J. Environ. Psychol.* **2015**, *42*, 123–127. [CrossRef]
17. Abbasi, R.; Martinez, P.; Ahmad, R. An ontology model to represent aquaponics 4, 0 system's knowledge. *Inf. Process. Agric.* **2021**, *9*, 514–532. [CrossRef]
18. Hemathilake, D.M.K.S.; Gunathilake, D.M.C.C. High-productive agricultural technologies to fulfill future food demands: Hydroponics, aquaponics, and precision/smart agriculture. In *Future Foods: Global Trends, Opportunities, and Sustainability Challenges*; Bhat, R., Ed.; Academic Press: London, UK, 2022; pp. 555–567. [CrossRef]
19. Grasso, S.; Asioli, D. Consumer preferences for upcycled ingredients: A case study with biscuits. *Food Qual. Prefer.* **2020**, *84*, 103951. [CrossRef]
20. Yilmaz, E.; Kahveci, D. Consumers' purchase intention for upcycled foods: Insights from Turkey. *Future Foods* **2022**, *6*, 100172. [CrossRef]
21. Oliveira, T.C.G.; Caleja, C.; Oliveira, M.B.P.P.; Pereira, E.; Barros, L. Reuse of fruits and vegetables biowaste for sustainable development of natural ingredients. *Food Biosci.* **2023**, *53*, 102711. [CrossRef]
22. Molnar, D.; Novotni, D.; Krisch, J.; Bosiljkov, T.; Ščetar, M. The optimisation of cookie formulation with grape and aronia pomace powders as cocoa substitutes. *Croat. J. Food Technol. Biotechnol. Nutr.* **2020**, *15*, 38–44. [CrossRef]
23. Jurendić, T.; Ščetar, M. *Aronia melanocarpa* products and byproducts for health and nutrition: A review. *Antioxidants* **2021**, *10*, 1052. [CrossRef]
24. Molnar, D.; Novotni, D.; Kurek, M.; Galić, K.; Iveković, D.; Bionda, H.; Ščetar, M. Characteristics of edible films enriched with fruit by-products and their application on cookies. *Food Hydrocoll.* **2023**, *135*, 108191. [CrossRef]

25. Sukboonyasatit, D. Prediction of Peoples' Intentions and Actual Consumption of Functional Foods in Palmerston North. Ph.D. Thesis, Massey University Palmerston North, Auckland, New Zealand, 2009.
26. Nystrand, B.T.; Olsen, S.O. Consumers' attitudes and intentions toward consuming functional foods in Norway. *Food Qual. Prefer.* **2020**, *80*, 103827. [CrossRef]
27. Sielicka-Różyńska, M.; Jerzyk, E.; GluzaInt, N. Consumer perception of packaging: An eye-tracking study of gluten-free cookies. *J. Consum. Stud.* **2021**, *45*, 14–27. [CrossRef]
28. Dean, M.; Shepherd, R.; Arvola, A.; Vassallo, M.; Winkelmann, M.; Claupein, E.; Lähteenmäki, L.; Raats, M.M.; Saba, A. Consumer perceptions of healthy cereal products and production methods. *J. Cereal Sci.* **2007**, *46*, 188–196. [CrossRef]
29. Kraus, A. Development of functional food with the participation of the consumer. Motivators for consumption of functional products. *Int. J. Consum. Stud.* **2015**, *39*, 2–11. [CrossRef]
30. Miklavec, K.; Pravst, I.; Grunert, K.G.; Klopcic, M.; Pohar, J. The influence of health claims and nutritional composition on consumers' yoghurt preferences. *Food Qual. Prefer.* **2015**, *43*, 26–33. [CrossRef]
31. Puerta, P.; Carrillo, E.; Badia-Olmos, C.; Laguna, L.; Rosell, C.M.; Tárrega, A. Coeliac consumers' expectations and eye fixations on commercial gluten-free bread packages. *LWT-Food Sci. Technol.* **2022**, *163*, 113622. [CrossRef]
32. Šedík, P.; Hudecová, M.; Predanócyová, K. Exploring consumers' preferences and attitudes to honey: Generation approach in Slovakia. *Foods.* **2023**, *12*, 1941. [CrossRef]
33. Čukelj, N.; Putnik, P.; Novotni, D.; Ajredini, S.; Voučko, B.; Ćurić, D. Market potential of lignans and omega-3 functional cookies. *Brit. Food J.* **2016**, *118*, 2420–2433. [CrossRef]
34. Savelli, E.; Murmura, F. The intention to consume healthy food among older Gen-Z: Examining antecedents and mediators. *Food Qual. Prefer.* **2023**, *105*, 104788. [CrossRef]
35. Narchi, I.; Walrand, S.; Boirie, Y.; Rousset, S. Emotions generated by food in elderly French people. *J. Nutr. Health Aging.* **2008**, *12*, 626–633. [CrossRef]
36. Tavolacci, M.P.; Déchelotte, P.; Ladner, J. Eating disorders among college students in France: Characteristics, help-and care-seeking. *Int. J. Environ. Res. Public. Health* **2020**, *17*, 5914. [CrossRef] [PubMed]
37. Priporas, C.V.; Vellore-Nagarajan, D.; Kamenidou, I. Stressful eating indulgence by generation Z: A cognitive conceptual framework of new age consumers' obesity. *Eur. J. Mark.* **2022**, *56*, 2978–3006. [CrossRef]
38. Kaylor, S.K.; Allen, I.; Crim, A.D.; Callihan, M.L. Calories and control: Eating habits, behaviors, and motivations of Generation Z females. *J. Am. Coll. Health* **2021**, *25*, 1–9. [CrossRef] [PubMed]
39. Durukan, A.; Gül, A. Mindful eating: Differences of generations and relationship of mindful eating with BMI. *Int. J. Gastron. Food Sci.* **2019**, *18*, 100172. [CrossRef]
40. Sitoayu, L.; Purwara Dewanti, L.; Melani, V.; Azahra Sumitra, P.; Rulina Marpaung, M. Differences in eating habits, stress, and weight changes among indonesian generations Y and Z students during the COVID-19 pandemic. *J. Res. Health* **2023**, *13*, 79–86. [CrossRef]
41. Lairon, D.; Bertrais, S.; Vincent, S.; Arnault, N.; Galan, P.; Boutron, M.C.; Hercberg, S. Dietary fibre intake and clinical indices in the French Supplementation en Vitamines et Minéraux AntioXydants (SU.VI.MAX) adult cohort. *Proc. Nutr. Soc.* **2003**, *62*, 11–15. [CrossRef]
42. The Everything Guide to Generation Z. Available online: https://The%20everything%20guide%20to%20gen%20z/the-everything-guide-to-gen-z.pdf (accessed on 18 August 2023).
43. Su, C.H.; Tsai, C.H.; Chen, M.H.; Lv, W.Q. US sustainable food market generation Z consumer segments. *Sustainability* **2019**, *11*, 3607. [CrossRef]
44. Alonso, A.; O'Neill, M.A. Consumption of muscadine grape by-products: An exploration among Southern US consumers. *Br. Food J.* **2012**, *114*, 400–415. [CrossRef]
45. Cattaneo, C.; Lavelli, V.; Proserpio, C.; Laureati, M.; Pagliarini, E. Consumers' attitude towards food by-products: The influence of food technology neophobia, education and information. *Int. J. Food Sci. Technol.* **2018**, *54*, 679–687. [CrossRef]
46. Murillo, S.; Ardoin, R.; Prinyawiwatkul, W. Factors Influencing Consumers' Willingness-to-Try Seafood Byproducts. *Foods* **2023**, *12*, 1313. [CrossRef]
47. Barrett, E.M.; Foster, S.I.; Beck, E.J. Whole grain and high-fibre grain foods: How do knowledge, perceptions and attitudes affect food choice? *Appetite* **2020**, *149*, 104630. [CrossRef] [PubMed]
48. Consumer Expectations for Brands in France for 2021. Available online: https://www.statista.com/statistics/1194944/consumer-expectations-brands-france/ (accessed on 1 August 2023).
49. FocusEconomics—France Archives. Available online: https://www.focus-economics.com/countries/france/ (accessed on 1 August 2023).
50. Cette, G.; Corde, S.; Lecat, R. Stagnation of productivity in France: A legacy of the crisis or a structural slowdown? *Econ. Stat.* **2017**, *494–496*, 11–36. [CrossRef]
51. World Bank Open Data. Available online: https://data.worldbank.org (accessed on 1 August 2023).
52. Kusumawardhany, P.A. Frugal lifestyle trend among generation Z. In Proceedings of the 19th International Symposium on Management (INSYMA 2022), Bali, Indonesia, 19–20 May 2022.
53. Liew, J.Y.; Ng, K.H.; Aziz, N.S.B.A. Antecedents of junk food purchase intention among generation Z: A case study of Kota Bharu, Kelantan. *AIP Conf. Proc.* **2023**, *2500*, 020042. [CrossRef]

54. Coderoni, S.; Perito, M.A. Sustainable Consumption in the Circular Economy. An Analysis of Consumers' Purchase Intentions for Waste-to-Value. *Food. J. Clean. Prod.* **2020**, *252*, 119870. [CrossRef]
55. République Française. Available online: https://www.strategie.gouv.fr/english-articles?page=1 (accessed on 21 October 2023).
56. IPSOS—Global Market Research and Public Opinion Specialist. Available online: https://www.ipsos.com/en-hr (accessed on 21 October 2023).

Chapter 3

General discussion

1. **Development of cookies and edible films enriched with fruit by-products**

   1.1. Development of cookies with grape- and aronia pomace

   1.2. Physical and sensory characteristics of cookies with grape- and aronia pomace

2. **Extending the shelf-life of cookies through the application of edible films**

   2.1. Accelerated and direct shelf-life method

3. **Improving the quality of cookies through the application of fruit by-products and edible films**

4. **Effects of the consumption of cookies containing GAP and KGAE on oxLDL in healthy women**

5. **Market potential and consumer attitude towards whole grain and sustainable cookies**

1.Development of cookies and edible films enriched with fruit by-products

In the initial phase (*Supplementary* and *Publication No. 1*), a formulation for whole grain cookies incorporating powdered grape- and aronia pomace as partial replacements for cocoa powder was developed. Additionally, edible films and coatings based on chitosan and GA, supplemented with grape seed extract, were formulated. This part also investigates the physical and sensory characteristics of the developed cookies, along with an assessment of the physicochemical, morphological, and the thermal properties of the edible coatings.

1.1. Development of cookies with grape- and aronia pomace

Identifying a suitable alternative to cocoa powder presents challenges, as existing substitutes often differ significantly from natural cocoa in color, solubility, and composition (Rikon and de Valle, 1982). While carob flour is described as a potential substitute for cocoa, it shows comparable positive aspects like phytochemicals but also additional negative aspects like astringent condensed tannins (Silanikove et al., 2006; Loullis and Pinakoulaki, 2018). Therefore, the primary objective was to discover a sustainable cocoa substitute with enhanced sensory attributes. It investigates, for the first time as far as it can be found in the literature, the potential of utilizing red grape- and aronia pomace as a partial cocoa substitute in cookie formulations.

Ten different formulations were developed using the mixture design and the Design Expert v.11 software (Stat-Ease, USA) (*Supplementary* – Table 1 and Figure S1). Cocoa powder was partially or completely replaced with powdered dried red grape (*Vitis vinifera L.* variety Frankovka and Syrah, with seeds) pomace and aronia (*Aronia melanocarpa L.*, without seeds) pomace in varying ratios from 0 to 100, maintaining similar particle size distribution as cocoa powder (*Supplementary* – Figure 1).

This dissertation examines the physical characteristics of cookies containing different proportions of cocoa powder, grape- and aronia pomace (*Supplementary* – Table 1). Despite the usual preference for a higher spread factor, cookie diameter and height serve as more reliable quality indicators (AACC Standard No 10-50.05, 1999). While the mixture composition did not significantly affect cookie height and spread ratio ($p>0.05$), interactions between aronia- and grape pomace, and aronia pomace and cocoa, negatively impacted the cookie diameter ($p<0.05$) (*Supplementary* - Figure 2a, Table 2). Comparable findings were reported by Karnopp et al. (2015) and Acun and Gül (2014), indicating no significant difference in spread ratio and diameter when grape pomace was incorporated into cookies by up to 30 %. In contrast, Ajila et al. (2008) observed a reduction in diameter and thickness with the addition of 15 % or 20 % mango peel powder, while Mildner-Szkudlarz et al. (2013) observed an increase in diameter and spread ratio with the inclusion of white grape pomace by up to 30 %. Discrepancies in the impact of pomace substitution on the cookie diameter may arise from different pomace types used, quantity, and composition, as well as differences in fat, sugar, and fiber content in other incorporated ingredients (Mamat and Hill, 2018).

The inclusion of grape- and aronia pomace powders had a significant effect on the color of the cookies, particularly evident in the L^* value, which increased when grape pomace constituted at least 50 % of the mixture, resulting in lighter cookies compared to those made with 100 % cocoa powder. The interaction of cocoa, grape- and aronia pomace powders notably affected a^* and b^* values, resulting in reduced color intensity (*Supplementary* - Table 1). Similarly, other studies observed that cookies become darker with increasing grape pomace content, exhibiting lower a^* and b^* values (Acun and Gül, 2014, Karnopp et al., 2015). The decline in a^* and b^* values may be linked to the anthocyanin composition in the pomace (Šarić et al., 2016) and the presence of cocoa in the control. The most significant color difference compared to the control (100 % cocoa) occurred when aronia dominated the mixture (*Supplementary* – Figure 2b). This suggests the potential of combining fruit pomaces containing varying levels of anthocyanin to achieve the desirable color similar to the control cookie (100 % cocoa).

In this study, we maintained uniform water addition across all formulations, and the inclusion of whole grain flour and oat flakes already contributed to a high fiber content. Furthermore, the incorporation of powdered pomaces might have altered the composition of fibers, potentially favoring more soluble forms. The cookies hardness, toughness, and flexibility remained unaffected by the mixture composition ($p>0.05$) (Supplementary - Table 1). In contrast, Kuchtova et al. (2016) observed a significant increase in hardness with 15 % grape seed powder, possibly due to lower a gluten content (Karnopp et al., 2015) and fiber composition of the added pomaces (Šarić et al., 2016).

The addition of grape- and aronia pomace powders significantly impacted the flavor, texture and overall acceptability of the cookies ($p<0.01$) (*Supplementary* – Tables 2 and 3). Texture liking was significantly influenced ($p<0.05$) by the interaction of all mixture components. Substituting cocoa with both grape- and aronia pomace, in small quantities of up to 30 %, positively influenced the texture preference. The addition of grape- and aronia pomace powders enhanced the cookies flavor, with cocoa powder exerting the strongest effect (*Supplementary* - Figure 2c). Overall acceptability was the highest when cocoa was dominant and the lowest when aronia was dominant (*Supplementary* - Figure 2d), while no significant differences were found in odor and appearance between the cookies ($p>0.05$). Mildner-Szkudlarz (2013) observed comparable outcomes regarding the influence of grape preparations on the odor and appearance of cookies. Consistent with our findings, Karnopp et al. (2015) showed that increased amounts of grape pomace can significantly affect the overall acceptability of cookies, emphasizing the need to improve formulations to increase consumption. The optimized mixture with a desirability of 0.725 was composed of 76.4 % cocoa powder, 17.5 % grape pomace, and 6.1 % aronia pomace. General opinions on the flavor, color, texture and overall acceptability of optimized cookies were mainly positive according to sensory analysis results (*Supplementary* – Table 3) suggesting that with utilizing powdered grape- and aronia pomaces as sustainable alternatives, up to 24 % of cocoa powder can successfully be substituted in the production of whole grain cookies. Therefore, the optimized cookie formulation identified in this study was used for further investigations detailed in this dissertation.

5.1. Physical and sensory characteristics of cookies with grape- and aronia pomace

This section summarizes the findings concerning the development and physicochemical, thermal, and structural properties of edible films formulated with chitosan and gum arabic, supplemented with grape seed extract (GSE). Two types of film-forming solutions were prepared: the first (KGA) comprised chitosan, GA, and glycerol, while the second (KGAE) included chitosan, GA, glycerol, and GSE.

The physical characteristics of the edible films are detailed in *Publication No. 1* - Table 1. Films containing GSE displayed approximately twice the thickness (104.4 μm) compared to those without GSE (61.9 μm). This aligns with findings from Oliveira Filho et al. (2020), who observed an increased thickness in chitosan-based films with the addition of citrus limonia essential oil (>0.75 %), and with Sogut and Seydim (2018), who reported similar trends with 5 %, 10 %, and 15 % inclusion of GSE in chitosan-based films.

Publication No. 1 (Table 1) shows that the water vapor permeability (WVP) was slightly higher in KGAE ($3.31 \pm 0.15 \times 10^{-1}$ g m^{-1} s^{-1} Pa^{-1}) than in films without extract ($2.38 \pm 0.15 \times 10^{-1}$ g m^{-1} s^{-1} Pa^{-1}). The introduction of hydrophilic GSE led to an increase in the WVP (Wu et al., 2014). As a result, applying chitosan-GA coatings with GSE to cookies requires a thicker layer and a prolonged drying time. Moreover, films enriched with GSE showed reduced water solubility (WS) with values of 12.44 ± 3.28 %, in contrast to the film without extract, with a solubility of 18.67 ± 0.56 %. The decreased WS observed in the film containing GSE may arise from the uneven and inadequate bonding of GSE within the film matrix, owing to its hydrophilic nature. This aligns with observations from Riaz et al. (2018), who observed similar findings when integrating apple pomace into a chitosan film. In contrast, Wang et al. (2013) documented increased WS in chitosan films infused with tea polyphenols and essential oils.

Packaging materials must effectively block visible and ultraviolet light to preserve light-sensitive food and to prevent nutrient and flavor losses (De Moraes Crizel et al., 2018). Films containing GSE showed a greater light-blocking ability at 600 nm compared to those without GSE (*Publication No. 1* - Table 1). This aligns with findings from Sogut and Seydim (2018) and Oliveira Filho et al. (2020). The reduced transparency may result from electrostatic interactions between GA and chitosan and the inclusion of colored phenolic compounds from GSE, altering the structure of the film matrix (Sogut and Seydim, 2018; Xu et al., 2021).

Since the majority of antioxidants in aronia and grape belong to the anthocyanins, the addition of GSE changed the films color due to the presence of colored antioxidant compounds and the acidic pH affecting the anthocyanins' red hue (*Publication No. 1* - Table 1). Increasing the GSE concentration led to higher redness (a^*) and yellowness (b^*) levels. Similar trends were observed by Sogut and Seydim (2018) and Moghadam et al. (2020), showing lower L^* values and higher a^* and b^* values in GSE-containing samples. The significant total color difference ΔE (45.65 ± 0.56) in GSE-containing films indicates noticeable color changes perceptible by the human eye. *Publication No. 1* shows that GAP with KGAE had higher L^* values and lower redness (a^*) and yellowness (b^*) compared to those with simple chitosan and GA films (*Publication No. 1* - Table 4). Additionally, the total color difference was smallest for GAP with KGAE compared to the control without a coating (*Supplementary* – Table 2), suggesting that GSE and grape- and aronia pomace could serve as viable replacement for cocoa powder in cookies.

All examined film-forming solutions (FFS) exhibited pH values within the acidic range, aligning with the optimal pH interval (3.5 to 5.0) favorable for the formation of a polyelectrolyte complex between GA and chitosan, as reported by Espinosa-Andrews et al. (2007) (*Publication No. 1* - Table 2). The chitosan solution had the lowest pH value (4.7), while the GA solution with GSE (GAE) displayed the highest pH value (4.96). Interestingly, the addition of GSE had a negligible effect on the pH value. Combining chitosan and GA solutions (KGA) resulted in a solution with a pH falling between those of the individual solutions.

Viscosity is a critical parameter for assessing the ability to spray FFS onto food, especially cookies in this study, and serves as an indicator of polymer stability within the solution. Chitosan solutions exhibited higher viscosity values compared to GA (*Publication No. 1* - Table 2). However, when the solutions were mixed, the viscosity significantly decreased, likely due to the formation of stable complexes between polysaccharides and proteins resulting from the interaction of positively charged chitosan ($-NH_3+$) and negatively charged GA (-COO). Interestingly, the addition of GSE increased the viscosity in simple formulations, with no significant difference observed in the chitosan-GA complex. These viscosity results were pivotal decisive in selecting the appropriate application method of FFS on cookies.

Thermal properties of edible films based on GA and chitosan, with and without GSE were also observed in *Publication No. 1* – Table 3. KGA films had endothermic peaks at 178.8 °C and 191.6 °C, while GSE-enriched films displayed a single, broad peak indicating structural changes.

The results indicate that both the KGA and KGAE films remained stable at higher temperatures, and therefore 80°C was chosen as the most suitable drying temperature for cookies after the FFS application.

Changes on film coatings were observed by Fourier transform infrared analysis (FTIR) and results given in *Publication No. 1* - Figure 1 show only slight changes in the pure chitosan film, with no significant differences between KGA and KGAE.

Surface topography of films based on chitosan and GA, both with and without GSE, was examined using scanning electron microscopy (SEM), and the results are presented in *Publication No. 1*. The chitosan-GA film surface appeared smooth, uniform, and free of irregularities, bubbles, or cracks. Conversely, films enriched with GSE (KGAE) displayed granular agglomerates (0.2–10 μm) within the film matrix (*Publication No. 1* - Figure 2), with minimal impact on film homogeneity. Despite the presence of agglomerates, the surface of the chitosan-GA film containing GSE remained smooth, indicating excellent structural integrity and compactness.

As flavor is the most important sensory characteristic to consumers, *Publication No. 1* – Table 4, shows that GAP with KGAE had the most favorable flavor (7.9 ± 0.9). Given the superior results in lightness, flavor, odor, and texture – KGAE edible films were chosen for further analysis on cookies.

2. Extending the shelf-life of cookies through the application of edible films

2.1. Accelerated and direct shelf-life method

In accordance with findings from the initial phase of the dissertation, the spraying technique was used to administer the chitosan and GA-based film-forming solution supplemented with the bioactive component (GSE) onto the cookies. Moreover, control cookies containing cocoa (CC), cookies with grape- and aronia pomace (GAP), and GAP with KGAE were chosen for subsequent analyses. *Publication No. 1* investigates the quality attributes of coated cookies stored under varying temperatures, along with examining the correlation between quality parameters (peroxide value) and sensory characteristics. Furthermore, Q10-values and activation energy (Ea) were calculated as part of the investigation.

Publication No. 1 shows that the application of edible coatings reduced peroxide formation in cookies during storage, thus extending the shelf-life. This finding was confirmed through accelerated (ASLT) and direct shelf-life tests. Despite slight moisture changes, remaining below 5 % in all cookies for 3 months, GAP with KGAE had the least moisture change, likely due to the additional drying (*Publication No. 1* – Table 5). Monitoring moisture content after applying edible films is crucial due to the hydrophilic nature of GSE. The peroxide value (PV) increased over 3 months at higher storage temperatures but stayed below the critical level of 10 mmol O_2 kg^{-1} (Niki, 2009). According to the ASLT, CC was expected to have the highest shelf-life due to the lowest increase in PV at 35°C (4.97 ± 0.04 mmol/kg). Despite experiencing the highest PV increase at 30°C among all groups, GAP with KGAE exhibited a higher activation energy, which slowed down the reaction and quality decline (*Publication No. 1* – Table 6). After six months, a rancid odor was detected only in CC (*Publication No. 1* – Figure 3a and b), indicating the development of secondary oxidation products and the end of its shelf-life.

The texture of cookies was altered by the application of coatings, significantly prolonging their shelf-life by at least 30 days compared to the CC, as confirmed by direct testing. Due to observed differences between the direct and the ASLT method, particularly regarding PV at 35°C in control cookies, future research could benefit from additional analyses like assessing anisidine values and conducting sensory evaluations.

3. Improving the quality of cookies through the application of fruit by-products and edible films

Publications No. 1 and *No. 2* examine the nutritional and antioxidant properties of whole grain cookies with 24 % cocoa powder substitution by grape- and aronia pomace, coated with edible films enriched with grape seed extract (GAP with KGAE).

The cookies showed non-significant differences in fat, protein, fiber, starch, glucose, and mineral content, indicating the successful utilization of fruit by-products as cocoa substitutes (*Publication No. 2* – Table 1).

The study investigates how grape- and aronia pomace, combined with edible films containing grape seed extract (GSE), influence the cookies total phenolic content (TPC) and antioxidant activity. Results show that cookies with GAP and KGAE had significant higher TPC levels (1.63 ± 0.03 μg GA g d.w^{-1}) compared to those with CC (1.39 ± 0.01 μg GA g d.w^{-1}). Besides, GAP in combination with KGAE demonstrated superior antioxidant potential, exceeding the levels of cookies containing only cocoa by 27 % to 73 % as well as exceeding GAP containing cookies by 5 % to 9 % (*Publication No. 2* - Table 2). Similar results were reported by Theagarajan et al. (2019) and Cádiz-Gurrea et al. (2017), suggesting an increase in phenolic content through grape pomace supplementation. Additionally, Cádiz-Gurrea et al. (2017) emphasized a higher antioxidant capacity and phenolic content of GSE compared to cocoa.

In addition to evaluating the antioxidant activity, starch digestibility was assessed *in vitro*, which revealed dominant levels of slowly digestible starch (SDS) compared to rapidly digestible starch (RDS). The cookies contained SDS levels 1.3 to 2 times higher than those reported by Garsetti et al. (2005), suggesting potential benefits for satiety and indicating a medium to low glycemic index. Furthermore, both modified cookies, GAP (2.65 ± 1.45% dry weight) and GAP with KGAE (1.88 ± 1.87% dry weight), showed an increased resistant starch (RS) content, although not significantly different from the CC (0.88 ± 0.43% dry weight). Noteworthy, GAP combined with KGAE had the highest RDS (21.06 ± 1.45% dry weight), indicating a need for a thicker layer of edible film to effectively reduce the final product's glycemic index.

Given their superior antioxidant potential and flavonoid content, GAP in combination with KGAE was chosen for the randomized controlled trial, where they were compared against commercially available cookies with similar composition.

4. Effects of the consumption of cookies containing GAP and KGAE on oxLDL receptor in healthy women

This dissertation investigates the effects of consuming GAP and KGAE containing cookies on oxLDL receptor formation in healthy women, detailed in *Publication No. 2*. A randomized controlled trial conducted involved twenty-five healthy women, aged 36 years on average, split into two groups consuming either 45 g of cookies containing GAP in combination with KGAE or commercial cookies for 10 days. Baseline characteristics, biochemical parameters, and Food Frequency Questionnaire results are outlined in *Publication No. 2* - Table 2 and Figure 1.

Throughout the study, oxLDL receptor concentrations did not significantly differ between the test- and the control group (0.29 ng/ml). No statistically significant predictors of changes in the oxLDL receptor concentration were observed on day 10 in either group. These results are consistent with Pokimica et al. (2019), who found no impact on oxLDL levels after 8 weeks of consuming aronia juice. In contrast, Naruszewicz et al. (2007) reported a 29 % decrease in oxLDL levels after 6 weeks of aronia flavonoid extract intake in post-myocardial infarction patients on statin therapy, suggesting a potential bias due to participant characteristics.

In *Publication No. 2* - Table 5, a link between waist circumference and oxLDL receptor levels was identified. The control group showed an inverse correlation between waist circumference and oxLDL receptor concentration on the 10th day ($r = -0.67$; $p = 0.034$) of cookie consumption, which was not observed in the test group, likely due to a diverse range of participants waist circumference. While a previous study by Sikora et al. (2014) reported modest reductions in BMI and waist circumference in obese individuals with metabolic syndrome after aronia extract supplementationtudies by Xie et al. (2017) and Jakovljevic et al. (2018) found no significant association between oxLDL levels and waist circumference changes in overweight or obese patients with metabolic syndrome.

In *Publication No. 2* – Table 6, contrasting results regarding the connection of the serum iron relationship with oxLDL receptor levels were documented. The control group showed a significant positive correlation between oxLDL receptor levels and serum iron ($r = 0.69$; $p = 0.012$) as well as oxLDL receptor levels and iron saturation ($r = 0.61$; $p = 0.037$), while the test group displayed an inverse correlation between oxLDL receptor levels and serum iron ($r = -0.62$; $p = 0.022$) and receptor levels and iron saturation ($r = -0.47$; $p = 0.105$) by day 10. Oral aronia extract supplementation (0.45 mL/kg/day) over a period of four weeks increased the serum iron levels in rats, as reported by Jakovljević et al. (2018). Additionally, the serum ferritin concentration and the haptoglobin phenotype (Hp) independently correlated with oxLDL levels in male participants (Brouwers et al., 2004), suggesting a link between iron availability and oxLDL levels. Variability in the haptoglobin phenotypes may have contributed to differing outcomes. Recent research linking iron-related oxidative stress to damage in metabolic syndrome patients suggests a connection between serum ferritin and oxLDL levels, potentially increasing the risk of metabolic syndrome in individuals with elevated levels of both markers (Leiva et al., 2013). Certain polyphenols may influence iron absorption, potentially worsening the cardiovascular disease risk, especially in the high-risk population (Lesjak et al., 2014).

A longer intervention period with more participants is needed to define the correlation between cookies consumption, waist circumferences, serum iron, and oxLDL receptors.

5. Market potential and consumer attitude towards whole grain and sustainable cookies

This dissertation is the first one to explore the market potential and consumer attitude toward whole grain and sustainable cookies made with food by-products and edible films. While comparing responses across different countries (Croatia, France and North Macedonia) and generations, parameters such as sustainability knowledge, nutritional awareness as well as the willingness to purchase environmentally friendly cookies enriched with food by-products were assessed in *Publication No. 3*. Participants were categorized into the following four generations according to a study conducted by Šedík et al. (2023): Generation Z (born between 2004 and 1997), Generation Y (born between 1996 and 1981), Generation X (born between 1980 and 1972), and Generation Silver (born between 1971 and 1952).

The study revealed distinct preferences between demographics. While chocolate-coated cookies remained popular overall, North Macedonian respondents favored whole grain and plain varieties (35 %), in contrast to the French who preferred chocolate-coated cookies (48 %), and the Croats who liked both (65 %) (*Publication No. 3* - Figure 1a). Generation Silver showed a preference towards whole grain cookies (37 %), while Generation Z favored chocolate-coated ones (49 %). These findings support previous research indicating a preference for chocolate and whole grain cookies in Croatia (Čukelj et al., 2016). Notably, Generation Z was found to prioritize flavor over nutrition, which is consistent with their lower emphasis on healthy eating reported by Savelli et al., 2023.

Generation Z represents a distinctive cohort of consumers characterized by suboptimal dietary patterns, potentially influenced by media exposure and a perceived inadequacy in culinary proficiency. Consequently, this cohort demonstrates a dependence on pre-packed convenience food.

French participants showed an increased cookie consumption attributed to habits, boredom, or stress ($p < 0.001$) (*Publication No. 3* - Figure 2a), indicating potential emotional eating tendencies. These results are in line with Narchi et al. (2008) findings on emotional eating behaviors among elderly French individuals, showing a positive correlation between positive emotions and food intake. Moreover, the prevalence of students among the French participants may be associated with an increased finding of stress-related eating behaviors, as stress is reported to significantly affect students dietary habits and may lead to eating disorders (Tavolacci et al., 2020). Cluster analysis confirmed emotional eating tendencies among the French participants, in contrast to the relatively uniform eating habits and the cookie preferences observed among Croats and North Macedonians grouped together (*Publication No. 3* – Figure 8). Generation Z showed a higher likelihood of consuming cookies due to habits, boredom, or stress compared to other generations (*Publication No. 3* - Figure 2b), consistent with findings by Priporas et al. (2022) and Čukelj et al. (2016), highlighting a link between habits and emotions. Kaylor et al. (2021) reported mixed attitudes towards eating habits among female Generation Z members, with some displaying signs of eating disorders. Additionally, Generation Z showed a lower eating discipline compared to previous generations (Durukan and Gul, 2019), with the recent COVID-19 pandemic increasing stress-related changes in this demographic (Sitoayu et al., 2019).

Publication No. 3 revealed that 86 % of the participants recognized the health benefits of regular whole grain consumption. French participants displayed a lower priority for cookie fiber content ($p < 0.001$), consistent with prior research highlighting insufficient fiber intake in France (Lairon et al., 2003). Additionally, Generation Y showed strong health-consciousness, especially regarding weight management. Significant differences were observed between Generation Z and other generations in fiber consumption ($p = 0.0002$) and intake of biologically active substances ($p < 0.001$), indicating potential gaps in the health education among Generation Z. This is in contrast with Su et al. (2019) findings, which reported effective health education among Generation Z individuals during their upbringing.

French participants scored lower on the dietary awareness index (0.67) than Croats (0.80) and North Macedonians (0.79). As expected, Generation Z showed the lowest level of nutritional awareness among the age groups, indicating a need for targeted health education.

Despite differences in country or generation, the majority of participants (84 %) demonstrated awareness regarding the importance of sustainable product development. However, there remains a lack of understanding regarding fruit by-products and edible films, leading to significant variations in sustainability knowledge scores across the different investigated countries (*Publication No. 3* – Figure 4a). These findings are in accordance with the conclusions of Cattaneo et al. (2018), highlighting that insufficient knowledge of food by-products contributes to a negative perception of their utilization. Additionally, Murillo et al. (2023) emphasized that familiarity prior consumption positively impacts the willingness to try food containing seafood by-products, while concerns related to sensory quality, safety, and nutrition serve as primary deterrents among consumers.

Across all demographics, only 29 % of participants showed immediate interest in acquiring novel, appealing cookies, with the majority displaying limited interest. Čukelj et al. (2016) observed a comparable trend, indicating that individuals with increased nutritional awareness were more willing to invest in functional cookies. These findings show the necessity for increased public education and promotion, particularly highlighting the benefits of whole grain products to stimulate whole grain consumption and enhance dietary fiber intake (Barrett, 2020). Regarding brand preference, French consumers tend to favor local brands or those they are familiar with, due to the association with higher quality (*Publication No. 3* – Figure 5). This corresponds with findings from a Statista survey (2021), which indicates that French consumers prioritize brands that emphasize products manufactured in France and support domestic manufacturers.

Besides the brand, the price was found to be an important factor across countries ($p < 0.001$) (*Publication No. 3* – Figure 5). Economic challenges such as recession and budget constraints are known drives towards affordability (Cette et al., 2017). Generation Y is demanding when it comes to brand selection, possibly favoring imports, while Generation Z is more open to trying new cookies ($p = 0.0199$) (*Supplementary* - Table S2 and Figure S2). Key drivers for their snack purchases include packaging, price, health information availability, product availability, and taste (Liew et al., 2023). Nevertheless, for Generation Z, higher prices were identified as a hurdle when purchasing cookies. French and Croatian consumers prioritized cookies with positive environmental impact (median test: chi-square = 7.291, $p = 0.026$) (*Supplementary* - Table S3, Figure S2), reflecting a broader preference for environmentally friendly food (Coderoni et al., 2020). However, the level of education was found to be a stronger predictor of environmentally friendly cookie purchases in contrast to the country of residence (*Publication No. 3* - Figure 6a).

Most participants demonstrated limited interest in purchasing cookies containing food by-products (*Supplementary* - Tables S2 and S3), potentially due to a lack of understanding, as reflected by the sustainability knowledge score, and due to an insufficient awareness of the health benefits associated with by-products. Residency and education significantly influenced the willingness to purchase cookies with food by-products (*Publication No. 3* - Figures 6b and Figure S2). French participants showed the highest interest in purchasing cookies containing by-products, while North Macedonians exhibited the least interest (median test: chi-square = 74.208, $p < 0.001$).

Yilmaz and Kahveci (2022) highlight that parameters including generation, gender, and household recycling habits play a central role in shaping a consumers attitude towards purchasing upcycled food. Consumers are more inclined to consider products incorporating by-products when they anticipate superior quality, enhanced taste, and perceive such products as contributing to addressing the issue of food waste.

The principal component loadings (PCA) analysis in *Publication No. 3* (Figure 7) confirmed significant differences between French and North Macedonian groups in various aspects, including their cookie preferences, the reasons for eating cookies, the tendency to prefer domestic or international brands, and the interest in buying cookies with added nutritional value. Moreover, it highlighted a noticeable distinction between Generation Y and Generation Z in regard to how they consume cookies (e.g. with coffee or as a snack), why they eat them, their intake of healthy compounds like bioactive substances and fiber, and how they read food labels.

Chapter 4

Conclusions

Given that cocoa powder is associated with increasing prices and the uncertainties regarding its production meeting the markets demand, coupled with Croatia's ample production of wine and aronia, substituting cocoa powder with by-products from grape- and aronia juice production not only supports reducing waste from juice manufacturing but also fosters the creation of functional and eco-friendly cereal products. Results from this dissertation demonstrate that grape- and aronia pomace can effectively substitute up to 24 % of cocoa powder in cookie formulations without compromising their sensory acceptability.

The edible films, containing chitosan and gum arabic enhanced with GSE, show favorable physico-chemical, morphological, and thermal characteristics. Incorporating GSE into the chitosan and gum arabic-based edible film resulted in a notable rise in total phenolic content and film thickness by 41 %, ensuring robust structural integrity without any adverse effects on appearance. While the addition of GSE led to reduced transparency by 92 %, it simultaneously bolstered the water vapor barrier by 30 %, all without inducing structural alterations at higher temperatures. These observations suggest that chitosan and gum arabic-based films infused with GSE are highly suitable for effective application, maintaining the quality standards of the intended product.

The edible film can be seamlessly integrated into cookies without compromising sensory properties or compromising the overall quality of the product. Based on texture and sensory evaluations, both an accelerated shelf-life test and a direct shelf-life method validated the safety and acceptable quality of the GAP with KGAE cookies after six months of storage. Nonetheless, for future studies, it is advisable to incorporate additional analyses such as measuring the anisidine value and conducting sensory evaluations for comprehensive assessment.

Fruit by-products and edible films had no adverse effect on the nutritional profile or *in vitro* starch digestibility of the cookies, despite the prevalence of SDS over RDS. Contrary, the addition of fruit by-products and edible films had a beneficial influence, increasing the flavonoid content by 22 % and the antioxidant potential by up to 73 % compared to the control cookies.

Moderate consumption of whole grain cookies containing fruit by-products did not result in the formation of oxLDL receptors in healthy women. However, a positive correlation between the concentration of oxLDL receptors and waist circumference was indicated. To validate the effectiveness of this potential preventive measure, further studies with a larger participant cohort and an extended intervention period are required.

Within Generation Z, nutritional awareness was found to be the lowest compared to other age groups, emphasizing the need for targeted health education initiatives aimed at this demographic group.

The study revealed clear differences in cookie preferences among participants: although chocolate-coated cookies remained popular overall, North Macedonian respondents favored whole grain and plain varieties. In contrast, the French showed a preference for chocolate-coated cookies, while Croats equally reported to enjoy both types.

Most consumers are familiar with sustainability concepts and express interest in purchasing cookies that promote both environmental welfare and personal health. However, their awareness of fruit by-products or edible films, as well as their willingness to purchase cookies containing food by-products, remains limited.

The insights garnered from the evaluation of market potential, nutritional awareness, and sustainability knowledge across Croatia, France, and North Macedonia will be crucial in formulating a marketing and education strategy aimed at effective communication of the benefits of utilizing upcycled ingredients in the production of nutritionally enriched food. Implementing these strategies holds the potential to enhance consumer acceptance of food products containing upcycled ingredients, thereby fostering the advancement of the circular economy.

The findings of this dissertation open up new possibilities in formulating innovative edible films. This research holds promise for the creation of novel cookie formulations with an extended shelf-life, offering unique qualities not found in existing cookies on the market. Moreover, these outcomes may offer valuable insights for the development of new products and the creation of marketing strategies aimed at promoting healthier and more sustainable cookie options.

Reference

AACC METHOD 10-50.05., 1999. Baking quality of cookie flour. Approved methods of analysis (11^{th} Ed.). American Association of Cereal Chemists AACC International St. Paul, MN, USA.

Acun, S., Gül, H., 2014. Effects of grape pomace and grape seed flours on cookie quality. Qual. Assur. Saf. Crop. Food. 6, 81–88. https://doi.org/10.3920/QAS2013.0264

Ahmed, A.A., Musa, H.H., Fedail, J.S., Sifaldin, A.Z., Musa, T.H., 2016. Gum arabic suppressed diet-induced obesity by alteration the expression of mRNA levels of genes involved in lipid metabolism in mouse liver. Bioact. Carbohydr. Diet Fibre. 7, 15–20. https://doi.org/10.1016/j.bcdf.2016.01.002

Ajila, C. M., Leelavathi, K., Prasada Rao U. J. S., 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. J. Cereal Sci. 48, 319-326. https://doi.org/10.1016/j.jcs.2007.10.001

Aksoylu, Z., Çağindi, Ö., Köse, E., 2015. Effects of blueberry, grape seed powder and poppy seed incorporation on physicochemical and sensory properties of biscuit. J. Food Qual. 38, 164–174. https://doi.org/10.1111/jfq.12133

Al-Jubori, Y., Ahmed, N.T.B., Albusaidi, R., Madden, J., Das, S., Sirasanagandla, S.R., 2023. The efficacy of gum arabic in managing diseases: A systematic review of evidence-based clinical trials. Biomolecules. 13(1), 138. https://doi.org/10.3390/biom13010138

Andújar, I., Recio, M.C., Giner, R.M., Ríos, J.L., 2012. Cocoa polyphenols and their potential benefits for human health. Oxidative Med. Cell. Longev. 2012, 906252. https://doi.org/10.1155/2012/906252

Apeda agriexchange (2020). Available online: Home: Agri Exchange (apeda.gov.in) on 25^{th} January 2024.

Arepally, D., Sudharshan, R.R., Goswami, T.K., Datta, A., 2020. Biscuit baking: A review. LWT. 131. 109726. https://doi.org/10.1016/j.lwt.2020.109726.

Ashour, M.A., Fatima, W., Imran, M., Ghoneim, M.M., Alshehri, S., Shakeel, F., 2022. A review on the main phytoconstituents, traditional uses, inventions, and patent literature of gum arabic emphasizing Acacia seyal. Molecules. 27, 1171. https://doi.org/10.3390/molecules27041171

Asp, N.G., Bjorck, I., 1992. Resistant starch. Trends Food Sci. Technol. 3, 111–114. https://doi.org/10.1016/0924-2244(92)90153-n

Baker, M.T., Lu, P., Parrella, J.A., Leggette, H.R., 2022. Consumer acceptance toward functional food: A scoping review. Int. J. Environ. Res. Public Health 19, 1217. https://doi.org/10.3390/ijerph19031217

Balbinoti, T.C.V., Stafussa, A.P., Haminiuk, C.W.I., Maciel, G.M., Sassaki, G.L., Jorge, L.M.D.M., Jorge R.M.M., 2020. Addition of grape pomace in the hydration step of parboiling increases the antioxidant properties of rice. Int. J. Food Sci. Technol. 55, 2370–2380. https://doi.org/10.1111/ijfs.14481

Barrett, E.M., Foster, S.I., Beck, E.J., 2020. Whole grain and high-fibre grain food: How do knowledge, perceptions and attitudes affect food choice? Appetite.149, 104630. https://doi.org/10.1016/j.appet.2020.104630

Bello-Perez, L.A., Flores-Silva, P.C., Agama-Acevedo, E-, Tovar, J., 2020. Starch digestibility: past, present, and future. J Sci Food Agric. 100(14), 5009-5016. https://doi.org/10.1002/jsfa.8955

Bender, A.B., Speroni, C.S., Salvador, P.R., Loureiro, B.B., Lovatto, N.M., Goulart, F.R., Lovatto, M.T., Miranda, M.Z., Silva, L.P., Penna, N.G., 2017. Grape pomace skins and the effects of its inclusion in the technological properties of muffins. J. Culin. Sci. Technol. 15, 143–157. https://doi.org/10.1080/15428052.2016.1225535

Benjamin, E.J., Muntner, P., Alonso, A., Bittencourt, M.S., Callaway, C.W., Carson, A.P., et al., 2019. Heart disease and stroke statistics-2019 update: a report from the American heart association. Circulation. 139 (10), e56–e528. https://doi.org/10.1161/CIR.0000000000000659

Bennato, F., Di Luca, A., Martino, C., Ianni, A., Marone, E., Grotta, L., Ramazzotti, S., Cichelli, A., Martino, G., 2020. influence of grape pomace intake on nutritional value, lipid oxidation and volatile profile of poultry meat. Food. 9, 508. https://doi.org/10.3390/food9040508.

Biscuit Market Outlook 2022-2026. Available online: Biscuit Market Outlook 2022 - 2026 (reportlinker.com). Accessed on 10th January 2024.

Blažanin, A., 2015. Inkapsulacija biljnih ekstrakata ionskim geliranjem emulzija. Final work. University of Zagreb. Faculty of Food Technology and Biotechnology, Zagreb, Croatia

Block, L.G., Grier, S.A., Childers, T.L., Davis, B., Ebert, J.E., Kumanyika, S., 2011. From nutrients to nurturance: A conceptual introduction to food well-being. J. Public Policy Mark. 30, 5–13. https://doi.org/10.1509/jppm.30.1.5

Bora, P., Ragaee, S., Abdel-Aal, E.S.M., 2019. Effect of incorporation of goji berry by-product on biochemical, physical and sensory properties of selected bakery products. Food Sci. Technol. 112, 108225. https://doi.org/10.1016/j.lwt.2019.05.123

Bordiga, M., Travaglia, F., Locatelli, M., 2019. Valorisation of grape pomace: An approach that is increasingly reaching its maturity—A review. Int. J. Food Sci. Technol. 54, 933–942. https://doi.org/10.1111/ijfs.14118

Borowska, S., Brzóska, M.M., 2016. Chokeberries (Aronia Melanocarpa) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. Compr. Rev. Food Sci. Food Saf. 15, 982–1017. https://doi.org/10.1111/1541-4337.12221

Brouwers, A., Langlois, M., Delanghe, J., Billiet, J., De Buyzere, M., Vercaemst, R., Rietzschel, E., Bernard, D., Blaton, V., 2004. Oxidized low-density lipoprotein, iron stores, and haptoglobin polymorphism. Atherosclerosis. 176, 189–195. https://doi.org/10.1161/HYPERTENSIONAHA.108.114439

Buerke, A., Straatmann, T., Lin-Hi, N., Müller, K., 2017. Consumer awareness and sustainability-focused value orientation as motivating factors of responsible consumer behavior. Rev. Manag. Sci.11, 959–991. https://doi.org/10.1007/s11846-016-0211-2

Buffière, C., Hiolle, M., Peyron, M.A., Richard, R., Meunier, N., Batisse, C., Rémond, D., Dupont, D., Nau, F., Pereira, B., Savary-Auzeloux, I. 2020. Food matrix structure (from biscuit to custard) has an impact on folate bioavailability in healthy volunteers. Eur. J. Nutr. 60, 411–423. https://doi.org/10.1007/s00394-020-02258-5

Büyükkaragöz, A., Baş, M., Sağlam, D., Cengiz, S.E., 2014. Consumers' awareness, acceptance and attitudes towards functional food in Turkey. Int. J. Consum. Stud. 38, 628–635. https://doi.org/10.1111/ijcs.12134

Cádiz-Gurrea, M.L., Borrás-Linares, I., Lozano-Sánchez, J., Joven, J., Fernández-Arroyo, S., Segura-Carretero, A., 2017. Cocoa and grape seed byproducts as a source of antioxidant and anti-inflammatory proanthocyanidins. Int. J. Mol. Sci. 18, 376. https://doi.org/10.3390/ijms18020376

Calame, W., Thomassen, F., Hull, S., Viebke, C., Siemensma, A.D., 2011. evaluation of satiety enhancement, including compensation, by blends of gum arabic. A Methodological Approach. Appetite. 57, 358–364. https://doi.org/10.1016/j.appet.2011.06.005

Canalis, M.S.B., Leon, A.E., Ribotta, P.D., 2017. Effect of inulin on dough and biscuit quality produced from different flours. Int. J. Food Stud. 6(1), 13–23. https://doi.org/10.7455/ijfs/6.1.2017.a2

Castro-Alayo, E.M., Idrogo-Vásquez, G., Siche, R., Cardenas-Toro, F.P., 2019. Formation of aromatic compounds precursors during fermentation of criollo and forastero cocoa. Heliyon. 5, e01157. https://doi.org/10.1016/j.heliyon.2019.e01157

Cattaneo, C., Lavelli, V., Proserpio, C., Laureati, M., Pagliarini, E., 2018. Consumers' attitude towards food by-products: The influence of food technology neophobia, education and information. Int. J. Food Sci. Technol. 54, 679–687. https://doi.org/10.1111/ijfs.13978

Cette, G., Corde, S., Lecat, R., 2017. Stagnation of productivity in France: A legacy of the crisis or a structural slowdown? Econ. Stat. 494–496, 11–36. https://doi.org/10.24187/ecostat.2017.494t.1916

Chatterjee, S., Sharma, S., 2019. Microplastics in our oceans and marine health. Field Actions Sci. Rep. 54-61.

Chen, X., Yang, H., Yan, N., 2016. Shell Biorefinery: Dream or Reality? Chem. Eur. J. 22, 13402-13421. https://doi.org/10.1002/chem.201602389

Chranioti, C., Tzia, C., 2014. Arabic gum mixtures as encapsulating agents of freezedried fennel oleoresin products. Food Bioproc. Tech. 7(4), 1057–106. https://doi.org/10.1007/s11947-013-1074-z

Cho, Y.W., Jang, J., Park, C.R., Ko, S.W., 2000. Preparation and solubility in acid and water of partially deacetylated chitins. Biomacromolecules. 1, 609–614. https://doi.org/10.1021/bm000036j

Cilli, L.P., Contini, L.R.F., Sinnecker, P., Lopes, P.S., Andreo, M.A., Neiva, C.R.P., Nascimento, M.S., Yoshida, C.M., Venturini, A.C., 2019. Effects of grape pomace flour on quality parameters of salmon burger. J. Food Process. Preserv. 44, e14329. https://doi.org/10.1111/jfpp.14329

Coderoni, S., Perito, M.A., 2020. Sustainable consumption in the circular economy. An analysis of consumers' purchase intentions for waste-to-value. Food. J. Clean. Prod. 252, 119870. https://doi.org/10.1016/j.jclepro.2019.119870

Codron, J. M., Siriex, L., Reardon, T., 2006. Social and environmental attributes of food products in an emerging mass market: Challenges of signaling and consumer perception, with European illustrations. Agric. Human Values 23(3), 283–297. https://doi.org/10.1007/s10460-006-9000-x

Comunian, T., Marluci, P.S., Clitor, F.S., 2021. The use of food by-products as a novel for functional food: Their use as ingredients and for the encapsulation process, Trends Food Sci. Technol. 108, 269-280. https://doi.org/10.1016/j.tifs.2021.01.003

Consumer Expectations for Brands in France for 2021. Available online: Consumer expectations for brands in France for 2021 | Statista Accessed on 20th August 2023.

Corredor, Z., Rodríguez-Ribera, L., Coll, E., Montañés, R., Diaz, J.M., Ballarin, J., Marcos, R., Pastor, S., 2016. Unfermented grape juice reduce genomic damage on patient undergoing hemodialysis. Food Chem. Toxicol. 92, 1–7. https://doi.org/10.1016/j.fct.2016.03.016

Crofton, E.C., Scannell, A.G., 2020. Snack food from brewing waste: Consumer-led approach to developing sustainable snack options. Br. Food J. 122, 3899–3916. https://doi.org/10.1108/BFJ-08-2018-0527

Čukelj, N., Putnik, P., Novotni, D., Ajredini, S., Voučko, B., Ćurić, D., 2016. Market potential of lignans and omega-3 functional cookies. Brit. Food J. 118, 2420–2433. https://doi.org/10.1108/BFJ-03-2016-0117

D'Amelio, P., Cristofaro, M.A., Tamone, C., Morra, E., Di Bella, S., Isaia, G., Grimaldi, A., Gennero, L., Gariboldi, A., Ponzetto, A., Pescarmona, G.P., Isaia, G.C. 2008. Role of iron metabolism and oxidative damage in postmenopausal bone loss. Bone. 43, 1010–1015. https://doi.org/10.1016/j.bone.2008.08.107

Daskalova, E., Delchev, S., Peeva, Y., Vladimirova-Kitova, L., Kratchanova, M., Kratchanov, C., Denev, P., 2015. Antiatherogenic and Cardioprotective Effects of Black Chokeberry (*Aronia Melanocarpa*) Juice in Aging Rats. Evid. Based Complement. Alternat. Med. 2015, e717439. https://doi.org/10.1155/2015/717439

De Moraes Crizel, T., de Oliviera Rios, A., Bandarra, N., Alves, V.D., 2018. Biodegradable films based on gelatin and papaya peel microparticles with antioxidant properties. Food Bioproc. Techn. 11, 536–550. https://doi.org/10.1007/s11947-017-2030-0

Dean, M., Shepherd, R., Arvola, A., Vassallo, M., Winkelmann, M., Claupein, E., Lähteenmäki, L., Raats, M.M., Saba, A., 2007. Consumer perceptions of healthy cereal products and production methods. J. Cereal Sci. 46, 188–196. https://doi.org/10.1016/j.jcs.2007.06.007

Defruyt, S., 2019. Towards a new plastics economy. Field Actions Science Reports. J. Field Actions. 78–81.

Demirkol, M., Tarakci, Z., 2018. Effect of grape (*Vitis labrusca* L.) pomace dried by different methods on physicochemical, microbiological and bioactive properties of yoghurt. LWT. 97, 770–777. https://doi.org/10.1016/j.lwt.2018.07.058

Denev, P., Číž, M., Kratchanova, M., Blazheva, D., 2019. Black chokeberry (Aronia melanocarpa) polyphenols reveal different antioxidant, antimicrobial and neutrophil-modulating activities. Food Chem. 284, 108-117. https://doi.org/10.1016/j.foodchem.2019.01.108

Desai, N., Rana, D., Salave, S., Gupta, R., Patel, P., Karunakaran, B., Sharma, A., Giri, J., Benival, D., Kommineni, N. 2023. Chitosan: A potential biopolymer in drug delivery and biomedical applications. Pharmaceuticdomards. 15(4),1313. http://doi.org/10.3390/pharmaceutics15041313

DiNicolantonio, J.J., Lucan, S.C., O'Keefe, J.H., 2016. The evidence for saturated fat and for sugar related to coronary heart disease. Prog. Cardiovasc. Dis. 58, 464–472. https://doi.org/10.1016/j.pcad.2015.11.006

Durukan, A., Gül, A., 2019. Mindful eating: Differences of generations and relationship of mindful eating with BMI. Int. J. Gastron. Food Sci. 18, 100172. https://doi.org/10.1016/j.ijgfs.2019.100172

Doaré, F., Ribeyre, F., Cilas, C., 2020. Genetic and environmental links between traits of cocoa beans and pods clarify the phenotyping processes to be implemented. Sci Rep. 10 (1), 9888. https://doi.org/ 10.1038/s41598-020-66969-9

Dutta, P.K., Tripathi, S., Mehrotra, G.K., Dutta, J., 2009. Perspectives for chitosan based antimicrobial films in food application. Food Chem. 114(4), 1173-1182. https://doi.org/10.1016/j.foodchem.2008.11.047

Dwyer, K., Hosseinian, F., Rod, M.R., 2014. The market potential of grape waste alternatives. J. Food Res. 3, 91. https://doi.org/10.5539/jfr.v3n2p91

Eltoum, Y. A., Babiker, E.E., 2014. Changes in antioxidant content, rehydration ratio and browning index during storage of edible surface coated and dehydrated tomato slices. J. Food Process. Preserv. 38, 1135–1144. https://doi.org/10.1111/jfpp.12073

Englyst, H.N., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr. 46, S33-50.

Eltoum, Y. A., Babiker, E.E., 2014. Changes in antioxidant content, rehydration ratio and browning index during storage of edible surface coated and dehydrated tomato slices. J. Food Process. Preserv. 38, 1135–1144. https://doi.org/10.1111/jfpp.12073

Englyst, H.N., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr. 46, S33-50.

Espinosa - Andrews, H., Baez - Gonzalez, J.G., Cruz - Sosa, F., Vernon - Carter, E.J., 2007. Gum Arabic-chitosan complex coacervation. Biomacromolecules. 8(4), 1313–1318. https://doi.org/10.1021/bm0611634

EU Directive 2019/904 of the European parliament and of the council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment. EU- European Union. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019L0904. Accessed on 14th October 2024.

European Commission (EC), 2022. Sustainable cocoa Initiative. Available online: The Sustainable Cocoa Initiative - European Commission (europa.eu) Accessed on 18th January 2024.

Ferreira A.R., Alves V.D., Coelhoso I.M., 2016. Polysaccharide-based membranes in food packaging applications. Membranes. 6, 22. https://doi.org/10.3390/membranes6020022.

Florence, S.P., Asna, U., Asha, M.R., Jyotona, R., 2014. Sensory, physical and nutritional qualities of cookies prepared from pearl millet (Opennisetum typhoideum). Food Process. Technol. 5, 377. https://doi.org/10.4172/2157-7110.1000377

Fontana, M., Murowaniecki Otero, D., Pereira, A.M., Santos, R.B., Gularte, M.A., 2022. Grape pomace flour for incorporation into cookies: Evaluation of nutritional, sensory and technological characteristics. J. Culin. Sci. Technol. 1–20. https://doi.org/10.1080/15428052.2022.2086956

Galanakis, C.M., 2017. Handbook of grape processing by-products sustainable solutions, Academic press, London.

Galić, K., Curić, D., Gabrić, D., 2009. Shelf-life of packaged bakery goods - a review. Critical Reviews in Food Science and Nutrition. 49(5), 405–426. https://doi.org/10.1080/10408390802067878

Ganglmair-Wooliscroft, A., Wooliscroft, B., 2022. An investigation of sustainable consumption behavior systems – Exploring personal and socio-structural characteristics in different national contexts. J. Bus. Res. 148, 161-173. https://doi.org/10.1016/j.jbusres.2022.04.049

Garcia, M.V., Milani, M.S., Ries, E.F., 2020. Production optimization of passion fruit peel flour and its incorporation into dietary food. Food Sci. Technol. Int. 26, 132–139. https://doi.org/10.1177/1082013219870011

García-Lomillo, J., González-SanJosé, M.L., 2017. Applications of wine pomace in the food industry: Approaches and functions. Compr. Rev. Food Sci. Food Saf. 16, 3–22. https://doi.org/10.1111/1541-4337.12238

Garrido, M.D., Auqui, M., Martí, N., Linares, M.B., 2011. Effect of two different red grape pomace extracts obtained under different extraction systems on meat quality of pork burgers. LWT-Food Sci. Technol. 44, 2238–2243. https://doi.org/10.1016/j.lwt.2011.07.003

Garsetti, M., Vinoy, S., Lang, V., Holt, S., Loyer, S., Brand-Miller, J.C., 2005. The glycemic and insulinemic index of plain sweet biscuits: Relationships to in vitro starch digestibility. J. Am. Coll. Nutr. 24, 441–447. https://doi.org/10.1080/07315724.2005.10719489

Ghormade, V., Pathan, E.K., Deshpande, M.V., 2017. Can fungi compete with marine sources for chitosan production? Int. J. Biol. Macromol. 104, 1415–1421. https://doi.org/10.1016/j.ijbiomac.2017.01.112

Gower, B.A., Bergman, R., Stefanovski, D., Darnell, B., Ovalle, F., Fisher, G., et al., 2016. Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women:

a randomized, controlled trial. Nutr. Metab. (London) 13, 2-8. https://doi.org/10.1186/s12986-016-0062-5

Gupta, M., Dey, S., Marbaniang, D., Pal, P., Ray, S., Mazumder, B., 2020. Grape seed extract: having a potential health benefit. J. Food Sci. Technol. 57(4), 1205–1215. https://doi.org/10.1007/s13197-019-04113-w

Hani Tabaie Zavareh, S.A., Ardestani, F., 2020. Antibacterial effects of chitosan coating containing *Mentha aquatica* L. essence against *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* in Iranian white cheese. Int. J. Dairy Technol. 73, 585–593. https://doi.org/10.1111/1471-0307.12678

Harugade, A., Sherje, A.P., Pethe, A., 2023. Chitosan: A review on properties, biological activities and recent progress in biomedical applications. React. Funct. Polym. 191, 105634.

Ibrahim, I.D., Hamam, Y., Sadiku, E.R., Ndambuki, J.M., Kupolati, W.K., Jamiru, T., Eze, A.A., Snyman, J. 2022. Need for Sustainable Packaging: An Overview. Polymers (Basel). 14(20), 4430. https://doi.org/10.3390/polym14204430

Jakovljevic, V., Milic, P., Bradic, J., Jeremic, J., Zivkovic, V., Srejovic, I., Nikolic Turnic, T., Milosavljevic, I., Jeremic, N., Bolevich, S., et al., 2018. Standardized Aronia melanocarpa extract as novel supplement against metabolic syndrome: A rat model. Int. J. Mol. Sci. 20, 6. https://doi.org/10.3390/ijms20010006

Jurendić, T., Ščetar, M., 2021. Aronia melanocarpa products and by-products for health and nutrition: A review. Antioxidants 10(7), 1052. https://doi.org/10.3390/antiox10071052

Jurikova, T., Mlcek, J., Skrovankova, S., Sumczynski, D., Sochor, J., Hlavacova, I., Snopek, L., Orsavová, J., 2017. Fruits of Black Chokeberry Aronia Melanocarpa in the Prevention of Chronic Diseases. Molecules. 22, 944. https://doi.org/10.3390/molecules22060944

Karagöz, Ş., Demirdöven, A., 2019. Effect of chitosan coatings with and without *Stevia rebaudiana* and modified atmosphere packaging on quality of cold stored fresh-cut apples. *LWT* Food Sci. Technol. 108, 332–337. https://doi.org/10.1016/j.lwt.2019.03.040

Kardum, N., Petrović-Oggiano, G., Takic, M., Glibetić, N., Zec, M., Debeljak-Martacic, J., Konić-Ristić, A., 2014. Effects of glucomannan-enriched, aronia juice-based supplement on cellular antioxidant enzymes and membrane lipid status in subjects with abdominal obesity. Sci. World J. 869250. https://doi.org/10.1155/2014/869250.

Karnopp, A.R., Figueroa, A.M., Los, P.R., Teles, J.C., Simões, D.R.S., Barana, A.C., Kubiaki, F.T., de Oliveiea, J.G.B., Granato, D., 2015. Effects of whole-wheat flour and bordeaux grape pomace (Vitis labrusca L.) on the sensory, physicochemical and functional properties of cookies. Cienc. Technol. Aliment. 35(4), 750-756. https://doi.org/10.1590/1678-457X.0010

Kasprzak-Drozd, K, Oniszczuk, T, Soja, J, Gancarz, M, Wojtunik-Kulesza, K, Markut-Miotła, E, Oniszczuk, A., 2021. The efficacy of black chokeberry fruits against cardiovascular diseases. Int. J. Mol. Sci. 22(12), 6541. https://doi.org/10.3390/ijms22126541

Kasprzak-Drozd, K, Oniszczuk, T, Soja, J, Gancarz, M, Wojtunik-Kulesza, K, Markut-Miotła, E, Oniszczuk, A., 2021. The efficacy of black chokeberry fruits against cardiovascular diseases. Int. J. Mol. Sci. 22(12), 6541. https://doi.org/10.3390/ijms22126541

Kautish, P., Paço, A., Thaichon, P., 2022. Sustainable consumption and plastic packaging: Relationships among product involvement, perceived marketplace influence and choice behavior. J. Retail. Consum. Serv. 67, 103032. https://doi.org/10.1016/j.jretconser.2022.103032

Kaylor, S.K., Allen, I., Crim, A.D., Callihan, M.L., 2021. Calories and control: Eating habits, behaviors, and motivations of Generation Z females. J. Am. Coll. Health. 25, 1–9. https://doi.org/10.1080/07448481.2021.1978465

Ke, C.L., Deng, F.S., Chuang, C.Y., Lin, C.H., 2021. Antimicrobial actions and applications of Chitosan. Polymers. 13, 904. https://doi.org/10.3390/ polym13060904

Kekalih, A., Sagung, I.O.A.A., Fahmida, U., Ermayani, E., Mansyur, M.A., 2019. A multicentre randomized controlled trial of food supplement intervention for wasting children in Indonesia-study protocol. BMC Public Health. 19, 305. https://doi.org/10.1186/s12889-019-6608-5

Kim, S.S., Shin, Y., 2020. Antibacterial and in vitro antidementia effects of aronia (Aronia Melanocarpa) leaf extracts. Food Sci. Biotechnol. 29, 1295–1300. https://doi.org/10.1007/s10068-020-00774-y

Kim, S.S., Shin, Y., 2020. Antibacterial and in vitro antidementia effects of aronia (Aronia Melanocarpa) leaf extracts. Food Sci. Biotechnol. 29, 1295–1300. https://doi.org/10.1007/s10068-020-00774-y

Kong, P., Cui, Z. Y., Huang, X. F., Zhang, D.D., Guo, R.J., Han, M., 2022. Inflammation and atherosclerosis: Signaling pathways and therapeutic intervention. Signal Transduct. Target. Ther. 7, 131. https://doi.org/10.1038/s41392-022-00955-7

Kraus, A., 2015. Development of functional food with the participation of the consumer. Motivators for consumption of functional products. Int. J. Consum. Stud. 39, 2–11. https://doi.org/10.1111/ijcs.12144

Kuchtova, V., Karovičova, J., Kohajdova, Z., Minarovičova, L., Kimličkova, V., 2016, Effects of white grape preparation on sensory quality of cookies. Acta. Chim. Slov. 9(2), 84-88. https://doi.org/10.1515/acs-2016-0014

Kulling, S.E., Rawel, H., 2008. Chokeberry (Aronia Melanocarpa)—A Review on the Characteristic Components and Potential Health Effects. Planta Med. 74, 1625–1634. https://doi.org/10.1055/s-0028-1088306

Lairon, D., Bertrais, S., Vincent, S., Arnault, N., Galan, P., Boutron, M.C., Hercberg, S., 2003. Dietary fibre intake and clinical indices in the French Supplementation en Vitamines et Minéraux AntioXydants (SU.VI.MAX) adult cohort. Proc. Nutr. Soc. 62, 11–15. https://doi.org/10.1079/PNS2002210

Laroche, M., Bergeron, J., Barbaro-Forleo, G., 2001. Targeting consumers who are willing to pay more for environmentally friendly products. J. Consum. Mark. 18(6), 503–520. https://doi.org/10.1108/EUM0000000006155

Lee, B.H., Bello-Pérez, L.A., Lin, A.H.M, Kim, C.Y., Hamaker, B.R., 2013. Importance of location of digestion and colonic fermentation of starch related to its quality. Cereal Chem. 90, 335- 343. https://doi.org/10.1094/CCHEM-05-13-0095-FI

Lee, H.J., Lee, J.J., Jung, M.O., Choi, J.S., Jung, J.T., Choi, Y.I., Lee, J.K., 2017. Meat Quality and Storage Characteristics of Pork Loin Marinated in Grape Pomace. Korean J. Food Sci. Anim. Resour. 37, 726. https://doi.org/10.5851/kosfa.2017.37.5.726

Lee, J., Cooke, J.P., 2011. The role of nicotine in the pathogenesis of atherosclerosis. Atherosclerosis. 215 (2), 281–283. https://doi.org/10.1016/j.atherosclerosis.2011.01.00

Leiva, E., Mujica, V., Sepúlveda, P., Guzmán, L., Núñez, S., Orrego, R., Palomo, I., Andrews, M., Arredondo, M.A., 2013. High levels of iron status and oxidative stress in patients with metabolic syndrome. Biol. Trace Elem. Res. 151, 1–8. https://doi.org/10.1007/s12011-012-9525-3

Leiva, E., Mujica, V., Sepúlveda, P., Guzmán, L., Núñez, S., Orrego, R., Palomo, I., Andrews, M., Arredondo, M.A., 2013. High levels of iron status and oxidative stress in patients with metabolic syndrome. Biol. Trace Elem. Res. 151, 1–8. https://doi.org/10.1007/s12011-012-9525-3

Leopold, J.A., Loscalzo, J., 2008. Oxidative mechanisms and atherothrombotic cardiovascular disease. Drug Discov. 5, 5–13. https://doi.org/10.1016/j.ddstr.2008.02.001

Lesjak, M., Hoque, R., Balesaria, S., Skinner, V., Debnam, E.S., Srai, S.K.S., Sharp, P.A., 2014. Quercetin inhibits intestinal iron absorption and ferroprotein transporter expression in vivo and in vitro. PLoS ONE. 9, e102900. https://doi.org/10.1371/journal.pone.0102900

Libby, P., Buring, J. E., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., Tokgözoğlu, L., Lewis, F.E., 2019. Atherosclerosis. Nat. Rev. Dis. Prim. 5 (1), 56. https://doi.org/10.1038/s41572-019-0106-z

Liew, J.Y., Ng, K.H., Aziz, N.S.B.A., 2023. Antecedents of junk food purchase intention among generation Z: A case study of Kota Bharu, Kelantan. AIP Conf. Proc. 2500, 020042. https://doi.org/10.1063/5.0110553

Lin, P., Ji, H. H., Li, Y.J., Guo, S.D., 2021. Macrophage plasticity and atherosclerosis therapy. Front. Mol. Biosci. 8, 679797. https://doi.org/10.3389/fmolb.2021.679797

Lindeboom, N., Chang, P.R., Tyler, R.T., 2004. Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. Starch/Stärke, 56, 89 – 99. https://doi.org/10.1002/star.200300218

Lipińska, P., Jóźwik, A., 2018. Hepatoprotective, Hypoglycemic, and Hypolipidemic Effect of Chokeberry Pomace on Polish Merino Lambs. Anim. Biotechnol. 29, 136–141. https://doi.org/10.1080/10495398.2017.1330209

Lockyer, S., Nugent, A.P., 2017. Health effects of resistant starch. Nutr. Bull. 42, 10- 41. https://doi.org/10.1111/nbu.12244

Lopez-Torrez, L., Costalat, M., Williams, P., Doco, T., Sanchez, C., 2015. Acacia Senegal vs. Acacia seyal gums – Part 1: Composition and structure of hyperbranched plant exudates. Food Hydrocoll. 51, 41–53. https://doi.org/10.1016/j.foodhyd.2015.04.019

Loullis, A., Pinakoulaki, E., 2018. Carob as cocoa substitute: a review on composition, health benefits and food applications. Eur. Food Res. Technol. 244, 959–977. https://doi.org/10.1007/s00217-017-3018-8

Lu, L.X., Xu, F., 2010. Effects of initial oxygen concentration and oxygen-barrier property of film on fat oxidation rate of packed cookies. Eur. Food Res. Technol. 231, 347–351. https://doi.org/10.1007/s00217-010-1282-y

MacArthur, E., Waughray, D., Stuchtey, M. 2016. Rethinking Plastics, Starting with Packaging. World Economic Forum; Cologne, Switzerland: pp. 1–206.

Mahdavi, V., Hosseini, S.E., Sharifan, A.,2018. Effect of edible chitosan film enriched with anise (*Pimpinella anisum* L.) essential oil on shelf-life and quality of the chicken burger. Food Sci. Nutr. 6, 269–279. https://doi.org/10.1002/fsn3.544

Malathi, A., Santhosh, K., Nidoni, U., 2014. Recent trends of biodegradable polymer: Biodegradable films for food packaging and application of nanotechnology in biodegradable food packaging. Curr. Trends Technol. Sci. 3, 73–79.

Mamat, H., Hill, S.E., 2018. Structural and functional properties of major ingredients of biscuit. Int. Food Res. J. 25(2), 462-471.

Manley, D. (2000) Technology of biscuits, crackers and cookies. 3rd ed., Woodhead Publishing Limited, Cambridge.

Marchiani, R., Bertolino, M., Ghirardello, D., McSweeney, P.L., Zeppa, G., 2016. Physicochemical and nutritional qualities of grape pomace powder-fortified semi-hard cheeses. J. Food Sci. Technol. 53, 1585–1596. https://doi.org/10.1007/s13197-015-2105-8

Mariod, A.A., 2018. Gum Arabic: Structure, properties, application and economics. Academic Press, London.

Mayer-Miebach, E., Adamiuk, M., Behsnilian, D., 2012. Stability of Chokeberry Bioactive Polyphenols during Juice Processing and Stabilization of a Polyphenol-Rich Material from the By-Product. Agriculture. 2, 244–258. https://doi.org/10.3390/agriculture2030244

Mendes, A.C., Pedersen, G.A. 2021. Perspectives on sustainable food packaging: is bio-based plastics a solution? Trends in Food Sci. Technol. 112, 839–846. https://doi.org/10.1016/j.tifs.2021.03.049

Merlo, T.C., Contreras-Castillo, C.J., Saldaña, E., Barancelli, G.V., Dargelio, M.D.B., Yoshida, C.M.P., Ribeiro Junior, E.E., Massarioli, A., Venturini, A.C., 2019. Incorporation of pink pepper residue extract into chitosan film combined with a modified atmosphere packaging: Effects on the shelf-life of salmon fillets. Food Res. Int. 125, 108633. https://doi.org/10.1016/j.foodres.2019.108633

Mildner-Szkudlarz, S., Bajerska, J., Zawirska-Wojtasiak, R., Górecka, D., 2013. White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. J. Sci. Food Agric. 93, 389–395 https://doi.org/10.1002/jsfa.5774

Moghadam, M., Salami, M., Mohammadian, M., Khodadadi, M., Emam – Djomeh, Z., 2020. Development of antioxidant edible films based on mung bean protein enriched with pomegranate peel. Food Hydrocoll. 104, 105735. https:// doi.org/10.1016/j.foodhyd.2020.105735

Mohamed, R.E., Gadour, M.O., Adam, I., 2015. The lowering effect of Gum Arabic on hyperlipidemia in Sudanese patients. Front. Physiol. 6, 160. https://doi.org/10.3389/fphys.2015.00160

Molnar, D., Gabaj, N.N., Vujić, L., Ščetar, M., Krisch, J., Miler, M., Štefanović, M., Novotni, D., 2023. Application of fruit by-products and edible film to cookies: Antioxidant activity and concentration of oxidized LDL receptor in women—A first approach. Appl. Sci. 13, 5513. https://doi.org/10.3390/app13095513

Molnar, D., Novotni, D., Krisch, J., Bosiljkov, T., Ščetar, M., 2020. The optimisation of cookie formulation with grape and aronia pomace powders as cocoa substitutes. Croat. J. Food Technol. Biotechnol. Nutr. 15, 38–44. https:// doi.org/10.31895/hcptbn.15.1-2.5

Murillo, S., Ardoin, R., Prinyawiwatkul, W., 2023. Factors Influencing Consumers' Willingness-to-Try Seafood Byproducts. Food.12, 1313. https://doi.org/10.3390/food12061313

Murmu, S.B., Mishra H.N., 2018. The effect of edible coating based on Arabic gum, sodium caseinate and essential oil of cinnamon and lemon grass on guava. Food Chem. 245, 820-828. https://doi.org/10.1016/j.foodchem.2017.11.104

Muthmainnah, N., 2019. Postharvest application of an edible coating based on chitosan and Gum Arabic for controlling respiration rate and vitamin C content of chill (*Capsicum frustecens L.).* In: Proceedings of the IOP conference series: Materials science and engineering, pp. 633.

Narchi, I., Walrand, S., Boirie, Y., Rousset, S., 2008. Emotions generated by food in elderly French people. J. Nutr. Health Aging. 12, 626–633. DOI: 10.1007/BF03008273

Naruszewicz, M., Łaniewska, I., Millo, B., Dłuzniewski, M., 2007. Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infraction (MI). Atherosclerosis. 194, e179–e184. https://doi.org/10.1016/j.atherosclerosis.2006.12.032

Nguyen, A.T., Parker, L., Brennan, L., Lockrey, S., 2020. A consumer definition of eco-friendly packaging. J. Clean. Prod. 252, 119792. https://doi.org/10.1016/j.jclepro.2019.119792

Niki, E. (2009). Lipid peroxidation: Physiological levels and dual biological effects. Free Radic. Biol. Med. 47, 469–484. https://doi.org/10.1016/j.freeradbiomed.2009.05.032

Nowak, D., Grąbczewska, Z., Gośliński, M., Obońska, K., Dąbrowska, A., Kubica, J., 2016. Effect of chokeberry juice consumption on antioxidant capacity, lipids profile and endothelial function in healthy people: A Pilot Study. Czech J. Food Sci. 34, 39–46. https://doi.org/10.17221/258/2015-CJFS.

Nystrand, B.T., Olsen, S.O., 2020. Consumers' attitudes and intentions toward consuming functional food in Norway. Food Qual. Prefer. 2020, 80, 103827. https://doi.org/10.1016/j.foodqual.2019.103827

Obermayer, G., Afonyushkin, T., Binder, C.J., 2018. Oxidized low-density lipoprotein in inflammation-driven thrombosis. J. Thromb. 16, 418–428. https://doi.org/10.1111/jth.13925

Ochmian, I.D., Grajkowski, J., Smolik, M., 2012. Comparison of Some Morphological Features, Quality and Chemical Content of Four Cultivars of Chokeberry Fruits (Aronia Melanocarpa). Not. Bot. Horti Agrobot. Cluj-Napoca. 40, 253. https://doi.org/10.15835/nbha4017181

OIV: Organisation Internationale de la vigne et du vin Global Economic Vitiviniculture Data, 2017. Statistics | OIV Accessed on 10th January 2019.

Oliveira Filho, J.G., Borges de Deus, I.P., Fernandes Valadares, A.C., Fernandes, C.C., Borges Estevam, E.B., Egea, M.G., 2020. Chitosan film with citrus LimoniaEssentil oil: Physical and morphological properties and antibacterial activity. Colloids Interfaces. 4(2), 18. https://doi.org/10.3390/colloids4020018

Ortega-Heras M., Gómez I., de Pablos-Alcalde S., González-Sanjosé M.L., 2019. Application of the Just-About-Right Scales in the Development of New Healthy Whole-Wheat Muffins by the Addition of a Product Obtained from White and Red Grape Pomace. Food. 8, 419. https://doi.org/10.3390/food8090419

Oszmiański, J., Wojdylo, A., 2005. Aronia melanocarpa phenolics and their antioxidant activity. Eur Food Res. Technol. 221, 809–813. https://doi.org/10.1007/s00217-005-0002-5

Otoni, C.G., Avena-Bustillos, R.J., Azeredo, H.M.C., Lorevice, M.V., Moura, M.R., Mattoso, L.H.C, Mchugh, T.H., 2017. Recent advances on edible films based on fruits and vegetables-A review. CRFSFS. 16, 1151-1169. https://doi.org/10.1111/1541-4337.12281

Pal, M., Devrani, M., Hadush, A., 2019. Recent developments in food packaging technologies. Beverage Food World. 46, 21–25.

Palocci, C.S., Chronopoulou, L., 2015. Fruit and Pomace Extracts: BiologicalActivity, Potential Applications and Benefical Health Effects: *Supercritical fluid extraction of pharmaceutic compounds from waste materials derived from vinification processes.* Nova Science Publishers, New York, str. 69-79.

Park, Y.K., Lee, S.H., Park, E., Kim, J.S., Kang, M.H., 2009. Changes in antioxidant status, blood pressure, and lymphocyte DNA damage from grape juice supplementation. Ann. N. Y. Acad. Sci. 1171, 385–390. https://doi.org/10.1111/j.1749-6632.2009.04907.x

Park, S., Kim, J.I., Lee, I., Lee, S., Hwang, M.-W., Bae, J.-Y., Heo, J., Kim, D., Han, S.-Z., Park, M.S., 2013. Aronia Melanocarpa and its components demonstrate antiviral activity against influenza viruses. Biochem. Biophys. Res. Commun. 440, 14–19. https://doi.org/10.1016/j.bbrc.2013.08.090

Park, H., Liu, Y., Kim, H.S., Shin, J.H., 2016. Chokeberry attenuates the expression of genes related to de novo lipogenesis in the hepatocytes of mice with nonalcoholic fatty liver disease. Nutr. Res. 36, 57–64. https://doi.org/10.1016/j.nutres.2015.10.010

Paucar-Menacho, L.M., Simpalo-López, W.D., Castillo-Martínez, W.E., Esquivel-Paredes, L.J., Martínez-Villaluenga, C., 2022. Improving nutritional and health benefits of biscuits by optimizing formulations based on sprouted pseudocereal grains. Food. 11(11), 1533. https://doi.org/10.3390/food11111533

Pavlović, A.N., Brcanović, J.M., Veljković, J.N., Mitić, S.S., Tošić, S.B., Kaličanin, B.M., Kostić, D.A., Đordević, M.S., Velimirović, D.S., 2015. Characterization of commercially available products of aronia according to their metal content. Fruits 70, 385–393. https://doi.org/10.1051/fruits/2015038

Plastic Europe, 2015. Plastics the Facts 2014/2015: An Analysis of European Plastics Production, Demand and Waste Data. Plastic Europe; Brussels, Belgium.

Pokimica, B., García-Conesa, M.T., Zec, M., Debeljak-Martačić, J., Ranković, S., Vidović, N., Petrović-Oggiano, G., Konić-Ristić, A., Glibetić, M., 2019. Chokeberry juice containing polyphenols does not affect cholesterol or blood pressure but modifies the composition of plasma phospholipids fatty acids in individuals at cardiovascular risk. Nutrients. 11, 850. https://doi.org/10.3390/nu11040850

Poznyak, V.A., Nikiforov, G.N., Markin, M.A., Kashirskikh, A.D., Myasoedova, A.V., Gerasimova, V.E., Orekhov, N.V., 2021. Overview of oxLDL and its impact on cardiovascular health: Focus on atherosclerosis. Front. Pharmacol. 11, 613780. https://doi.org/10.3389/fphar.2020.613780

Pradeep, A.R., Agarwal, E., Bajaj, P., Naik, S.B., Shanbhag, N., Uma, S.R., 2012. Clinical and microbiologic effects of commercially available gel and powder containing Acacia arabica on gingivitis. Aust. Dent. J. 57, 312–318. https://doi.org/10.1111/j.1834-7819.2012.01714.x

Official Gazette on biscuits and cookies related products (2005) Narodne novine 73, Zagreb.

Priporas, C.V., Vellore-Nagarajan, D., Kamenidou, I., 2022. Stressful eating indulgence by generation Z: A cognitive conceptual framework of new age consumers' obesity. Eur. J. Mark. 56, 2978–3006. https://doi.org/10.1108/EJM-06-2021-0386

Prokopov, T., Goranova, Z., Baeva, M., Slavov, A., 2015. Effects of powder from white cabbage outer leaves on sponge cake quality. Int. Agrophys. 29(4), 493–500. https://doi.org/10.1515/intag-2015-0055

Puerta, P., Laguna, L., Tárrega, A., Carrillo, E., 2022a. Relevant elements on biscuits purchasing decision for coeliac children and their parents in a supermarket context. Food Qual. Prefer. 98, 104496. https://doi.org/10.1016/j.foodqual.2021.104496

Puerta, P., Carrillo, E., Badia-Olmos, C., Laguna, L., Rosell, C.M., 2022b. Tárrega, A. Coeliac consumers' expectations and eye fixations on commercial gluten-free bread packages. LWT-Food Sci. Technol. 163, 113622. https://doi.org/10.1016/j.lwt.2022.113622

Putro, J.N., Lunardi, V.B., Soetaredjo, F.E., Yuliana, M., Santoso, S.P., Wenten, I.G., Ismadji, S. A., 2021. Review of Gum Hydrocolloid Polyelectrolyte Complexes (PEC) for Biomedical Applications: Their Properties and Drug Delivery Studies. *Processes*. *9*, 1796. https://doi.org/10.3390/pr9101796

Raafat, D., Sahl, H.G., 2009. Chitosan and its antimicrobial potential—A critical literature survey. Microb. Biotechnol. 2, 186–201. https://doi.org/10.1111/j.1751-7915.2008.00080.x

Raczkowska, E, Nowicka, P, Wojdyło, A, Styczyńska, M, Lazar, Z., 2022. Chokeberry pomace as a component shaping the content of bioactive compounds and nutritional, health-promoting (anti-diabetic and antioxidant) and sensory properties of shortcrust pastries sweetened with sucrose and erythritol. Antioxidants (Basel). 11(2), 190. https://doi.org/10.3390/antiox11020190

Rani, V., Sangwan, V., Rani, V., Malik, P., 2020. Orange peel powder: A potent source of fiber and antioxidants for functional biscuits. Int. J. Curr. Microbiol. Appl. Sci. 9, 1319–1325. https://doi.org/10.20546/ijcmas.2020.909.167

Rao, M. S., Kanatt, S. R., Chawla, S. P., Sharma, A., 2010. Chitosan and guar gum composite films: Preparation, physical, mechanical and antimicrobial properties. Carbohydr. Polym. 82(4), 1243–1247. https://doi.org/10.1016/j.carbpol.2010.06.058

Rasines-Perea, Z., Teissedre, P.L., 2017. Grape polyphenols' effects in human cardiovascular diseases and diabetes. *Molecules. 22*, 68. https://doi.org/10.3390/molecules22010068

Reichert, C.L., Bugnicourt, E., Coltelli, M.B., Cinelli, P., Lazzeri, A., Canesi, I., Braca, F., Martínez, B.M., Alonso, R., Agostinis, L., Verstichel, S., Six, L., De Mets, S., Gomez, E.C., Ißbrücker, C., Geerinck, R., Nettleton, F.D., Campos, I., Sauter, E., Pieczyk, P., Schmid, M., 2020. Bio-based packaging: Materials, modifications, industrial applications and sustainability. Polymers. 12, 1558. https://doi.org/10.3390/polym12071558

Riaz, A., Lei, S., Akhtar, H.M.S., Wan, P., Chen, D., Jabbar, S., Abid, M., Hashim, M.M., Zeng, X., 2018. Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols. Int. J. Biol. Macromol. 114, 547–555. https://doi.org/10.1016/j. ijbiomac.2018.03.126

Rikon, S.M., del Valle, F., 1982. imitation cocoa powder and method of preparing same. U.S. patent 4,335,153.

Rinsky, L.H., Rinsky, G., 2009. The Pastry Chef's Companion: A Comprehensive Resource Guide for the Baking and Pastry Professional, John Wiley and Sons, Chichester, pp. 1-134.

Ríos-Covian, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de los Reyes-Gavilan, C.G., Salazar, G., 2016. Intestinal short chain fatty acids and their link with diet and human health. Front. Microbol. 7, 1-9. https://doi.org/10.3389/fmicb.2016.00185

Ryu, K.S., Shim, K.S., Shin, D., 2014. Effect of grape pomace powder addition on TBARS and color of cooked pork sausages during storage. Korean J. Food Sci. Anim. Resour. 34, 200. https://doi.org/10.5851/kosfa.2014.34.2.200

Salah, S., Salih, A., Sabir, O.M., Mshelbwala, M., Gadour, M.O.E.H., 2012. Gum Arabic a superb anti-diarrheal agent. Sudan J. Med. Sci. 7, 83–88.

Sakloetsakun, D., Preechagoon, D., Bernkop-Schnùrch, A., Pongjanyakul, T., 2015. Chitosan-gum Arabic polyelectrolyte complex films: Physicochemical, mehanical and mucoadhesive properties. Pharm. Dev.Technol 1-10. https://doi.org/10.3109/10837450.2015.1035727

Savelli, E., Murmura, F., 2023. The intention to consume healthy food among older Gen-Z: Examining antecedents and mediators. Food Qual. Prefer. 105, 104788. https://doi.org/10.1016/j.foodqual.2022.104788

Sayago-Ayerdi, S., García-Martínez, D.L., Ramírez-Castillo, A.C., Ramírez-Concepción, H.R., Viuda-Martos, M., 2021. Tropical fruits and their co-products as bioactive compounds and their health effects: A review. Food. 10, 1952. https://doi.org/10.3390/food10081952

Selani, M.M., Contreras-Castillo, C.J., Shirahigue, L.D., Gallo, C.R., Plata-Oviedo, M., Montes-Villanueva, N.D., 2011. Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. Meat Sci. 88, 397–403. https://doi.org/10.1016/j.meatsci.2011.01.017

Shafi, A., Ahmad, F., Mohammad, Z.H., 2022. Effect of the addition of banana peel flour on the shelf-life and antioxidant properties of cookies. ACS Food Sci. Technol. 2, 1355–1363. https://doi.org/10.1021/acsfoodcitech.2c00159

Sielicka-Rózynska, M., Jerzyk, E., GluzaInt, N., 2021. Consumer perception of packaging: An eye-tracking study of gluten-free cookies. J. Consum. Stud. 45, 14–27. https://doi.org/10.1111/ijcs.12600

Sikora, J., Broncel, M., Mikiciuk-Olasik, E., 2014. Aronia melanocarpa Elliot reduces the activity of angiotensin I-converting enzyme—In vitro and ex vivo studies. Oxid. Med. Cell. Longev. 2014, 739721. https://doi.org/10.1155/2014/739721

Silanikove, N., Landau, S., Or, D., Kababya, D., Bruckental, I., Nitsan, Z., 2006. Analytical approach and effects of condensed tannins in carob pods (Ceratonia siliqua) on feed intake, digestive and metabolic responses of kids. Livest. Sci. 99 (1) 29-38. https://doi.org/10.1016/j.livsci.2005.05.018

Sitoayu, L., Purwara Dewanti, L., Melani, V., Azahra Sumitra, P., Rulina Marpaung, M., 2023. Differences in eating habits, stress, and weight changes among indonesian generations Y and Z students during the COVID-19 pandemic. J. Res. Health. 13, 79–86. https://doi.org/10.32598/JRH.13.2.2076.1

Sogut, E., Seydim, A. C., 2018. The effects of Chitosan and grape seed extract-based edible films on the quality of vacuum packaged chicken breast fillets. Food Packag. Shelf-life, 18, 13–20. https://doi.org/10.1016/J.FPSL.2018.07.006

Sonoda, K., Aoi, W., Iwata, T., Li, Y., 2013. Anthocyanin-rich Aronia Melanocarpa extract improves body temperature maintenance in healthy women with a cold constitution. SpringerPlus 2, 1–5. https://doi.org/10.1186/2193-1801-2-626

Sörqvist, P., Haga, A., Holmgren, M., Hansla, A., 2015. An eco-label effect in the built environment: Performance and comfort effects of labeling a light source environmentally friendly. J. Environ. Psychol. 42, 123–127. https://doi.org/10.1016/j.jenvp.2015.03.004

Stenmarck, Å., Jensen, C., Quested, T., Moates, G., 2016. Estimates of European food waste levels. FUSIONS EU Project – Reducing food waste through social innovation. https://doi.org/10.13140/RG.2.1.4658.4721

Strader, A.D., Woods S.C., 2005. Gastrointestinal hormones and food intake. Gastroenterology. 128, 175-191. https://doi.org/10.1053/j.gastro.2004.10.043

Su, C.H., Tsai, C.H., Chen, M.H., Lv, W.Q., 2019. US sustainable food market generation Z consumer segments. Sustainability 11, 3607. https://doi.org/10.3390/su11133607

Sukboonyasatit, D., 2009. Prediction of peoples' intentions and actual consumption of functional food in Palmerston North. Ph.D. Thesis, Massey University Palmerston North, Auckland, New Zealand.

Szopa, A., Kokotkiewicz, A., Kubica, P., Banaszczak, P., Wojtanowska-Krośniak, A., Krośniak, M., Marzec-Wróblewska, U., Badura, A., Zagrodzki, P., Bucinski, A., et al., 2017. Comparative analysis of different groups of phenolic compounds in fruit and leaf extracts of Aronia sp.: A. melanocarpa, A. arbutifolia, and A. ×prunifolia and their antioxidant activities. Eur. Food Res. Technol. 243, 1645–1657. https://doi.org/10.1007/s00217-017-2872-8

Šarić, B., Mišan, A., Mandić, A., Nedeljković, N., Pojić, M., Pestorić, M., Đilas, S., 2016. Valorisation of raspberry and blueberry pomace through the formulation of value-added gluten-free cookies. J. Food Sci. Technol. 53 (2) 1140–1150. https://doi.org/10.1007/s13197-015-2128-1

Šedík, P., Hudecová, M., Predanócyová, K., 2023. Exploring consumers' preferences and attitudes to honey: Generation approach in Slovakia. Food. 12, 1941. https://doi.org/10.3390/food12101941

Šporin, M., Avbelj, M., Kovač, B., Možina, S.S., 2018. Quality characteristics of wheat flour dough and bread containing grape pomace flour. Food Sci. Technol. Int. 24, 251–263. https://doi.org/10.1177/1082013217745398

Tagliani, C., Perez, C., Curutchet, A., Arcia, P., Cozzano, S., 2019. Blueberry pomace, valorization of an industry by-product source of fibre with antioxidant capacity. Food Sci. Technol. 39, 644–651. https://doi.org/10.1590/fst.00318

Taheri, R., Connolly, B.A., Brandm M.H., Bollingm B.W., 2013. Underutilized chokeberry (Aronia melanocarpa, Aronia arbutifolia, Aronia prunifolia) accessions are rich sources of anthocyanins, flavonoids, hydroxycinnamic acids, proanthocyanidins. J. Agric. Food Chem. 61, 8581 – 8. https://doi.org/10.1021/jf402449q

Tahir, H.E., Xiaobo, Z., Jiyong, S., Mahunu, G.K., Zhai, X., Mariod, A.A., 2018. Quality and postharvest-shelf-life of cold-stored strawberry fruit as affected by Gum Arabic (Acacia senegal) edible coating. J. Food Biochem. 42 e12527. https://doi.org/10.1111/jfbc.12527

Tarasevičienė, Ž., Čechovičienė, I., Jukniūtė, K., Šlepetienė, A., Paulauskienė, A., 2021. Qualitative properties of cookies enriched with berries pomace. Food Sci. Technol. 41, 474–481. https://doi.org/10.1590/fst.02120

Tavolacci, M.P. Déchelotte, P., Ladner, J., 2020. Eating disorders among college students in France: Characteristics, help-and care-seeking. Int. J. Environ. Res. Public. Health. 17, 5914. https://doi.org/10.3390/ijerph17165914

Theagarajan, R., Malur Narayanaswamy, L., Dutta, S., Moses, J.A., Chinnaswamy, A., 2019. Valorisation of grape pomace (cv. Muscat) for development of functional cookies. Int. J. Food Sci. Technol. 54, 1299–1305. https://doi.org/10.1111/ijfs.14119

Tiamiyu, Q.O., Adebayo, S.E., Yusuf, A.A., 2023.Gum Arabic edible coating and its application in preservation of fresh fruits and vegetables: A review. Food. Chem. Adv. 2, 100251.

Toaldo, I.M., Cruz, F.A., de Alves, T.L., de Gois, J.S., Borges, D.L., Cunha, H.P., da Silva, E.L., Bordignon-Luiz, M.T., 2015. Bioactive potential of Vitis labrusca L. grape juices from the Southern Region of Brazil: Phenolic and elemental composition and effect on lipid peroxidation in healthy subjects. Food Chem. 173, 527–535. https://doi.org/10.1016/j.foodchem.2014.09.171

Tolić, M.T., Jurčević, I.L., Krbavčić, I.P., Marković, K., Vahčić, N., 2015. Phenolic content, antioxidant capacity and quality of chokeberry (Aronia melanocarpa) products. Food Technol. Biotechnol. 53(2), 171-179. https://doi.org/10.17113/ftb.53.02.15.3833

Tolić, M.T., Krbavčić, I.P., Vujević, P., Milinović, B., Jurčević, I.L.; Vahčić, N., 2017. Effects of weather conditions on phenolic content and antioxidant capacity in juice of chokeberries (Aronia Melanocarpa L.). Pol. J. Food Nutr. Sci. 67, 67–74. https://doi.org/10.1515/pjfns-2016-0009

Topolska, K., Florkiewicz, A., Filipiak-Florkiewicz, A., 2021. Functional Food—Consumer Motivations and Expectations. Int. J. Environ. Res. Public Health 18, 5327. https://doi.org/10.3390/ ijerph18105327

Usman, M., Ahmed, S., Mehmood, A., Bilal, M., Patil, P.J., Akram, K., Farooq, U., 2020. Effect of apple pomace on nutrition, rheology of dough and cookies quality. J. Food Sci. Technol. 57, 3244–3251. https://doi.org/10.1007/s13197-020-04355-z

Vecchio, R., Annunziata, A., 2015. Willingness-to-pay for sustainability-labelled chocolate: An experimental auction approach. J. Clean. Prod. 86, 335–342. https://doi.org/10.1016/j.jclepro.2014.08.006

Verbeken, D., Dierckx, S., Dewettinck, K., 2003. Exudate gums: occurrence, production, and applications. Appl. Microbiol. Biotechnol. 63, 10–21. https://doi.org/10.1007/s00253-003-1354-z

Vital, A.C.P., Santos, N.W., Matumoto-Pintro, P.T., da Silva Scapim, M.R., Madrona, G.S., 2018. Ice cream supplemented with grape juice residue as a source of antioxidants. Int. J. Dairy Technol. 71, 183–189. https://doi.org/10.1111/1471-0307.12412

Vugrinec, K., 2018. Inkapsulacija polifenola maslačka (Taraxacum officinale L.) u pektinske sustave nosača primjenom različitih tehnika. Final work. University of Zagreb. Faculty of Food Technology and Biotechnology, Zagreb, Croatia.

Vuillemin, M.E., Michaux, F., Muniglia, L., Linder, M., Jasniewski, J., 2019. Gum Arabic and chitosan self-assembly: Thermodynamic and mechanism aspects. Food Hydrocolloids, 96, 463-474. https://doi.org/10.1016/j.foodhyd.2019.05.048

Vujković, I., Galić, K., Vereš, M., 2007. Ambalaža za pakiranje namirnica, Tectus d.o.o., Zagreb.

Walker, R., Tseng, A., Cavender, G., Ross, A., Zhao, Y., 2014. Physicochemical, nutritional, and sensory qualities of wine grape pomace fortified baked goods. J. Food Sci. 79, S1811–S1822. https://doi.org/10.1111/1750-3841.12554

Walther, B.A., Kusui, T., Yen, N., Hu, C.S., Lee, H., 2020. Plastic pollution in East Asia: Macroplastics and microplastics in the aquatic environment and mitigation efforts by various actors. In: Plastic Aquatic Environ—Part I, Springer International Publishing, pp. 353–403.

Wang, L., Dong, Y., Men, H., Tong, J., Zhou, J., 2013. Preparation and characterization of active films based on chitosan incorporated tea polyphenols. Food Hydrocoll. 32(1), 35–41. https://doi.org/10.1016/J. FOODHYD.2012.11.034

Wawer, I., Wolniak, M., Paradowska, K., 2006. Solid state NMR study of dietary fiber powders from aronia, bilberry, black currant and apple. Solid State Nucl. Magn. Reson. 30, 106–113. https://doi.org/10.1016/j.ssnmr.2006.05.001

WHO (2021). Cardiovascular diseases (CVDs). WHO – World Health Organization. Available online: Cardiovascular diseases (CVDs) (who.int). Accessed on 10th March 2023.

Williams, P.A., Phillips, G.O., 2021. Introduction to food hydrocolloids. In: Handbook of Hydrocolloids; Elsevier: Amsterdam, The Netherlands, pp. 3–26.

Wu, J., Ge, S., Liu, H., Wang, S., Chen, S., Wang, J., Li, J., Zhang, Q., 2014. Properties and antimicrobial activity of silver carp (Hypophthalmichthys molitrix) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. Food Packag. Shelf-life. 2, 7–16. https://doi.org/10.1016/j. fpsl.2014.04.004

Xie, L., Vance, T., Kim, B., Lee, S.G., Caceres, C., Wang, Y., Hubert, P.A., Li, J.Y., Chun, O.K., Bolling, B.W., 2017. Aronia berry polyphenol consumption reduces plasma total and low-density lipoprotein cholesterol in former smokers without lowering biomarkers of inflammation and oxidative stress: a randomized controlled trial. Nutr. Res. 37, 67-77. https://doi.org/10.1016/j.nutres.2016.12.007

K., Bolling, B.W., 2017. Aronia berry polyphenol consumption reduces plasma total and low-density lipoprotein cholesterol in former smokers without lowering biomarkers of inflammation and oxidative stress: A randomized controlled trial. Nutr. Res. 37, 67–77. https://doi.org/10.1016/j.nutres.2016.12.007

Xu, L., Zhang, X., Chu, Z., Wang, H., Li, Y., Shen, X., Cai, L., Shi, H., Zhu, C., Pan, J., Pan, D., 2021. Temperature-responsive multilayer films based on block copolymercoated silica nanoparticles for long-term release of favipiravir. ACS Appl. Polym. Mater. 4, 14014–14025. https://doi.org/10.1021/acsomega.2c03591

Yamane, T., Kozuka, M., Yamamoto, Y., Nakano, Y., Nakagaki, T., Ohkubo, I., Ariga, H., 2016. effectiveness of aronia berries for reduction of mild fibrosis and gene expression analysis in livers from mice fed a high-fat diet with aronia berries. Funct. Food Health Dis. 6, 144–157. https://doi.org/10.31989/ffhd.v6i3.245

Yilmaz, E., Kahveci, D., 2022. Consumers' purchase intention for upcycled food: Insights from Turkey. Future Food. 6, 100172. https://doi.org/10.1016/j.fufo.2022.100172

Yu, J., Ahmedna, M., 2013. Functional components of grape pomace: Their composition, biological properties and potential applications. Int. J. Food Sci. Technol. 48, 221–237. https://doi.org/10.1111/j.1365-2621.2012.03197.x

Zern, T.L., Fernandez, M.L., 2005. Cardioprotective effects of dietary polyphenols. J. Nutr. 135, 2291–2294. https://doi.org/10.1093/jn/135.10.2291

Zhang, G., Hamaker, B.R., 2009. Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. Crit. Rev. Food Sci. Nutr. 49, 852-867. https://doi.org/10.1080/10408390903372466

Autobiography

Dunja Molnar is currently working as R&D Portfolio Data Assistant Manager for the Adriatic cluster at Ledo Plus d.o.o. part of Nomad Food, where she has been employed since 2018 as R&D technologist for ice cream. Her main responsibilities include implementing and executing product strategies concerning sustainability, nutrition, sensory analysis, clean labeling, and benchmarking for the Adriatic region. Moreover, she is responsible for harmonizing processes and documentation within the cluster to ensure compliance with Nomad Food' policies and procedures.

In 2011, Dunja obtained a Bachelor's degree, followed by a Master's degree in Nutritional Sciences from the Faculty of Food Technology and Biotechnology, University of Zagreb in 2013. In February 2017, she began her doctoral studies in Biotechnology and Bioprocess Engineering, Food Technology and Nutrition, at the University of Zagreb, Faculty of Food technology and Biotechnology.

Her scientific research is focused on the development of upcycled cookies using sustainable raw materials and reducing waste in the food industry. This involves substituting cocoa powder with by-products from grape- and aronia juice production, as well as developing edible films enriched with these by-products. Dunja's scientific work has resulted in the publication of three A1 (Q1 and Q2) scientific papers and three A2 scientific papers, as well as numerous posters with international reviews. She financed her doctoral studies entirely independent and has shown a great example of domestic and international scientific research cooperation with numerous institutions including the Faculty of Food Technology and Biotechnology, the Faculty of Pharmacy and Biochemistry, the Vinograd Hospital, the Laboratory for Analytical Research Sample Control, the Food Industry Design company (Serbia), the University of Szeged (Hungary), the University of Skopje (North Macedonia), and the ONIRIS University (France).

Additionally, since 2023, she has been involved in the *Zdrovlje prije svega* project in Međimurje County, aiming to educate and raise awareness among residents about the importance of healthy nutrition and physical activity. Moreover, Dunja Molnar won scholarships for the EIT Food Summer School on New Product Development for the Food Industry (2020) and the EIT Food Innovator Fellowship (2021).

List of authors publications

Original scientific papers indexed in Web of Science (Current Contents Connect)

- **Molnar, D.,** Novotni, D., Kurek, M., Galić, K., Iveković, D., Bionda, H., Ščetar, M. (2023) Characteristics of edible films enriched with fruit by-products and their application on cookies. *Food Hydrocoll.* **135**, 108191. IF=10.7 (Q1)
- **Molnar, D.,** Velickova, E., Prost, C., Temkov, M., Ščetar, M., Novotni, D. (2023) Consumer Nutritional Awareness, Sustainability Knowledge, and Purchase Intention of Environmentally Friendly Cookies in Croatia, France, and North Macedonia. *Food* **12**, 3932. IF=5.2 (Q1)
- **Molnar, D.,** Gabaj, N.N., Vujić, L., Ščetar, M., Krisch, J., Miler, M., Štefanović, M., Novotni, D. (2023) Application of fruit by-products and edible film to cookies: Antioxidant activity and concentration of oxidized LDL receptor in women—A first approach. *Appl. Sci.* **13**, 5513. 513. IF=2.7 Q(2)

Original scientific papers indexed in other database

- **Molnar, D.,** Novotni, D., Krisch, J., Bosiljkov, T., Ščetar, M. (2020) The optimisation of cookie formulation with grape and aronia pomace powders as cocoa substitutes. *Cro. J. Food Technol. Biotechnol. Nutr*. **15**, 38–44. CAB: Accession Number 20113064817
- **Molnar, D.,** Rimac Brnčić, S., Vujić, L., Gyimes, E., Krisch, J. (2015) Characterization of biscuits enriched with black currant and jostaberry powder. *Cro. J. Food Technol. Biotechnol. Nutr*. **10(1-2)**, 31-36

Scientific papers in conference proceedings with an international review

- Sorić, T., **Molnar, D.,** Dolanc, I., Bočkor, L., Šarac, J., Čoklo, M. (2019) The relationship between processed food dietary pattern and depression in adults. In: Proceedings of the 12th International Scientific and Professional Conference WITH FOOD TO HEALTH (Jozinović, A., Ačkar, Đ., Babić, J. et al. Ed). Osijek, str. 41-51.

Book of Abstracts

- **Molnar, D.,** Novotni, D., Ščetar, M., Bosiljkov; T. (2019) the optimization of biscuit formulation with grape and aronia pomace as cocoa substitutes. In: *10th International Congress Flour-bread 19 and 12th Croatian Congress of Cereal Technologists Brašno- kruh Book of Abstracts,* 71.
- Buljan, J., **Molnar, D.,** Ščetar, M., Kurek, M., Galić, K. (2021) Barrier and mechanical properties of chitosan films with grape seed extract. In: *10th Central European Congress on Food (CEFood) Book of Abstracts* (Mujčinović, A., ed.). Sarajevo: Poljoprivredno-prehrambeni fakultet Univerziteta u Sarajevu, 122-122
- Ščetar, M., Lenard, I., Kurek, M., **Molnar, D.,** Galić, K. (2022) Characterisation of chitosan films with Vitamin C In: *Book of Abstracts of the 10th International Congress of Food Technologists, Biotechnologists and Nutritionists* (Komes, D., ed.), Croatian Society of Food Technologists, Biotechnologists and Nutritionists, Zagreb, Croatia, 44-45.
- **Molnar, D.,** Novotni, D., Ščetar, M., Vujić, L., Krisch, J. (2023) Consumers attidutes and nutritional profile of with fruit by-products and edible films enriched cookies. In: *14th International Scientific and Professional Conference WITH FOOD TO HEALTH Book of Abstracts,* online, str. 57.

Supplementary

1. Publication No 4.: Molnar, D., Novotni, D., Krisch, J., Bosiljkov, T., Ščetar, M. (2020) The optimisation of cookie formulation with grape and aronia pomace powders as cocoa substitutes. *Cro. J. Food Technol. Biotechnol. Nutr*. **15**, 38–44.

2. **Table S1:** Optimization and validation of a powdered mixture of aronia (%) and grape pomace (%) for partial replacement of cocoa powder (%) in cookie formulation

3. **Table S2:** Purchase intention of whole grain, nutritionally improved and sustainable cookies by generations.

4. **Table S3:** Purchase intention of whole grain, nutritionally improved and sustainable products by country

5. **Figure S1:** The appearance of biscuits depending on the mixture composition (cocoa, aronia pomace and grape pomace)

ORIGINAL SCIENTIFIC PAPER

The optimisation of biscuit formulation with grape and aronia pomace powders as cocoa substitutes

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Abstract

By-products of fruit processing, such as grapes and aronia pomace, are rich in fibre and polyphenols, and their application in bakery products could significantly improve nutritional value, bioactive potential and shelf life of final product. This research aimed at optimising wholemeal biscuit formulation with grape and aronia pomaces as partial substitutes for cocoa powder, using the desirability function. Ten formulations with the mixture of cocoa powder, grape and aronia pomace powders in different ratios (0-100%) were tested. The physical properties (texture, colour, thickness, diameter, spread factor), the sensory acceptability of biscuits, and microbiological safety were investigated. The interaction of all three mixture components significantly affected the instrumentally measured redness a and liking of texture. Biscuit diameter, yellowness b*, and liking of flavour were affected by the interaction of aronia and grape pomace. Biscuit height, spread ratio, hardness, flexibility, toughness, appearance, and odour were not significantly influenced by the mixture composition. The optimized mixture composition contained cocoa, grape pomace, and aronia pomace in the ratio 76.4 : 17.5 : 6.1 (respectively), with the desirability of 0.78. After confirmation, biscuits with that mixture were proven better than the control, that contained 100% cocoa, in terms of decreased hardness (-23%) and toughness (-19%), having the same sensory acceptability, and were microbiologically safe for 5 months. The findings of this study indicate that aronia and grape pomace could be used as partial substitutes for cocoa powder up to 24.3% in the production of wholemeal biscuits.*

Keywords*: aronia pomace, grape pomace, biscuit formulation optimisation, cocoa substitute*

Introduction

Biscuits are popular cereal based products, due to their rich nutrition content, different shapes and flavours, ready-to-eat form, and long shelf life (Ajila et al., 2008). They are known as indulgent food although there is growing interest in healthier and functional biscuits (Crofton et al., 2013; Konstantas et al., 2019). Biscuits could be used as medium for incorporation of various ingredients and nutrients into the diet (Čukelj et al., 2016) such as dietary fibre, proteins, vitamins, minerals, polyphenols, β-glucans, and sterols.

Cocoa powder is one of the most popular additional ingredients in bakery industry due to its unique flavour (Manley, 2000; Loullis and Pinakoulaki, 2018). Demand for cocoa is continuously increasing, and it is very likely that the production will not be able to maintain with demand, which would lead to the rise of cocoa price (Loullis and Pinakoulaki, 2018). Moreover, biscuits containing chocolate/cocoa are environmentally the least sustainable in terms of cocoa production (Konstantas et al., 2019). These are the reasons why finding a replacement for cocoa is becoming an imperative. Many studies investigated the substitutes for cocoa butter, but there is only a limited number of studies trying to find substitution for cocoa powder (Barroso et al., 2015, Rosa et al., 2015; Fadel et al., 2006). The main limitation of commercially available *imitation cocoa powders* based on starch, legumes, soy flour, chicory root, chocolate flavour, and different additives is that they differ significantly from natural cocoa powder, mostly in colour, water solubility and chemical composition (Rikon and de Valle, 1982). Most recent studies showed that carob powder may be successfully used as low-cost cocoa substitute (Pawlowska et al., 2018; Loullis and Pinakoulaki, 2018). Carob flour may substitute up to 30% of the cocoa powder in biscuits without appreciable change in flavour (Manley, 2000). Similar to cocoa, carob flour contains various phytochemicals but is also rich in condensed tannins which tend to give an astringent aftertaste to products (Loullis and Pinakoulaki, 2018; Silanikove et al., 2006). Thus, finding a cocoa substitute with better sensory characteristics is needed.

Dried fruits (grapes in particular) are common, but rather expensive raw material for making biscuits. Recently, fruit pomaces from wine industry such as white grape pomace (Mildner-Szkudlarz et al., 2013) and red grape pomace (Acun and Göl, 2014), as well as pomaces from juice industry, such as apple pomace (Singh et al., 2012) and blueberry pomace (Curutchet et al., 2019) were suggested as alternative sources of dietary fibre and phenolics for the novel formulation of biscuits. Pomace may account from 20% up to 50% of the original fruit weight (Khanal et al., 2009) and is disposed as waste from juice and wine industry. The disposal of such waste materials is environmentally and economically problematic (Palocci and Chronopoulou, 2015). Growing interest has been raised to safe disposing of fruit processing waste and recovering of valuable compounds such as phenolics and dietary fibre remained in the by-products (Galanakis, 2012; Khanal et al., 2009). Nevertheless, pomaces being a rich source of valuable components, could be used as low-cost functional ingredients in food production (Galanakis, 2012; Karnopp et al., 2015; Prokopov et al., 2015). To our knowledge, the potential of using red grape and aronia pomaces as partial cocoa substitutes for making biscuits formulation with great sensory and physical properties has not been investigated yet. Thus, this research aimed to optimize the wholemeal biscuit formulation with powdered grape and aronia pomaces as partial substitutes for cocoa powder using a mixture design and desirability function. The pomaces were finely ground to approximate particle size of the cocoa powder. Physical and sensory properties as well as the microbiological safety of biscuits were determined.

Materials and methods

Raw materials

Wholemeal spelt flour (Siladi, Croatia) containing 12% protein and fine oat flakes (Crownfield, Germany) containing 13.5% proteins were bought on a local market. Cocoa powder (Kraš, Croatia) contained 20% fat and margarine (with butter) contained 70% fat (Zvijezda, Croatia). Dried red grape (*Vitis vinifera*, Frankovka and Syrah variety, with seeds) pomace and aronia (*Aronia melanocarpa,* without seeds) pomace were obtained as by-products from juice production from local producers (Natkrižovljan, Cestica Community and Demit, Dugo Selo, Croatia, respectively). The following properties were taken into account: dark colour, high antioxidant capacity, and good flavour. The moisture level was below 10%. Dried grape and aronia pomace were ground in a laboratory ball mill (Cryomill, Retsch, Austria), in order to reach the average particle size assembling to cocoa powder. Pomaces (7 g) were 3 min ground in a 50 mL stainless steel container with 12 steel balls (10 mm diameter), and vibration frequency of 30 Hz. Particle size distribution of cocoa powder and ground pomaces was determined in triplicates by laser diffraction method with Malvern Mastersizer 2000 instrument and a Scirocco 2000 dry dispersion unit (Malvern Instruments Ltd., UK). The mean particle size distribution of cocoa, grape and aronia pomaces powder is shown on Figure 1. The median diameter of 50th percentile of cocoa powder was 41 ± 3 µm, grape pomace powder 37 ± 2 µm and aronia pomace powder 35 ± 3 µm.

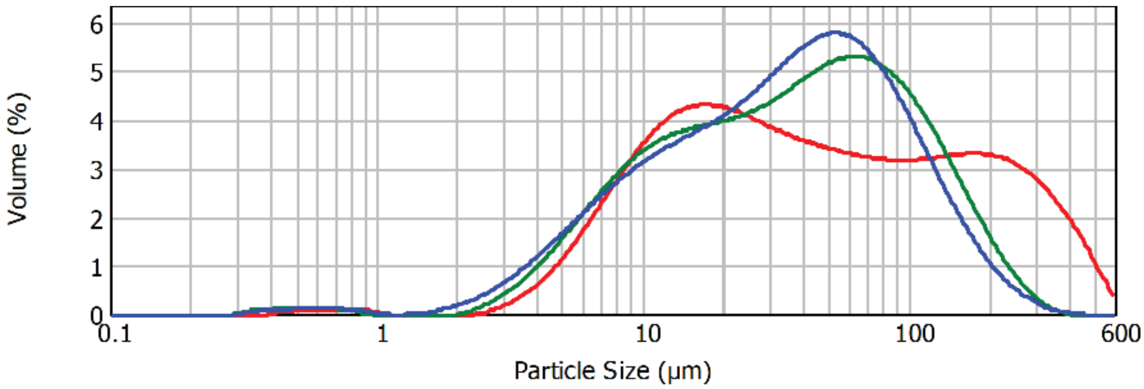

Figure 1. *Particle size distribution of cocoa powder (red line), grape (green line) and aronia (blue line) pomace powder*

Experimental design and biscuit formulation

Ten formulations were prepared according to mixture design using Design Expert v.11 software (Stat-Ease, USA). The following formulation for the control biscuit (100% cocoa) was designed in preliminary experiments (weight on total dough weight, in percentage): fine oat flakes 30%; wholemeal spelt flour 25.2%; margarine 20%; brown sugar 13.5%; cocoa powder 4.8%; vanilla sugar 3.5%; salt 2%; tap water 0.4%; baking powder 0.3%; sodium bicarbonate 0.3%. Cocoa powder was completely or partially replaced with grape and/or aronia pomace powder in different ratios (0-100%) (Table 1). Biscuits were made according to AACC (1999) method 10-50.05. Dough was prepared by mixing all the ingredients together, and then it was rolled out to thickness of 5 mm and shaped with round cutter 5 cm diameter. Thirty pieces of each biscuit formulation were baked in two batches in an oven at 180°C for 12 minutes. Biscuits were cooled and packed in biaxially oriented polypropylene (BOPP) coated with acrylic/polyvinylidene chloride (AcPVDC) to obtain composite 32 µm thick BOPPAcPVDC film (ExxonMobil, Belgium). Pouches with biscuits were hermetically sealed using welding machines Audion Sealkid 421 (Viro, Croatia) and stored at room temperature (22 °C ±1 °C) for analyses.

Determination of biscuit physical characteristics

Biscuit physical characteristics were determined the day after baking in at least 10 replicates. The diameter was measured with a calliper across the biscuit length, height was measured on 6 biscuits stacked together and biscuit spread factor was calculated following AACC 10-50.05 (1999). Texture was determined with HD.Plus Texture Analyser using three point bending rig with HDP/3PB knife-edge probe, and 30-kg load cell (Stable Micro System, UK). The analysis was performed with a test speed of 2 mm/sec and distance of 5 mm. Hardness, flexibility and toughness were calculated according to American Institute of Baking (AIB) protocol. The colour of biscuit's upper surface was measured using a calibrated spectrophotometer Model CM-3500d (Konica Minolta, Japan). The L^*, a^*, b^* components of the CIELab space were recorded, where L^* indicates lightness (L=0 (black), L=100 (white), a^* indicates chromaticity on a green (−) to red (+) axis, and b^* chromaticity on a blue (−) to yellow (+) axis. Colour difference (dE) from the control sample (100% cocoa) was calculated according to Pathare et al. (2013): $dE = ((\Delta L)^2+(\Delta a)^2+(\Delta b)^2)^{1/2}$.

Sensory analysis

Nine-point hedonic scale (Svensson, 2012) was used to evaluate biscuits appearance, odour, flavour, texture, and overall acceptability; with 1 dislike extremely and 9 like extremely. Sensory evaluations were carried out in two different sessions. In the first session, experienced panellists (9 female and 2 male) were asked to evaluate ten formulations of biscuits with the mixture of cocoa powder, grape and aronia pomace powders in different ratios (0-100%). In the second session, 27 female and 10 male untrained panellists, using the same nine-point hedonic scale, determined how they liked the biscuits with optimized mixture composition and the control. For all sessions, biscuits were coded with numbers on a white plate and cold tap water was provided for cleansing palate before tasting each sample. Each panellist received whole biscuits on a plate.

Microbiological analysis of biscuits

The microbiology of the control and optimized biscuits was examined in five parallel determinations according to the Commission regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs and

Croatian National Guidelines on microbiological criteria for food stuffs (2011). Microbiological analyses were done for seven months in order to determine the shelf life of biscuits. Total cell number was determined by plate count method on plate count agar (peptone 5 g, glucose 10 g, yeast extract 2.5 g, agar - agar 25 g in 1000 ml distilled water). Mould count was determined by plate count method on malt extract medium (malt extract 5 g; yeast extract 5 g, glucose 5 g, agar-agar 20 g in 1000 ml distilled water). Coagulase positive *Staphylococcus* was determined by plate count method on Baird-Parker agar (Sigma-Aldrich, Hungary). The presence of *Enterobacteriaceae* was determined in Brillant Green Bile Lactose broth (Sigma-Aldrich, Hungary). The number of *Salmonella* was determined according to ISO 6579-1:2017.

Data analyses and formulation optimisation

Design Expert v.11 (Stat-Ease, USA) software was used to generate the experimental design, evaluate raw data by analysis of variance (ANOVA) at $p<0.05$, and the numerical optimisation using desirability function. Different models were tested for their ability to fit the experimental response values, and the backward elimination procedure ($p<0.05$) was conducted for fitting the experimental data. The criteria for optimisation were to minimise hardness and colour difference (with importance 3) and to maximise diameter (importance 1), taste, odour and texture (importance 5) as well as overall acceptability of biscuits (importance 3) in order to develop biscuits with the most similar sensory properties as biscuits with 100 % cocoa powder. The same quality parameters were determined for the biscuit baked with the optimised mixture of cocoa, grape and aronia pomace powders as for the control (100% cocoa). After confirmation experiment, the prediction error (%) was calculated as (experimental - predicted) / predicted × 100.

Results and discussion

Physical characteristics of biscuits

The physical characteristics of biscuits formulations with different cocoa powder, grape and aronia pomace powders ratios are presented in table 1. Higher spread factor (higher diameter and lower height) is considered a positive biscuit characteristic. Still, diameter and height are better for estimation of quality than spread ratio since diameter and height can vary in different ways giving the same spread factor (AACC Standard No 10-50.05, 1999). Biscuit height and spread ratio were not significantly ($p>0.05$) influenced by the mixture composition, but biscuit diameter was negatively affected ($p<0.05$) depending on the linear and nonlinear interactions between aronia and grape pomace, and aronia and cocoa (Figure 2a, Table 2). In accordance with our results, Karnopp et al. (2015) found no significant difference in spread ratio of biscuits with added grape pomace (up to 30%). Ajila et al. (2008) reported a decrease in the diameter but also in the thickness of biscuits with addition of 15% or 20% mango peel powder. Dissimilar, Acun and Gül (2014) found that there was no significant difference between diameters of biscuits with added white grape pomace and Mildner-Szkudlarz et al. (2013) found an increase in diameter and spread ratio of biscuits with incorporated white grape pomace. Different findings of pomace effect on biscuits diameter could be due to differences in pomace type, amount and composition used as well as in fat sugar and fibre content of other ingredients used for making biscuit (Mamat and Hill, 2018).

Colour is one of the key factors affecting consumer's choice and preferences regarding biscuit acceptability (Chakraborty et al., 2010). The colour of biscuits is influenced by the formulation as well as by the caramelization, dextrinization and Maillard reactions that occur during the baking process (Walker et al., 2014). The interaction of grape and aronia pomace powders significantly influenced L^* values ($p=0.004$) (Table 2). When grape pomace composed at least 50% of the mixture, L^* value was higher which means that biscuits became lighter than the

Table 1. *Mixture composition and physical properties of biscuits with the mixture of cocoa powder, grape and aronia pomace powders in different ratios*

Mixture composition			*Physical properties of biscuits*								
Aronia (%)	**Grape (%)**	**Cocoa (%)**	Diameter (cm)	Height (cm)	Spread ratio	Hardness (N)	Flexibility (mm)	Toughness (N)	L^*	a^*	b^*
0	0	100.00	5.02 ± 0.06	0.77 ± 0.04	6.5 ± 0.2	26.9 ± 1.0	3.62 ± 0.45	48.3 ± 2.0	32.15 ± 1.1	9.92 ± 0.4	11.40 ± 0.6
16.67	16.67	66.67	4.98 ± 0.05	0.79 ± 0.12	6.3 ± 0.1	19.7 ± 2.1	4.0 ± 0.18	40.3 ± 3.6	30.72 ± 1.3	7.20 ± 0.6	8.99 ± 0.5
0	100.00	0	4.99 ± 0.05	0.84 ± 0.04	5.9 ± 0.2	25.7 ± 0.2	3.4 ± 0.56	44.3 ± 0.8	36.08 ± 1.6	6.74 ± 0.5	9.32 ± 1.0
100.00	0	0	5.06 ± 0.04	0.81 ± 0.04	6.3 ± 0	22.9 ± 1.8	3.7 ± 0.26	40.2 ± 3.7	27.46 ± 1.0	5.01 ± 0.3	4.49 ± 0.9
16.67	66.67	16.67	4.99 ± 0.08	0.84 ± 0.04	6.0 ± 0.2	27.3 ± 2.4	4.6 ± 0.58	48.9 ± 2.4	31.07 ± 1.1	6.73 ± 0.5	7.14 ± 0.6
0	50.00	50.00	4.98 ± 0.06	0.84 ± 0.04	6.0 ± 0.2	27.4 ± 1.6	4.2 ± 0.72	52.1 ± 4.7	33.89 ± 1.6	8.20 ± 0.6	11.20 ± 0.8
50.00	50.00	0	4.98 ± 0.04	0.80 ± 0.04	6.3± 0	22.1 ± 2.4	4.2 ± 0.32	44.9 ± 0.4	28.18 ± 1.0	6.28 ± 0.3	4.70 ± 0.9

50.00	0	50.00	4.95 ± 0.03	0.84 ± 0.04	5.9 ± 0.2	26.9 ± 1.0	3.6 ± 0.79	47.2 ± 1.2	29.62 ± 1.8	5.14 ± 0.5	6.50 ± 1.0
66.67	16.67	16.67	4.97 ± 0.06	0.78 ± 0.04	6.4 ± 0.1	24.6 ± 0.6	3.6 ± 0.57	42.5 ± 2.1	29.06 ± 0.8	5.02 ± 0.5	5.41 ± 1.8
33.33	33.33	33.33	4.97 ± 0.05	0.74 ± 0.07	6.7 ± 0.4	26.6 ± 0.8	3.6 ± 0.82	45.8 ± 0.2	30.32 ± 1.5	5.74 ± 0.6	7.00 ± 0.8

control sample with 100% cocoa powder. The interaction of all three mixture components (cocoa, grape and aronia pomace powders) significantly ($p<0.05$) affected a^* and b^* values (Table 2), whose intensities were lower after adding pomaces. Similary, Acun and Göl (2014) observed that the biscuits with addition of red grape pomace and grape seed flour are darker and that grape pomace addition in biscuits led to decrease in b^* value. Also, Karnopp et al. (2015) observed that biscuits with higher grape pomace content are darker and have lower a^* and b^* values. A noticed decrease in a^* and b^* values might be the reflection of the anthocyanins composition from pomace (Šarić et al., 2016), but also due to the fact that our control sample contained cocoa powder.

Overall, the colour difference from the control formulation (100% cocoa) was the biggest when aronia prevailed in the mixture (Figure 2b). Our study indicates the possibility of combining fruit pomaces with different concentrations of *anthocyanins* to obtain desirable colour as it is in control formulation (100 % cocoa).

Hardness, toughness and flexibility of biscuits were not significantly ($p>0.05$) affected by the mixture composition (Table 1). Hardness and toughness were significantly correlated ($r=0.8$). Contrary, Kuchtova et al. (2016) reported that biscuits with 15% grape seed powder became significantly harder compared with control sample. In previous studies the hardening effect was attributed mostly to the lower content of gluten (Karnopp et al., 2015) and the composition of fibres from the added pomaces (Šarić et al., 2016) which possess high water holding capacity resulting in harder biscuits (Turksoy and Ozkaya, 2011). In our study, the water addition was consistent between formulations, and the amount of fibre was already high since we used wholemeal flour and oat flakes. Moreover, we used powdered pomaces, which might have shifted the fibre composition toward more soluble.

Consumers liking

The sensorial quality of food products plays an important role in the choice of food and hedonic testing are often used to determine consumer's attitude towards the food by assessing a degree of acceptance of a new product or by enhancing the existing food product (Curutchet et al., 2019). The addition of grape and aronia pomace powders significantly influenced the flavour and overall acceptability of biscuits ($p<0.01$) (Table 3). The flavour of biscuits was dependent on all mixture components and their interactions (Table 2). The addition of grape and aronia pomace powders had positive impact on biscuits flavour, despite of their fruity taste, although cocoa powder had the greatest impact on liking the flavour (Figure 2c). The liking of texture was significantly ($p<0.05$) influenced by the interaction of all mixture components (Tables 2 and 3). The balanced replacement of cocoa with both grape and aronia in small amounts (up to 30%) positively influenced the liking of texture. Just like a flavour, the overall acceptability of biscuits was the highest when cocoa prevailed in the mixture, whereas the lowest when aronia prevailed (Figure 2d). The odour and appearance were no significantly different between biscuits ($p>0.05$). Similar effects of grape preparations on odour and appearance of biscuits were observed by Mildner-Szkudlarz (2013). In accordance with our study, Karnopp et al. (2015) showed that the addition of grape pomace in bigger amounts significantly influence the overall acceptability of biscuits and that biscuits formulation needs to be optimised in order to stimulate consumption.

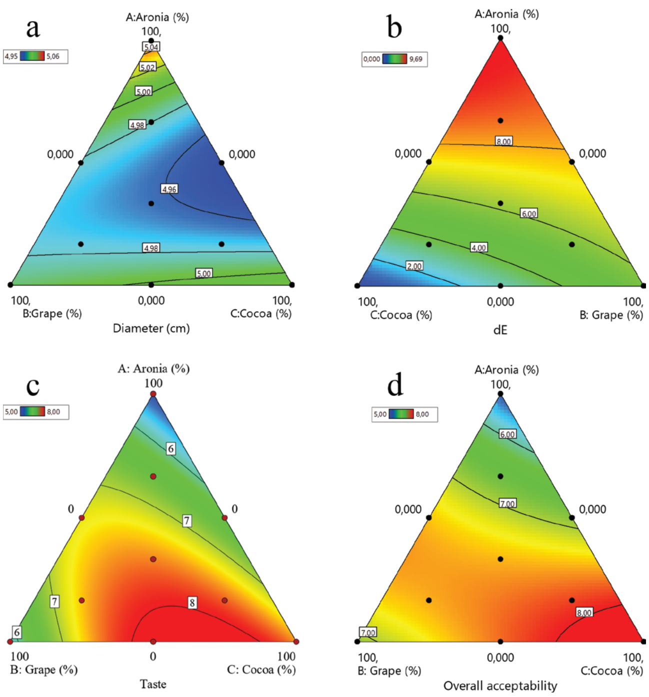

***Figure 2.** Contour plots of selected responses (biscuits diameter, colour difference, flavour, and overall acceptability) in relation to the mixture composition*

Table 2. *Significant coefficients of the fitted equations for the different responses depending on the linear and nonlinear interactions between aronia pomace, grape pomace and cocoa (actual factors)*

Response	Linear mixture	A×B	A×C	B×C	A×B×C	AB^{2}C	ABC^2	R^2
Diameter		-0.174	**-0.328**					0.892
Flavour	5.11×A+5.79×B+7.57×C	7.042		5.951				0.909
Texture	*6.51×A+6.97×B+7.01×C*	**2.993**	1.051		-7.941			0.784
Overall acceptability	*5.22×A+6.89×B+8.49×C*	5.451						0.858
Lightness L^*	**26.38×A+34.22×B+31.97×C**							0.955
Redness a^*	**5.02×A+6.78×B+9.90×C**	1.683	**-9.349**		-14.313			0.984
Yellowness b^*	**4.70×A+9.14×B+11.36×C**	-9.300	*-5.950*					0.977
Colour difference dE	**9.95×A+5.60×B-0.03×C**		**9.473**					0.986

Note: **bold** *significant at $p<0.001$; regular $p<0.05$; italic $p<0.010$; A, aronia; B, grape; C, cocoa.*

Table 3. *Results of sensory analysis of biscuits with the mixture of cocoa powder, grape and aronia pomace in different ratios*

Mixture composition			*Sensory properties of biscuits*				
Aronia (%)	**Grape (%)**	**Cocoa (%)**	Appearance	Odour	Flavour	Texture	Overall acceptability
0	0	100.00	8 ± 0.7	8 ± 1.8	8 ± 1.6	7± 1.9	8 ± 0.6
16.67	16.67	66.67	7 ± 1.1	8 ± 1.2	8 ± 0.3	7± 1.0	8 ± 0.8
0	100.00	0	7 ± 1.7	7 ± 1.2	6 ± 1.2	7 ± 0	6 ± 1.1
100.00	0	0	7 ± 1.3	7 ± 1.2	5 ± 2.7	7 ± 2.1	5 ± 2.5
16.67	66.67	16.67	7 ± 0.9	7 ± 1.2	7 ± 0.5	7± 1.6	8 ± 0.8
0	50.00	50.00	8 ± 0.8	9 ± 0.7	8 ± 0.6	7± 1.4	8 ± 0.9
50.00	50.00	0	8 ± 0	8 ± 1.3	7 ± 1.4	8 ± 1.4	7 ± 1.3
50.00	0	50.00	8 ± 0.9	8 ± 1.7	6 ± 1.5	7 ± 1.8	7 ± 1.2
66.67	16.67	16.67	8 ± 0.6	7 ± 1.2	7 ± 0.6	7 ± 1.3	7 ± 0.8
33.33	33.33	33.33	8 ± 0.5	8 ± 1.0	8 ± 0.6	7 ± 1.0	8 ± 0.8

Formulation optimisation and validation

The optimised mixture composition with a desirability of 0.725 was: cocoa powder 76.4%, grape pomace 17.5% and aronia pomace 6.1%. The model prediction of properties of biscuits made with optimised mixture were: diameter 4.99 cm, height 0.66 cm, spread ratio 7.56, hardness 23.6 N, flexibility 3.8 mm, toughness 47.3 N, colour L^* 32.02, a^* 8.45, b^* 10.51, appearance 8, flavour 8, texture 8, and overall acceptability 8. After baking biscuits with the optimized mixture composition, measured values were in good agreement with predicted values (with prediction error in brackets) for diameter 4.95 cm (-1%), L^* 31.01 (-3 %), a^* 7.91 (-6 %), b^* 9.82 (-7 %), appearance 8 (-2 %), flavour 8 (-2 %), liking of texture 8 (0 %) and overall acceptability 8 (-1 %). Although the models were nonsignificant, measured values for height 0.61 cm (-8 %), spread ratio 8.06 (7 %), hardness 22.1 N (-6 %), flexibility 3.5 mm (-8 %), toughness 42.1 N (-11 %), and odour 9 (8 %) were in a reasonable agreement with predicted values. Thus, biscuits with optimised mixture of cocoa, grape and aronia pomaces powders were proven better than the control in terms of lower hardness (-23%) and toughness (-19%), bigger spread ratio, and the same overall acceptability. General opinions on the flavour, colour, texture and overall acceptability of optimised biscuits were highly positive (in category 'liked very much') indicating that aronia and grape pomace powders could be used as partial substitutes for cocoa powder in biscuits.

Microbiological evaluation

The shelf-life of food including biscuits may be compromised by the growth of food spoilers and foodborne pathogens. Fruit pomaces and cocoa powder possess different phenolic compounds, which have antimicrobial activity (Galanakis, 2017). For example, grape pomace, used for preparation of this biscuits, was proposed as an interesting source of natural products to extend the shelf-life of different foodstuff such as meat and meat products, fish products, seafood, and cheese (Galanakis, 2017). *Salmonella, Enterobacteriaceae,* and *Staphylococcus* were not detected in the optimised and control biscuits during 6 months of storage. Moreover, no moulds or yeasts were found after six months of storage. Total plate count was growing gradually from 9.2×10^1 cfu/g after one month of storage to 5×10^4 cfu/g after six months of storage in both control and optimised biscuits. This depicts that total viable count of the biscuits was a bit higher than 10 000 cfu/g which is recommended in Croatian National Guidelines on microbiological criteria for foodstuffs (2011). Still, we can conclude that both samples were within the microbial limit standard and thus safe for consumption during six months of storage.

Conclusions

In this research, we optimised wholemeal biscuit formulation with grape and aronia pomaces as partial substitutes for cocoa powder using mixture design and desirability function. Biscuits height, spread ratio, hardness, flexibility, toughness, appearance, and odour were not significantly influenced by the mixture composition. On the other hand, the diameter of biscuits was significantly dependent on interactions between all three mixture components. Overall acceptability of biscuits was mostly driven by their flavour which was better liked when cocoa powder prevailed in the mixture. Still, biscuits with the optimised mixture of cocoa (76.4%), grape pomace (17.5%) and aronia pomace (6.1%) were proven better than the control (100% cocoa) in terms of decreased hardness (-23%) and toughness (-19%), having the same sensory acceptability. The findings of this study indicate that powdered grape and aronia pomaces as sustainable resources could successfully substitute up to 24% of cocoa powder in the production of wholemeal biscuits. Future studies should investigate different sustainable sources for substituting cocoa powder in fine bakery products.

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References

Acun S., Göl, H. (2014) Effects of grape pomace and grape seed flours on cookie quality. *Quality Assurance and Safety of Crops and Foods,* 6 (1) 81- 88.

Ajila C.M., Leelavathi K., Prasada Rao, U.J.S. (2008) Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of Cereal Science,* 48 (2) 319-326.

AACC (1999) Method 10-50.05. Baking quality of cookie flour. Approved methods of analysis (11th Ed.). American Association of Cereal Chemists AACC International, St. Paul, MN, USA.

Barroso L.S., de Oliveira V.R., Garcia A.V., Doneda D., Ourique L.A., Vieira M.M. (2015) Physicochemical and sensory evaluation of sandwich cookies made with carob powder. *Advance Journal of Food Science and Technology,* 9 (4) 290-295.

Chakraborty S.K., Kumbhar B.K., Chakraborty S, Yadav P., Prakash Sharma H. (2010) Colour characteristics and overall acceptability of fibre enriched biscuits: a response surface approach. *International Journal of Food Engineering*, 6 (6) 6.

COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

Croatian National Guidelines on microbiological criteria for food stuffs (2011) 3rd ed, Ministry of Agriculture, Zagreb, Croatia.

Crofton E.C., Markey A., Scannell A.G.M. (2013) Consumers' expectations and needs towards healthy cereal based snacks: An exploratory study among Irish adults*. British Food Journal*, 115 (8) 1130-1148.

Curutchet A., Cozzano S., Tárrega A., Arcia P. (2019) Blueberry pomace as a source of antioxidant fibre in cookies: Consumer's expectations and critical attributes for developing a new product. *Food Science and Technology International,* 25 (8) 642-648.

Čukelj N., Putnik P., Novotni D., Ajredini S., Voučko B., Ćurić, D. (2016) Market potential of lignans and omega-3 functional cookies. *British Food Journal*, 118 (10) 2420-2433.

Fadel H.M., Abdel Mageed M.A., Abdel Samad A.K.M.E., Lotfy S.N. (2006) Cocoa substitute: Evaluation of sensory qualities and flavour stability. *European Food Research and Technology*, 223 (1) 125–131.

Galanakis C.M. (2012) Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, 26 (2) 68–87.

Galanakis C.M. (2017) *Handbook of grape processing by products sustainable solutions*.1st ed. London: Academic press.

ISO 6579-1:2017 (2017) Horizontal method for the detection, enumeration and serotyping of Salmonella spp. Geneva, Switzerland: International Organization for Standardization (ISO).

Karnopp A. R., Figueroa A. M., Los P. R., Teles J. C., Simões D. R. S., Barana A. C., Kubiaki F. T., de Oliveiea J. G. B., Granato D. (2015) Effects of whole-wheat flour and bordeaux grape pomace (*Vitis labrusca* L.) on the sensory, physicochemical and functional properties of cookies. *Ciência e Tecnologia de Alimentos*, 35(4) 750-756.

Khanal R.C., Howard L.R., Brownmiller C.R., Prior, R.L. (2009) Influence of extrusion processing on procyanidin composition and total anthocyanin contents of blueberry pomace. *Journal of Food Science*, 74 (2) H52–H58.

Konstantas A., Stamford L., Azapagic A. (2019) Evaluation of environmental sustainability of biscuits at the product and sectoral levels. *Journal of Cleaner Production,* 230 1217-1228.

Kuchtova V., Karovičova J., Kohajdova Z., Minarovičova L., Kimličkova, V. (2016) Effects of white grape preparation on sensory quality of cookies.

Acta Chimica Slovaca, 9 (2) 84-88.

Loullis A., Pinakoulaki E. (2018) Carob as cocoa substitute: a review on composition, health benefits and food applications. *European Food Research and Technology*, 244 (6) 959–977.

Mamat H., Hill S.E. (2018) Structural and functional properties of major ingredients of biscuit. *International Food Research Journal* 25(2) 462-471.

Manley D. (2000) *Technology of biscuits, crackers and cookies*. 3rd ed. Cambridge: Woodhead Publishing Limited.

Mildner-Szkudlarz S., Bajerska J., Zawirska-Wojtasiak R., Górecka D. (2013) White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. *Journal of the Science of Food and Agriculture,* 93 (2) 389-95.

Palocci C.S., Chronopoulou L. (2015) *Supercritical fluid extraction of pharmaceutic compounds from waste materials derived from vinification processes*. In: Fruit and Pomace Extracts: Biological Activity, Potential Applications and Beneficial Health Effects, J.P. Owen (Ed). Future Medicine Ltd., p. 69–79. London: Nova Science Publishers.

Pathare P.B., Opara U.L., Al-Said F.A.J. (2013) Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food and Bioprocess Technology,* 6 (1) 36-60.

Pawłowska K., Kuligowski M., Jasińska-Kuligowska I., Kidon M., Siger A., Rudzinsk M., Nowak, J. (2018) Effect of replacing cocoa powder by carob powder in the muffins on sensory and physicochemical properties. *Plant Foods for Human Nutrition,* 73 196–202.

Prokopov T., Goranova Z., Baeva M., Slavov A., Galanakis M.C. (2015) Effects of powder from white cabbage outer leaves on sponge cake quality. *International Agrophysis,* 29 (4) 493–500.

Rikon S.M., del Valle F. (1982) US Patent No. 4,335,153. Imitation cocoa powder and method of preparing same. Washington, DC: U.S. Patent Office.

Rosa C.S., Tessele K., Prestes R.C., Silveira M., Franco F. (2015) Effect of substituting of cocoa powder for carob flour in cakes made with soy and banana flours. *International Food Research Journal,* 22 (5) 2111-2118.

Silanikove N., Landau S., Or D., Kababya D., Bruckental I., Nitsan, Z. (2006) Analytical approach and effects of condensed tannins in carob pods (*Ceratonia siliqua*) on feed intake, digestive and metabolic responses of kids. *Livestock Science,* 99 (1) 29-38.

Singh A., Rana I., Sahi N.C., Lohani U.C., Chand, K. (2012) Optimization of process variables for preparation of apple pomace - black soyflour based biscuits. International Journal of Food, *Agriculture and Veterinary Sciences,* 2 (1) 101-106.

Svensson L. (2012) Design and performance of small scale sensory consumer tests. University of Agricultural Sciences: The Faculty of Natural Resources and Agricultural Sciences, Uppsala. Retrived from Uppsala website: http://stud.epsilon.slu.se/50 45/1

Šarić B., Mišan A., Mandić A., Nedeljković N., Pojić M., Pestorić M., Đilas S. (2016) Valorisation of raspberry and blueberry pomace through the formulation of value-added gluten-free cookies. *Journal of Food Science and Technology,* 53 (2) 1140–1150.

Turksoy S. and Ozkaya B. (2011) Pumpkin and carrot pomace powders as a source of dietary fiber and their effects on the mixing properties of wheat flour dough and cookie quality. *Food Science and Technology Research*, 17 (6) 545–553.

Walker R., Tseng A., Cavender G.A., Zhao Y., Ross, A. 2014. Physicochemical, nutritional, and sensory qualities of wine grape pomace fortified baked goods. *Journal of Food Science,* 79 (9) 1811-1822.

Table S1. Optimization and validation of a powdered mixture of aronia (%) and grape pomace (%) for partial replacement of cocoa powder (%) in cookie formulation

Cookie property	Predicted Value	Measured Value	Prediction Error
Diameter (cm)	4.99	4.95	-1 %
Height (cm)	0.66	0.61	-8 %
Spread Ratio	7.56	8.06	7 %
Hardness (N)	23.6	22.1	-6 %
Flexibility (mm)	3.8	3.5	-8 %
Toughness (N)	47.3	42.1	-11 %
Color L*	32.02	31.01	-3 %
Color a*	8.45	7.91	-6 %
Color b*	10.51	9.82	-7 %
Appearance	8	8	-2 %
Flavor	8	8	-2 %
Texture	8	8	0 %
Overall Acceptability	8	8	-1 %
Odor	N/A	9	8 %

Note: Prediction error is calculated as (Measured Value - Predicted Value) / Predicted Value * 100%. Negative values indicate an underestimation in prediction, while positive values indicate an overestimation.

Table S2. Purchase intention of whole grain, nutritionally improved and sustainable cookies by generation (number with percentage in brackets).

	Generation Z, n = 191	Generation Y, n = 337	Generation X, n = 153	Silver Generation, n = 76
I buy whole grain cookies because they are good for health				
I completely agree	16 (8)	49 (14)	25 (16)	23 (30)
I partially agree	57 (30)	129 (38)	62 (40)	25 (33)
I neither agree nor disagree	51 (27)	100 (30)	28 (18)	18 (24)
I somewhat disagree	22 (12)	21 (6)	10 (7)	2 (3)
I do not agree at all	31 (16)	29 (9)	18 (12)	7 (9)
Did not answer	14 (7)	9 (3)	10 (7)	1 (1)
Would you be attracted to a cookie with a changed or improved nutritional value (e.g., less sugar and/or fat, added minerals, added fiber, added antioxidants) if you knew that it had a beneficial effect on your health?				
Yes	155 (81)	266 (79)	119 (78)	61 (80.3)
No	24 (13)	62 (18)	26 (17)	14 (18.4)
Did not answer	12 (6)	9 (3)	8 (5)	1 (1.3)
Would you be willing to pay a higher price for cookies with altered or improved nutritional value (compared to a conventional cookie)?				
Yes	107 (56)	243 (72)	104 (68)	51 (67.1)
No	72 (38)	86 (26)	41 (27)	24 (31.6)
Did not answer	12 (6)	8 (2)	8 (5)	1 (1.3)
Would you be interested in buying cookies that, in addition to having a positive impact on human health, also has a positive impact on the environment?				
Yes	184 (96)	317 (94)	145 (95)	73 (96)
No	7 (4)	20 (6)	8 (5)	3 (4)
Would you be willing to buy cookies with by-products of the food industry?				
Yes	152 (80)	252 (75)	96 (63)	50 (66)
No	39 (20)	85 (25)	57 (37)	26 (34)

Table S3. Purchase intention of whole grain, nutritionally improved and sustainable products by country (number with percentage in brackets).

	Croatia, n = 472	France, n = 166	North Macedonia, n = 119
I buy whole grain cookies because they are good for health			
I completely agree	77 (16)	18 (11)	18 (15)
I partially agree	189 (40)	46 (28)	38 (32)
I neither agree nor disagree	123 (26)	48 (29)	26 (22)
I somewhat disagree	35 (7.5)	17 (10)	3 (2.5)
I do not agree at all	48 (10.5)	25 (15)	12 (10)
Did not answer		12 (7)	22 (18.5)
Would you be attracted to a cookie with a changed or improved nutritional value (e.g. less sugar and/or fat, added minerals, added fiber, added antioxidants) if you knew that it had a beneficial effect on your health?			
Yes	385 (82)	129 (78)	87 (73)
No	87 (18)	26 (15.5)	13 (11)
Did not answer		11 (6.5)	19 (16)
Would you be willing to pay a higher price for cookies with altered or improved nutritional value (compared to a standard cookie)?			
Yes	352 (75)	78 (47)	75 (63)
No	120 (25)	78 (47)	25 (21)
Did not answer		10 (6)	19 (16)
Would you be interested in buying cookies that, in addition to having a positive impact on human health, also has a positive impact on the environment?			
Yes	453 (96)[a]	158 (95)[a]	108 (91)[b]
No	19 (4)[a]	8 (5)[a]	11 (9)[b]
Would you be willing to buy cookies with by-products of the food industry?			
Yes	361 (76) [a]	140 (84) [a]	49 (41) [b]
No	111 (24) [a]	26 (16) [a]	70 (59) [b]

Figure S1. The appearance of biscuits depending on the mixture composition (cocoa, aronia pomace and grape pomace)

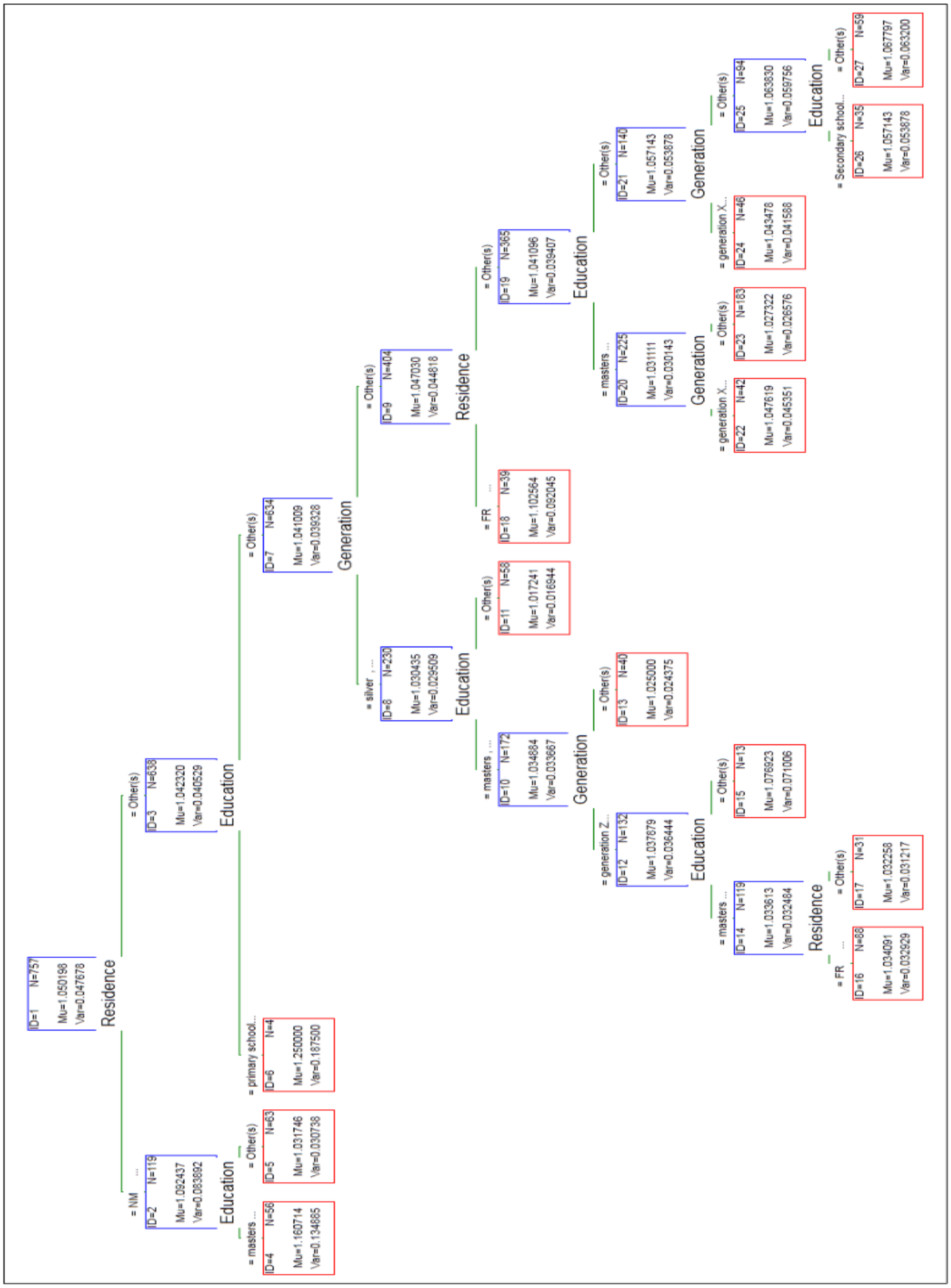

Figure S2. The classification and regression tree on the consumers interest in buying cookies that in addition to having a positive impact on human health, also have a positive impact on the environment.