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DOCTORAL DISSERTATION

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Supervisor:

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Zagreb, 2022

Prehrambeno-biotehnološki fakultet

Matea Habuš

RAZVOJ TRODIMENZIJSKI TISKANIH PROIZVODA OD ŽITARICA OBOGAĆENIH OBRAĐENIM PŠENIČNIM POSIJAMA

DOKTORSKI RAD

Mentor:
izv. prof. dr. sc. Dubravka Novotni

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Matea Habuš

Development of 3D-printed cereal-based products enriched with pre-processed wheat bran

Supervisor:
Dubravka Novotni, Ph.D., Associate professor (the University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Food Engineering)

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Development of 3D-printed cereal-based products enriched with pre-processed wheat bran

Matea Habuš, 390/PT

Short abstract: Wheat bran is an edible by-product rich in dietary fibre and polyphenolic compounds, whose addition in cereal foods diminishes their technological and sensory properties. Three-dimensional (3D) printing technology could provide consumers with a more diverse range of healthier snacks with added bran. This study focused on bran pre-processing (using micronisation, lactic acid fermentation, enzymes, high-intensity ultrasound, microwaves or pulsed light) and their incorporation into the 3D-printed oat snacks or breakfast cereals. Bran pre-processing with high-intensity ultrasound, microwaves and pulsed light reduced polyphenol oxidase activity (by 93%, 83%, or 78%, respectively) and enzymatic darkening of dough. Ultrasonication also lessened the activity of lipase (by 64%) and peroxidase (by 90%) and prolonged bran shelf-life. Bran fermentation degraded fructans by 93%. Pre-processing of the bran contributed to the high printing precision (up to 95%) and minimized deformation in baking (to 5%), and resulted in snack products and breakfast cereals of the desired properties.

Keywords: *wheat bran, high-intensity ultrasound, 3D printing, snack, enzyme treatment, sourdough fermentation*
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Razvoj trodimenzijski tiskanih proizvoda od žitarica obogaćenih obrađenim pšeničnim posijama

Matea Habuš, 390/PT

Sažetak: Pšenične posije su jestivi nusproizvod i bogat izvor prehrambenih vlakana i polifenolnih spojeva čiji dodatak rezultira nepoželjnim promjenama tehnoloških i senzorskih svojstava proizvoda od žitarica. Tehnologija trodimenzijskog (3D) tiska bi potrošačima mogla pružiti raznovrsniju ponudu zdravijih snack-proizvoda s dodanim posijama. U ovoj disertaciji istražena je prethodna brada posija (usitnjavanje, kisela fermentacija, enzimski tretman, ultrazvuk visokog intenziteta, mikrovalovi ili pulsirajuće svijetlo) i njihov dodatak u 3D tiskani zobeni snack-proizvod ili žitarice za doručak. Prethodna obrada posija ultrazvukom visokog intenziteta, mikrovalovima i pulsirajućim svjetlom smanjila je aktivnost polifenol oksidaze (za 93%, 83%, odnosno 78%) te enzimatsko tamnjenje tijesta. Ultrazvučna obrada rezultirala je i smanjenjem aktivnosti lipaze (za 64%) i peroksidaze (za 90%) te produljenom trajnošću posija. Fermentacija posija razgradila je udio fruktana za 93%. Prethodna obrada posija pridonijela je visokoj preciznosti tiska (do 95%) i smanjenju deformacije proizvoda pečenjem (na 5%), te rezultirala snack-proizvodima i žitaricama za doručak željenih svojstava.

Ključne riječi: *pšenične posije, ultrazvuk visokog intenziteta, 3D tisak, snack, enzimski tretman, fermentacija kiselog tijesta*
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I would like to dedicate this dissertation to the biggest fighter in the whole world, my Mother.

The dissertation topic was accepted at the 11th regular session of the Faculty Council of the Faculty of Food Technology and Biotechnology, the University of Zagreb in the academic year 2021/2022 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 March 16th, 2021 at the 8th regular session in the 352nd academic year (2020/2021).

Extended Abstract

Consumers show increased interest in a wider offer of healthier snack foods with reduced fat, sugar and salt content, but high content of bioactive compounds and dietary fibre. Extrusion-based 3D printing is an emerging technology that enables the development of healthier snack using alternative ingredients such as wheat bran, but also presents challenges such as dough darkening and post-process deformations. The aim of the present dissertation was to investigate the possibility of 3D printing of oat snack and breakfast cereals enriched with wheat bran, focusing on bran pre-processing while defining 3D printing conditions and a post-processing method. For pre-processing, non-thermal techniques (micronization, high-intensity ultrasound, pulsed light, and vacuum microwave cooking) were explored for the inactivation of undesirable enzymes and preservation of bioactive compounds in wheat bran, while bioprocessing with yeast, lactic acid bacteria or enzyme inulinase was investigated for the reduction of fructans in bran. The combining effect of adding glucose oxidase and xylanase in dough of reduced oil content was investigated for preventing its darkening or shrinkage. Wheat bran was high in dietary fibre (36.1%.), total phenolics (0.681 mg GAE/g d.w.) but also contained fructans (2.64%) that trigger symptoms in people with irritable bowel syndrome and enzymes such as polyphenol oxidase (68 U). Pre-processing of wheat bran with high-intensity ultrasound successfully reduced the activity of the polyphenol oxidase (by 93%), lipase (by 64%) and peroxidase (by 90%), and prolonged the oxidative stability of wheat bran during ambient storage by up to 12 months. Vacuum microwave cooking and pulsed light also reduced the activity of polyphenol oxidase (by 83 and 78%, respectively) of wheat bran and prevented dough darkening similarly as glucose oxidase. The bioprocessing of bran reduced its high content of fructans (up to 93%). At the optimum xylanase amount (37 U/g), shrinkage of snacks during baking was minimized from 32 to 5%, whereby the structure scanned by electron microscopy was more complex and compact. Pre-processing of bran improved the rheological properties of the dough, the quality of 3D printing, as well as the colour, textural and sensory properties of enriched snacks and breakfast cereals

Keywords: *wheat bran, high-intensity ultrasound, 3D printing, snack, enzyme treatment, sourdough fermentation*

Proširenи sažetak

Potrošači sve više traže raznolikiju ponudu zdravijih snack-proizvoda sa smanjenim udjelom masti, šećera i soli te velikim udjelom bioaktivnih tvari i prehrambenih vlakana. 3D tisak na principu ekstruzije inovativna je tehnologija koja omogućuje razvoj zdravijih snack-proizvoda koristeći alternativne sastojake kao što su pšenične posije, ali i postavlja izazove u proizvodnji poput tamnjenja tijesta i deformacije nakon završne obrade. Cilj ove disertacije bio je ispitati potencijal 3D tiska zobenih snack-proizvoda i žitarica za doručak obogaćenih pšeničnim posijama, pritom stavljajući naglasak na definiranje uvjeta procesa 3D tiskanja te metode završne obrade. Istražile su se netoplinske metode obrade (mikronizacija, ultrazvuk visokog intenziteta, pulsirajuće svjetlo i mikrovalno kuhanje pod vakuumom) za inaktivaciju nepoželjnih enzima i očuvanje sadržaja bioaktivnih spojeva u pšeničnim posijama, te fermentacija kvascima, bakterijama mliječne kiseline ili enzimima za smanjenje udjela fruktana. Ispitao se sinergijski učinak glukoza oksidaze i ksilanaze na sprječavanje tamnjenja i deformacije tijesta sa smanjenim udjelom ulja. Pšenične posije su sadržavale visok udio prehrambenih vlakana (36,1%), ukupnih fenola (0,681 mg GAL/g s.tv.), ali i fruktana (2,64%) koji izazivaju simptome kod pojedinaca oboljelih od sindroma iritabilnog crijeva te enzima poput polifenol oksidaze (68 U). Predobrada pšeničnih posija ultrazvukom visokog intenziteta uspješno je smanjila aktivnost enzima polifenol oksidaze (za 93%), lipaze (za 64%) i peroksidaze (za 90%), a uz to i produljila vrijeme skladištenja pšeničnih posija pri sobnoj temperaturi za 12 mjeseci. Mikrovalno kuhanje pod vakuumom i pulsirajuće svjetlo također su rezultirali smanjenjem aktivnosti polifenol oksidaze (za 83 i 78%) te sporijim tamnjenjem tijesta slično kao i dodatak glukoza oksidaze. Fermentacija posija dovela je do smanjenog udjela fruktana (za do 93%). Pri optimalnom udjelu dodane ksilanaze (37 U/g) smanjena je deformacija snack-proizvoda tijekom pečenja sa 32% na 5%, pri čemu je struktura slikana elektronskim mikroskopom nakon tretmana bila kompleksnija i kompaktnija. Obrada posija poboljšala je reološka svojstva tijesta kao i kvalitetu 3D tiska te boju, teksturalna i senzorska svojstva obogaćenih snack-proizvoda i žitarica za doručak.

Ključne riječi: *pšenične posije, ultrazvuk visokog intenziteta, 3D tisak, snack, enzimski tretman, fermentacija kiselog tijesta*

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

DUBRAVKA NOVOTNI is an associate professor at the Faculty of Food Technology and Biotechnology of the University of Zagreb. In 2011 she obtained a Ph.D. in Nutrition from the Faculty of Food Technology and Biotechnology, University of Zagreb. Since 2018, at the same faculty, she works as an Associate Professor teaching courses related to the 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 wheat and gluten-free 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 30 peer reviewed journal articles with more than 400 citations (h-index 12) and authored several book chapters with recognized scientific publishers like Elsevier and CRC Press. She presents her work continually at international scientific conferences in different countries. She reviewed numerous scientific papers for peer review journals. She is a member of the Technical Committee of International Association for Cereal Science and Technology, European Chemical Society, Division of Food Chemistry, 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), and editorial board of Journal of Food, Nutrition and Dietetics Research. Since 2006 she was 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), Croatia, and participates in „Flat bread of Mediterranean area; innovation & emerging process & technology“, Partnership for Research and Innovation in the Mediterranean Area (PRIMA, 2031) as well as in COST action SOURDOugh biotechnology network towards novel, healthier and sustainable food and bIoproCesseS (18101), Belgium.

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List of Abbreviations

ΔE^*	Total colour difference
3D	Three-dimensional
a^*	Redness
AB	Amaranth bran
ANN	Artificial neural networks
ANOVA	Analysis of variance
AO	Antioxidant activity
b^*	Yellowness
BI	Browning index
DPPH	2,2-diphenyl-1-picrylhydrazyl
FODMAP	Fermentable oligo- di- and monosaccharides and polyols
FRAP	Ferric reducing antioxidant power
G’	Storage modulus
G’’	Loss modulus
GOS	Galacto-oligosaccharides
GOX	Glucose oxidase
HIU	High-intensity ultrasound
IBS	Irritable bowel syndrome
L^*	Lightness
LAB	Lactic acid bacteria
NCWS	Non-celiac wheat sensitivity
PLS	Partial least squares
PPO	Polyphenol oxidase
RSM	Response surface methodology
TPC	Total phenolic content
WB	Wheat bran
XYL	Xylanase

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General introduction

Wheat bran (WB), a by-product of wheat milling, consists of the grain's outer layers, making it rich in fibre, phenolic acids, flavonoids and carotenoids (Prückler et al., 2014). Although it is commonly used as a livestock feed, interest in its use in the food industry is increasing due to its many beneficial effects on human health. Antioxidant and anti-inflammatory properties are attributed to the bioactive compounds in bran. In addition, adequate intake of cereal fibre and polyphenols protects against colon cancer, neurological and cardiovascular diseases. Still, WB contains high levels of fructans which are classified as fermentable oligo- di- and monosaccharides and polyols which trigger and aggravate the symptoms in people with irritable bowel syndrome (IBS) (Laurent et al., 2020; Nyyssölä et al., 2020). Although hydrolysis of cereal fructans and galacto-oligosaccharides (GOS) can occur by fermentation with microbial cultures, or with various enzymes, no study has investigated the effect of inulinase, *S. cerevisiae*, *K. marxianus,* or *L. fermentum* on the removal of fructan from WB. Due to the high content of glutathione and fibre, as well as the high activity of enzymes, the addition of WB results in products with impaired technological and sensory properties, such as altered appearance, colour, taste and hardness (Prückler et al., 2014; Laddomada et al., 2015). However, adding bran up to a maximum of 20% gives the product the desired sensory properties (Laddomada et al., 2015). Insoluble fibre predominates in WB, of which arabinoxylans are the most abundant. After xylanase treatment, they change into a soluble form, leading to an increase in the viscosity and stability of the dough (Santala et al., 2011).

Bran is associated with high activity of enzymes such as polyphenol oxidase (PPO), peroxidase, lipoxygenase, lipase and others, limiting its addition to food (Rani et al., 2001; Laddomada et al., 2015). PPO is a group of enzymes that catalyses the hydroxylation of monophenols and oxidation of *o*-diphenols to *o*-quinones in the presence of molecular oxygen, which can then react with phenolic groups of phenolic acids (Niu et al., 2014a). These reactions have a negative effect on the taste and texture and lead to an undesirable browning or discoloration of cereal foods (Hidalgo et al., 2013; Niu et al., 2014a), as well as to a reduction in their antioxidant activity (AO) (Laddomada et al., 2015).

Cryomilling is a method of the mechanical micronization of a material under cryogenic conditions. The negative effect of wheat bran on the dough's rheological properties and the final products' sensory properties is also attributed to the particle size of the bran. Its micronization improves cereal products' textural properties and sensory quality (Niu et al., 2014b). Micronization also increases AO and a desirable ratio of insoluble and soluble fibre (Čukelj

Mustač et al., 2020). In addition to micronization and enzymatic treatment, bran is treated by various thermal techniques to mitigate and eliminate the negative effects of adding bran to bakery products. While these treatments reduce enzymes activity and stabilize bran, they can lead to negative changes in taste, colour and nutritional composition of the food (Niu et al., 2014a). New technologies such as high-intensity ultrasound (HIU), pulsed light and microwaves are being investigated as non-aggressive alternatives. Ultrasound treatment has been shown effective in reducing the activity of amylase (Kadkhodaee and Povey, 2008), lipase, peroxidase (Ercan, 2013) and PPO in whole wheat flour (Hidalgo et al., 2013). Since most of the PPO is concentrated in the bran, HIU treatment applied to wheat bran, instead of whole wheat flour could be more efficient. However, the effect of HIU on bran PPO seems to depend on the type of cereal, as Čukelj Mustač et al. (2019) showed that HIU treatment leads to activation of millet bran PPO. Furthermore, microwaves inactivate lipases and peroxidases in wheat bran and germ (Ertaş, 2015; Kubo et al., 2020). Vacuum microwave cooking can influence the enzymatic activity of the raw material and contribute to the sensory profile of food and reduce the risk of microbial contamination during storage (Renna et al., 2017). In addition, pulsed light treatment can inactivate PPO of fungi (Pellicer et al., 2018) and horseradish peroxidases (Wang et al., 2017). However, there is no study investigating the influence of HIU or combined effect of HIU and micronization on PPO, bioactive compounds and technological properties of WB for direct food applications. Also, the effect of vacuum microwave cooking and pulsed light on PPO in WB remains unexplored.

There is a need to develop novel food manufacturing techniques for providing ready-to-eat, safe, and all-time accessible food products showing a beneficial effect on human health (Grasso, 2020; Hess and Slavin, 2018). An emerging technology that offers the possibility of producing food with complex and customized structure, texture and composition is three-dimensional (3D) printing (Sun et al., 2015). This refers to the layer-by-layer printing of the sample according to a given 3D-model (Sun et al., 2015; Lille et al., 2018). Among the various 3D printing techniques, extrusion-based 3D printing has been the most successful since it offers high precision and industrialization of the production of carbohydrate-rich food such as bread, biscuits and snacks (Liu et al., 2019). It was also proven advantageous in developing and producing healthy and customized snack products (Derossi et al., 2020; Keerthana et al., 2020; Lille et al., 2020). The quality of 3D printing depends on the pre-processing, as well as 3D printing and post-processing conditions. Food materials must possess suitable physical and chemical properties, including particle size, flow properties, rheological properties and

mechanical strength (Liu et al., 2019). Thereat, it is crucial to determine the rheological properties of the materials used, as it contributes to the printing performance and stability of the 3D-printed shape. The material must have a sufficiently low viscosity at high shear rates to flow through the 3D printer nozzle, but also can quickly increase viscosity after 3D printing to allow the next layer of material to be deposited (Lille et al., 2018). Due to its consistency, rheology and ability to solidify after printing, dough is the most suitable material for 3D printing food. Its flow behaviour and viscosity affect extrudability, while its elasticity, gel strength, fracture resistance and adhesive properties affect the ability to obtain the desired 3D structures (Jiang et al., 2019). Studies on the production of 3D-printed snacks have used wheat flour and starch, characterized by low nutritional value and rapid digestibility (Liu et al., 2019). To improve and increase the nutritional value of cereal products, white wheat flour can be replaced with whole oats or barley, which represent better sources of fibre, especially β-glucan (Rieder et al., 2012). To the best of our knowledge, there is no published report on the undesirable colour changes of 3D-printed dough over time, or on the effect of fructans removal techniques or fermentation, as well as pre-processing with HIU, vacuum microvawe cooking, and pulsed light on 3D printability of snacks.

In order to maintain a stable structure and shape, preserve nutritional value and sensory properties and prolong their stability, 3D-printed cereal foods need to be post-processed. Post-processing techniques include drying, cooking, baking, roasting, steaming or cooling (Sun et al., 2015; He et al., 2019), with the most common technique being a combination of freezing and drying in a conventional way or with microwaves and vacuum (He et al., 2019).

This research involves the pre-processing of WB using various methods and techniques to give an insight into the possibilities of its application in the production of 3D-printed snacks and breakfast cereals. Not only to achieve a lower activity of enzymes in bran but also to obtain desirable and/or improved rheological properties of the dough, as well as physical and sensory properties of baked snacks or breakfast cereals. Pre-processed bran can also benefit human health, due to its bioactive profile and lower level of fermentable oligosaccharides.
This dissertation in the form of published papers and a final comprehensive review aims to provide new insights into the effects of micronization, HIU, pulsed light and vacuum microwave cooking on the bioactive compounds and activity of WB enzymes. Degradation of fructans is necessary to make bran suitable for consumption by people with digestive disorders. The development of 3D-printed cereal products with improved nutritional value will contribute

to further extrusion-based 3D printing technology research. It will show the importance of optimizing the conditions of pre-processing, 3D printing and post-processing. The general discussion of this dissertation gives valuable information on the pre-processing of WB, from its enzyme activity to its final incorporation into 3D-printed snacks and breakfast cereals.

Chapter 1

Theoretical background

1. Ready-to-eat food – snacks and breakfast cereals
2. Wheat bran – rich in bioactive compounds, fibre, fructans and enzymes
3. Pre-processing of bran – conventional and emerging techniques
4. 3D printing of cereal food
5. Hypothesis, research objectives, and expected scientific contributions

1. Ready-to-eat food – snacks and breakfast cereals

The rapid increase of the competitive food market resulted in the development of various ready-to-eat snack food products, with the main aim of making them tasty for consumers (Grasso, 2020; Saleh et al., 2019). To achieve an appealing taste, adding natural or synthetic food flavours and taste enhancers such as sugars, alternative sweeteners, fat, and sodium is inevitable (Grasso, 2020). Therefore, the term "snack food", usually called "snacks", is mostly connected with energy-dense, nutrient-poor foods high in sugar, sodium, and/or saturated fat (Hess and Slavin, 2018). Overeating of snacks may increase the risk of chronic and food consumption-related diseases. Compared to main meals, snacks have an opposite influence on energy balance and may contribute to fat deposition (Saleh et al., 2019). However, although snacks are often considered detrimental to health, they could also be a useful tool to promote healthy eating habits (Beets et al., 2015) and be helpful for energy and blood glucose regulation (Bellisle, 2014). The foods categorized as snacks are based on grains, potato, meat or fruit. Salty snacks include crackers, popcorn, pretzels, crisp bread, puffed cereal cakes, chips and nuts (Chapelot, 2011; Green et al., 2017).

Besides snacks, breakfast cereals are another ready-to-eat food category (Devi et al., 2014) consumed daily (Lee and Ryu, 2015). Although breakfast is recommended as part of healthy diet, breakfast cereals are frequently linked with high amount of added sugar and saturated fat, as well as low amount of proteins (Angelino et al., 2019; Devi et al., 2014; Prada et al., 2021). Indeed, various studies have reported that the majority of breakfast cereals contribute to salt and sugars intakes in all groups of the population (Angelino et al., 2019; Chepulis et al., 2017; Nieto et al., 2017; Pombo-Rodrigues et al., 2017; Soo et al., 2016).

Nowadays, consumers aspire for healthier snack foods and breakfast cereals that are high in protein, dietary fibre and bioactive compound. Cereal grains and their processed foods represent the key of the human diet. Several studies have connected the intake of whole grains and reducing the risk of chronic diseases such as cancer, cardiovascular diseases, type II diabetes, and gastrointestinal disorders (Saleh et al., 2019). Whole grains contain all the important seed parts in their original proportions, including germ, bran and endosperm (Adebo and Medina-Meza, 2020). Their health benefits are mainly attributed to the micronutrients, phytochemicals, and dietary fibre, which are concentrated in the outer bran layer and the germ (Saleh et al., 2019). During the preparation and processing treatments, whole grains are faced with a substantial reduction in the content of nutrients, dietary fibre and bioactive compounds. Well

known strategies to avoid this problem are protein enrichment with legumes or the usage of bran to increase dietary fibre and bioactive compounds content (Onipe et al., 2019; Saleh et al., 2019). Legumes contain proteins, carbohydrates, dietary fibre, minerals, vitamins, enzyme inhibitors, lectins, phytosterols, and phenolic compounds (Singh and Basu, 2012). So far, navy bean, pinto bean, green lentil, or commercial yellow pea flour were successfully used for enrichment of cookies (Zucco et al., 2011). Further, wheat flour can be substituted or replaced with barley, oat, rye, and millet flour in highly nutritious breads (Collar and Angioloni, 2014; Ragaee et al., 2011; Saleh et al., 2019; Serpen et al., 2012; Sharma and Gujral, 2014). Moreover, the addition of WB to wheat flour resulted in enhanced AO of extruded snacks (Fleischman et al., 2016; Laus et al., 2017). Still, the incorporation of whole grain flour or bran can negatively affect the technological quality and sensory properties of foods, resulting in minor expansion and harder and darker products with lingering aftertaste and lower sensory scores (Onipe et al, 2015).

2. Wheat bran – rich in bioactive compounds, fibre, fructans and enzymes

Wheat bran is commonly used as a livestock feed, but the interest for its food uses is increasing due to its several potential benefits on human health. Complex structure of bran makes it a rich source of dietary fibre and various phytochemicals such as phenolic acids, flavonoids and carotenoids (Anson et al., 2012; Brewer et al., 2014; Elleuch et al., 2011; Onipe et al., 2015; Rico et al., 2020). WB, containing pericarp (inner and outer), seed coat, hyaline layer, aleurone layer, germ and starch endosperm residues, accounts for up to 25% of the total wheat grain weight (Anson et al., 2012). The outer and inner pericarp are rich in insoluble dietary fibre (heteroxylans and cellulose), while the seed coat contains lipid components, namely alkylresorcinols and sterols. The aleurone layer carries wheat bran's bioactive content, surrounded by thick cell walls constructed of arabinoxylans, *β*-glucans, and proteins (Anson et al., 2012; Hemery et al., 2010). It is rich in lignans, phytic acid, vitamins and minerals (Onipe et al., 2015).

The content of bioactive components in wheat covers a wider range due to different ecological factors, geographical areas of cultivation, genetic factors and evolution, as well as different methods of extraction and quantification (Anson et al., 2012). Numerous studies have shown that an adequate intake of cereal polyphenols including phenolic acids, lignans, flavonoids and carotenoids leads to protection against colon cancer, neurological and cardiovascular diseases (Anson et al., 2012; Brewer et al., 2014; Laddomada et al., 2015; Onipe et al., 2015; Rico et al., 2020).

The major part of WB AO can be attributed to its phenolic acids content (Laddomada et al., 2015; Rico et al., 2020). Phenolic acids belong to the group of polyphenols, and their content depends on climatic conditions, growing conditions and genetics, or varieties of different plant species (Laddomada et al., 2015). Their structure differs depending on the number and position of hydroxyl groups in the aromatic ring (Kim et al., 2006). They are divided into two groups: hydroxybenzoic and hydroxycinnamic acids. In whole grain, endosperm, bran, germ, and flour, they can be found in free, bound, and conjugated form (Laddomada et al., 2015). However, a higher proportion of phenolic acids is mainly located in the outer parts of the grain, especially the pericarp and the aleurone layer. In WB, derivatives of hydroxycinnamic acid are found in higher concentrations, primarily ferulic, *p*-coumaric acid, synapic, and vanilic acid. They are predominantly responsible for high antioxidant activity of WB (Laddomada et al., 2015; Rosa et al., 2013), due to the presence of CH=CH-COOH group which shows greater antioxidant

capacity compared to COOH group of hydroxybenzoic acid derivatives (Kim et al., 2006). The most abundant phenolic acid in wheat is ferulic acid and accounts for more than 70 to 90% of total phenolic acids of wheat bran (Brewer et al., 2014; Kim et al., 2006; Laddomada et al., 2015; Prückler et al., 2014). It shows antioxidant, anti-inflammatory and anti-cancer properties, and can inhibit lipid peroxidation by removing superoxide, as well as inhibit LDL-cholesterol oxidation (Anson et al., 2012).

The fibre content in all cereals decreases from the outer layers to the endosperm inside the grain (Dong et al., 2019). WB contains from 33% to 63% of dietary fibre, of which 46% are non-starch polysaccharides (Apprich et al., 2014; Stevenson et al., 2012). The most common are arabinoxylans (the main component of hemicellulose) (70%), cellulose (24%) and β-glucans (6%), while a slightly smaller portion falls on lignin, galactans and fructans (Apprich et al., 2014; Maes and Delcour, 2002; Stevenson et al., 2012). The outer layers of WB (pericarp) are constructed of hydrophobic, thick cell walls containing cellulose and complex xylans with a high ratio of arabinose and xylose. The primary role of the outer layers is to protect the wheat grain from various external factors (Charmet et al., 2007). Additionally, the pericarp and seed coat contain significant amounts of lignin (Prückler et al., 2014). On the other hand, the aleurone layer contains bioactive components surrounded by thick cell walls constructed of relatively linear arabinoxylans with a low ratio of arabinose and xylose (Charmet et al., 2007). Dietary fibre of cereals exhibit a numerous physiological functions; soluble fibre delay gastric emptying, improve gut health, control serum total and low-density cholesterol, as well as glycaemic index, whereas insoluble fibre increase stool volume, reduce appetite, food intake and blood glucose levels, and protect against type-2 diabetes (Elleuch, Bedigian, Roiseux, Besbes, & Blecker, 2011; Zhang, Li, Li, & Liu, 2019). Compared to barley and oats, wheat contains a small proportion of β-glucans, about 2 %, that are mainly found in the aleurone layer. However, wheat β-glucans have special properties of low solubility and formation of viscous solutions and gels (Prückler et al., 2014).

Various metabolic disorders among the population in developed countries are in direct correlation with poor nutrition habits. In people with irritable bowel syndrome and non-celiac wheat sensitivity (NCWS), different gastrointestinal symptoms (e.g. abdominal pain, swelling, constipation and diarrhoea) are caused by fermentable oligo- di- and monosaccharides and polyols, abbreviated as FODMAP, which include fructans and galacto-oligosaccharides, lactose, fructose when in excess to glucose, sorbitol, mannitol and xylitol (Ispiryan et al., 2020; Nyyssölä et al., 2020). These symptoms occur because FODMAPs are not digestible in the

human small intestine, but they are rapidly fermented by the intestinal microbiota (Atzler et al., 2020; Fang et al., 2021; Nyyssölä et al., 2020). Whole grain wheat, rye and barley contain a high amount of dietary fibre, but also fructans and GOS (Atzler et al., 2020; Haskå et al., 2008; Ispiryan et al., 2021; Prückler et al., 2015) which are mainly concentrated in the bran (Haskå et al., 2008; Laurent et al., 2020; Verspreet et al., 2015; Ziegler et al., 2016), whereas gluten-free grains such as oats or rice contain small amounts of FODMAPs (Biesiekierski et al., 2011; Ispiryan et al., 2021). The main function of fructans in plants is the storage of their carbohydrates. Plant fructans are structurally diverse molecules and can be divided into inulin (linear polymer with β (1–2)-linkages between the fructose molecules), levan (linear polymer with β (2–6)-linked fructose units) and mixed levan (containing both β (1–2) and β (2–6)-links). Mixed levan is present in wheat and barley (Nyyssölä et al., 2020). In addition, fructans are for healthy people considered as prebiotics and are an important source of sugar for yeast fermentation in bread making (Laurent et al., 2020). The market for low FODMAPs products is mainly based on gluten-free products that are not sensory attractive and have low nutritional value (Ispiryan et al., 2020). Therefore, there is a need to develop high-fibre but low-FODMAPs food.

The functionality of WB is largely determined by the high activities of the naturally occurring enzymes, including hydrolases (α-amylase, β-amylase, peptidase, endoxylanases, xylosidase), oxidoreductase (lipase, lipoxygenase, peroxidase, PPO), and isomerase (Hemdane et al., 2016; Hu et al., 2018; Rani et al., 2001). These bran-related enzymes have the potential to interact with specific wheat flour constituents and, therefore, affect the bread making process (Hemdane et al., 2016). Wheat α-amylase is located mainly in the pericarp with small quantities present in aleurone layer and the seed coat. The scutellum and embryo are rich in lipoxygenase, while PPO and peroxidase are predominant in all bran layers (Rani et al., 2001). Excess α-amylase activity can cause starch breakdown during dough mixing and fermentation, resulting in bread that is generally unacceptable to consumers. Regarding peptidases, endopeptidases are most relevant for the functionality in bread making as these enzymes can weaken the gluten network through cleaving its proteins. Endoxylanases can have a positive effect in bread making, converting arabinoxylans into a soluble form, thereby increasing the viscosity of the dough and its stability. Depending on the endoxylanases activity, the addition of WB can increase the stickiness of the dough, or the volume of the final product (Hemdane et al., 2016; Liu et al., 2017; Rani et al., 2001).

However, a major challenge in the food industry represents PPO due to the involvement in the darkening of wheat products and other cereals, vegetables and fruits (Hemdane et al., 2016; Saqlan Naqvi et al., 2013). It is a group of enzymes that catalyses two types of reactions in the same time. First one is the hydroxylation of monophenols into *o*-diphenols and second one is the oxidation of *o*-diphenols to *o*-quinons in the presence of molecular oxygen, which can then react with phenolic groups of phenolic acids (Fan et al., 2020; Niu et al., 2014b; Pathare et al., 2013; Pellicer and Gómez-López, 2017; Saqlan Naqvi et al., 2013; X. tian Zhang et al., 2019; Zhao et al., 2020). Resulting *o*-quinons can potentially further react non-enzymatically with other phenolic compounds, amino acids and proteins, and result in formation of brown complexes, i.e. melanins, or a decrease in antioxidant activity (Laddomada et al., 2015; Saqlan Naqvi et al., 2013; Silva et al., 2015; Zhao et al., 2020). These reactions negatively affect the flavour and texture and can also lead to undesirable discoloration of wheat foods, such as noodles and chapattis (Niu et al., 2014b; Yadav et al., 2010; Zhao et al., 2020). Due to PPO activity being easily controlled by pH, temperature, and processing (Ghazal et al., 2019; Niu et al., 2014b; Yadav et al., 2010) various thermal and non-thermal processing techniques that can inactivate PPO are being developed.

Still, rapid deterioration of its lipids is the main reason for WB being unsuitable for most food applications (Hu et al., 2018; Kong et al., 2021; Sharma et al., 2014; Sudha et al., 2011). A combination of high amounts of unsaturated fatty acids and enzymes that cause chemical spoilage results in bran with short shelf-life (Hu et al., 2018; Poudel and Rose, 2018). Lipase action initiates release of polyunsaturated fatty acids from triglycerides during the storage under ambient conditions (Hemdane et al., 2016; Hu et al., 2018). Following lipolysis, peroxidase promotes oxidation of free fatty acids, which further contributes to oxidative rancidity (Hemdane et al., 2016; Piechowiak et al., 2018; Poudel and Rose, 2018). The bran lipid oxidation rate is influenced by the fatty acid composition, the amount of antioxidants, metals and water, and the storage temperature (He et al., 2020; Kong et al., 2021; Sharma et al., 2014). The main strategies for extending the shelf life of bran are aimed at controlling the activity of lipid-degrading enzymes (Liao et al., 2020; Piechowiak et al., 2018; Tolouie et al., 2018). Although much is known about autoxidation and enzymatic oxidation of pure oils and fats, the oxidation rate of lipids in wheat by-products is weakly understood.

Because of its composition and possible isolation of its valuable ingredients (Apprich et al., 2014), WB is increasingly used in producing cereal-based products (Laddomada et al., 2015).

However, its application is mainly in the composition of whole wheat flour, since it negatively affects the technological quality, sensory features and oxidative stability of resulting product, leading to consumption of bran products being lower than the recommended doses.

3. Pre-processing of bran - conventional and emerging techniques

Various pre-treatments before milling, as well as milling, fractionation, and processing techniques are developing in order to modify the technological, sensorial, and biochemical properties of bran (Anson et al., 2012; Parenti et al., 2020).

Soaking and fermentation are commonly used to process bran and can decrease phytic acid and improve the bioavailability of minerals (Aivaz M and Mosharraf L, 2013; Mohammadi et al., 2021). Soaking is frequently used for grain-based beverages like wheat bran tea (Wang et al., 2019). Moreover, it showed a positive effect on the extractability of bound phenolic in rice bran (Zhao et al., 2022). It was successfully used for maize bran conversion to biomethane (Cayetano et al., 2019). Further, the most common fermentation processes are yeast fermentation and sourdough. When compared to non-fermented, fermented wheat grains contain higher levels of antioxidants. Bioprocessing of WB increases the bioavailability of ferulic acid and other phenolic compounds (Anson et al., 2012). The action of endogenous enzymes in favourable pH value adjusted due to the fermentation causes hydrolysis and solubilisation of proteins and fibre, and thus affect the bioavailability of minerals and phytochemicals in bran (Anson et al., 2012).

Application of numerous enzymes can target specific linkages in the arabinoxylans (Anson et al., 2012). So, for example, endo-β-(1,4)-D-xylanases cleave the xylans backbone internally, β-D-xylosidase remove xylose monomers from the non-reducing end of xylo-oligosaccharides, α-L-arabinofuranosidase remove arabinose substituents from the xylans backbone, and ferulic acid esterase remove ferulic acid groups from arabinose substituents. These enzymes' actions can release compounds bound to arabinoxylans, i.e. increase their bioaccessibility and bioavailability. Moreover, treatment with xylanase (XYL) increases the soluble fibre and water extractable ferulic acid content (Anson et al., 2012). Enzymatic treatment with XYL affects dough rheology by increasing the content of soluble fibre, especially arabinoxylans, (Onipe et al., 2019), and the water-binding capacity of the dough (Park et al., 2019), due to which it could potentially lead to better stability and strength of bran-enriched dough (Dai and Tyl, 2021).

Also, in order to mitigate and eliminate the negative effects of adding bran to bakery products various thermal techniques are applied. Partial or complete inactivation of WB lipase or peroxidase was achieved by conventional heating, autoclaving, toasting, extrusion cooking, microwave, dielectric heating (Sharma et al., 2014; Sudha et al., 2011), superheated steam treatment, and steam explosion (Hu et al., 2018; Kong et al., 2021). Ultraviolet irradiation, infrared radiation, ultrasound, ohmic, radio frequency, and microwave heating, as emerging

technologies, have been investigated for extending the shelf life of rice bran (He et al., 2020; Lakkakula et al., 2004; Liao et al., 2020; Ling et al., 2018; Mohammadi et al., 2021; Yu et al., 2020). Although successful in enzyme inactivation and stabilization of bran, these processing techniques result in negative changes in taste, colour and nutritional composition of the food (Niu et al., 2014a).

Ultrasound is novel non-thermal processing technique with low environmental impact, that offers high speed and process efficiency, better quality product due to retaining its natural characteristics (texture, nutritional value, organoleptic properties) and extended shelf life (Bhargava et al., 2021; Viell et al., 2020). The mechanism of its action is based on the formation of high-frequency ultrasonic waves that are capable of causing cavitation due to the expansion and contraction cycles experienced by the material, thereby enhancing mass transport by disrupting the plant cell walls (Espada-Bellido et al., 2017; Viell et al., 2020). It has been widely used for the extraction of bioactive compounds from numerous plant materials (Cengiz et al., 2021; Torres and Domínguez, 2020). Ultrasound treatment reduced the activity of lipase and peroxidase in tomato (Ercan, 2013). Recently, HIU was also found effective in reducing PPO activity of vegetables (Fan et al., 2020; X. tian Zhang et al., 2019) and fruit (Costa et al., 2013; Rodríguez et al., 2015). Moreover, HIU successfully reduced the activity of whole wheat flour PPO (Hidalgo et al., 2013), but combined with the usage of ascorbic acid (Niu et al., 2014). Still, in case of millet bran, HIU treatment resulted in activation of PPO. It could be pointed out that the effect of HIU on bran PPO is dependent on the type of cereal, and that it is necessary to optimize the parameters of HIU processing.

Microwave heating of a particular material is caused by the ability of the material to absorb microwave energy and convert it into heat (Chandrasekaran et al., 2013). Its application on barley (Sharma and Gujral, 2011), pear snacks (Devi et al., 2021), apple juice (Siguemoto et al., 2018) and strawberry purée (Marszałek et al., 2015) resulted in a successful inactivation of PPO. Microwave processing in the food industry is increasingly combined with vacuum because it contributes to faster water evaporation at temperatures below boiling point, thus enabling a shorter processing time (Song et al., 2018). Due to the removal of air the process of oxidation of compounds present in the treated food is prevented, which further preserves the colour and texture of the food (Chandrasekaran et al., 2013). During treatment with microwave vacuum cooking, which is carried out at a lower temperature compared to microwave heating at atmospheric pressure, the preservation of antioxidant compounds is also preserved (Song et

al., 2018). Also, the use of microwave vacuum cooking reduces the possibility of creating burns on the surface of treated products and improves the energy efficiency of the process (Zhang et al., 2007). Therefore, vacuum microwave cooking can influence the enzymatic activity of the raw material, contribute to the sensory profile of food and reduce the risk of microbial contamination during storage (Renna et al., 2017). However, its effect on WB has not yet been investigated.

Pulsed light technology is a non-thermal food processing method based on applying one or more pulses of high-intensity wide-spectrum light encompassing from UV to infrared (Pellicer et al., 2018). Its application has been mainly focused on microbial inactivation, but in the last years it has been also used for enzyme inactivation (Pellicer and Gómez-López, 2017; Vollmer et al., 2020; Wang et al., 2017). Pulsed light has so far effectively inactivated PPO of fungi (Pellicer et al., 2018), pineapple juice (Vollmer et al., 2020), and horseradish peroxidases (Wang et al., 2017).

Another emerging technology, micronization, resulting in particles with median diameter of 50^{th} percentile (d (50)) smaller than 50 μm, drastically decreases the particle size of materials and produces powder with improved surface characteristics for improving its dispersibility and solubility (Lin et al., 2021). Micronization of WB improves the textural properties and sensory quality of cereal products (Niu et al., 2014b), resulting in increased AO and a desirable ratio of insoluble and soluble fibre (Čukelj Mustač et al., 2020; Lin et al., 2021). Micronization performed at temperatures below 0 °C using liquid nitrogen is known as cryogenic grinding (Anson et al., 2012). Cryogenic grinding aims particles fragmentation and preservation of thermo-labile compounds. As it causes a decrease in bran particle size and a disruption of the cell walls, it could possibly lead to higher bioaccessibility and bioavailability of bran bioactive compounds (Anson et al., 2012; Hemery et al., 2010).

To give a better overview, in Table 1 examples of application of HIU, vacuum microwave cooking, pulsed light or micronization aiming at investigating their effect on the reduction of enzymes activity or bioactive compounds content are presented.

Table 1. Latest examples of different bran processed to investigate its enzyme activity, antioxidant activity (AO) or polyphenol content (TPC)

Material	Processing method	Conditions	Purpose	Results	Literature
Wheat bran	- steam explosion and conventional thermal sterilization	- steam explosion: reactor chamber at 0.3, 0.5 and 0.8 mPa for 5 min - sterilization: vertical autoclave at 121°C for 20 min	- stabilization of bran - maintenance or improvement of nutritional profile of bran with steam explosion	- more successful inactivation of lipase with steam explosion - complete inactivation of peroxidase - increase of peroxide value of wheat bran after both treatments - a decrease of fatty acid value in treated bran after 28 days of storage in PE bags at 50°C	Kong et al., 2021
Wheat bran	- microwave	- microwave power of 2.5, 5.0, 7.5, or 10.0 kW - time of 15, 30, 60, 90, or 120 s	- investigation on the stability and antioxidant capacity of bran	- decreased lipase activity, free fatty acid and moisture content - stronger TPC and AO - optimal conditions for stabilizing the bran: 7.5 kW and 120 s	Liu et al., 2021
Wheat and oat bran	- ultrasound	- frequency of 20, 25, and 45 kHz - time of 10 min - temperature of 25 ± 1°C	- assistance of the extraction of phenolics - conduct the kinetics of ultrasound-assisted extraction	- application of ultrasound altered the microscopic structure of the bran tissue and enhanced the extraction of free phenolics - introduction of a new constant related to the extraction time of phenolic	Milićević et al., 2021
Wheat bran	- microwave	- power 700 W - time 60, 90, 120, 150, and 180 s	- stabilization and extension of shelf life of whole wheat flour	- reduced lipase activity - retarded rancidity - maintained quality of whole wheat flour dough and steamed bread	Qu et al., 2021
Wheat bran	- ultrasound	- ultrasonic treatment: frequency of 100 Hz, power 120 W, and	- comparison of the effect of ultrasonic and thermal treatment with	- ultrasound more effective in achieving higher polyphenol extraction yield	Cherif et al., 2020

		acoustic energy density 120 W L^{-1} - thermal treatment: 3, 6, and 24 h at 50, 70, and 90°C	tertiary deep eutectic solvent in the polyphenol extraction yield and AO	- obtained extracts exhibited improved antioxidant characteristics	
Proso millet bran	- micronization	- ball mill - grinding with or without liquid nitrogen cooling (frequency 30 Hz, time 2, 4, 8 or 12 min)	- investigation of the potential of micronized proso millet bran as a functional ingredient for gluten-free bread	- bran diameter of 50^{th} percentile ranging between 171 and 26 µm - content of bioactive compounds does not highly depend on the bran particle size -increased percentage of the smallest particles with d (50) between 30 and 40 µm gives higher AO	Čukelj Mustač et al., 2020
Wheat bran	- ultrasound	- temperature 30, 50, or 70°C - time 20, 40, or 60 min - ultrasonic frequency 20, 35, or 50 kHz - ultrasonic power 200, 300, or 400 W	- maximization of phenolic compounds yield and antioxidant activity -optimization of the ultrasound-assisted extraction conditions	- wheat bran with increased TPC and AO - optimal conditions: temperature 50°C, time 40 min, 35 kHz and 300 W for ultrasonic frequency and power	Fan et al., 2020
Rice bran	- hot-air assisted radio frequency heating and extrusion	- (1) low temperature: heating to 100°C, held at 100-105°C for 15 min - (2) high temperature: heating to 110°C, held at 110-115°C for 6 min - heating rate ~9.4°C/min	- comparison of hot-air assisted radio frequency heating and extrusion - reduction of lipase and PPO activity - stabilization of bran	- reduced lipase and PPO activities - similar fatty acid composition and protein, lipid, total tocotrienol, and total tocopherol content in untreated and bran treated with hot-air heating - stronger AO and free flavonoid content in hot-air heated bran than after extrusion	Liao et al., 2020
Proso millet bran	- HIU		- increase of the amount of freely	- enhancement of AO and TPC at 80% amplitude for 12.5 min	Čukelj Mustač et al., 2019

			available bioactive compounds - analysis of the effect of HIU on enzymatic browning	- improvement of physical properties and browning at 100% amplitude for 5 min - HIU activates PPO - HIU treatment needs to be optimized depending on the bran purpose	
Rice bran	- microwave	- microwave power at 850, 925, and 1000 W - time 3, 4.5, and 6 min - temperature of 70°C - belt speed 10 mm/s	- investigation of microwave heating effect on rancidity of bran	- reduced free fatty acid, acid value, and peroxide value at higher power and treatment time - optimal conditions: 925 W for 3 min	Lavanya et al., 2019
Wheat bran	- conventional hot air and superheated steam explosion	- temperature of 170°C, time of 1-20 min at 1 min intervals	- inactivation of peroxidase and lipolytic enzymes - evaluation of nutritional attributes of bran	- superheated steam explosion inactivates enzymes faster and thus preserves lipid, protein, ash, and dietary fibre content - superheated steam explosion gives higher AO, increased extractable phenolic compounds content, lower peroxide value, and stronger unsaturated fatty acids content	Hu et al., 2018

4. 3D printing of cereal food

Three-dimensional (3D) food printing, known as additive manufacturing, is an emerging technology with a great potential in producing customized food with appealing forms, complex structure, tailored texture, and personalized nutritional values (Guo et al., 2021; Le-Bail et al., 2020; Y. Liu et al., 2020; Pulatsu et al., 2021; Wang et al., 2021). It combines three steps: modelling by using computer-aided design software, 3D printing and post-processing (Vieira et al., 2020; Wang et al., 2021). The main disadvantage of the 3D-printing process is the long production time for a large number of objects (Huang et al., 2019; Le-Bail et al., 2020; Z. Liu et al., 2020; Liu and Zhang, 2021). On the other hand, various food designs, mass customization, and the inclusion of alternative ingredients are the main advantages of 3D food printing (Lille et al., 2018; Theagarajan et al., 2020; Wang et al., 2021; Zhang et al., 2018). Among different 3D food printing methods, the extrusion-based 3D printing technology (Fig. 1) was proven as effective in the development of healthy, customized snack products from food pastes made mostly of wheat flour, protein, starch or fibre-rich materials (Derossi et al., 2021; Keerthana et al., 2020; Lille et al., 2018; Severini et al., 2018; Uribe-Wandurraga et al., 2020).

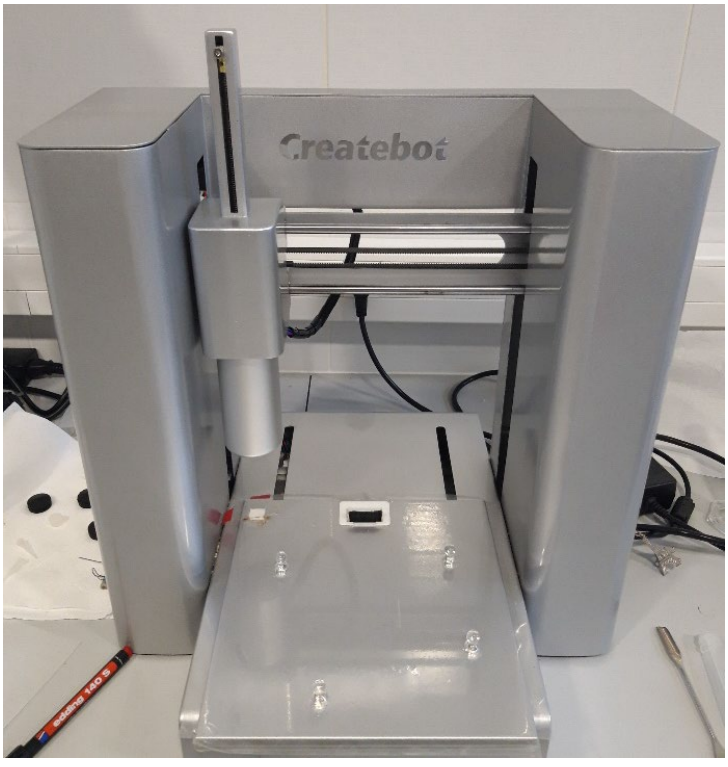

Figure 1. An example of 3D printer used in this study (Authors photograph)

Extrusion-based 3D printing is based on a material being extruded through a nozzle moving in x-, y- and z-direction, creating a given shape layer-by-layer (Kim et al., 2019; Sun et al., 2018;

Vieira et al., 2020). To achieve a desirable printing quality, a material to be printed needs to be of sufficiently low viscosity in order to be easily extruded through a nozzle (i.e. to flow during printing) and support its given structure during and after printing without deformation of the printed shape (Lille et al., 2018; Vieira et al., 2020). 3D food printing is strongly affected by the physicochemical properties of the ingredients used, i.e. by their particle size, flow properties, rheological properties and mechanical strengths. Also, it is necessary to define the process parameters, such as printing speed, extrusion and printing rate, infill percentage, nozzle diameter, and layer and nozzle heights (Derossi et al., 2020; Guénard-Lampron et al., 2021; Huang et al., 2019; Jagadiswaran et al., 2021; Krishnaraj et al., 2019; Liu et al., 2019; Theagarajan et al., 2020). The synergy of the recipe and printing parameters defines the printing precision and plays a key role in the 3D-printed product quality (Dankar et al., 2018; Vieira et al., 2020). The quality of printed food is also determined by post-processing (Wang et al., 2021) which mainly includes drying, cooking, baking, frying, and steaming (He et al., 2019; Sun et al., 2015). In this way 3D-printed food becomes microbiologically safe and gets its desired shape, texture, sensory and nutrition properties. However, high temperatures during post-processing can lead to deformations of printed shape (Krishnaraj et al., 2019; Pulatsu et al., 2020; Vieira et al., 2020). Generally, the most pliable material for 3D food printing is dough due to its consistency, rheology and ability to solidify after printing. Dough, however, inevitably requires post-processing (Severini et al., 2016). Different studies were conducted aiming at suppressing shape deformation of the dough while being exposed to high-temperatures during baking, cooking, or drying. Two main strategies are known; recipe modification or pre-processing of ingredients which mainly includes heating, blanching, hot air drying, grinding, ultrasound, or microwaves (Kim et al., 2019; Sun et al., 2020).

Regardless of the type of the food being 3D-printed, optimizing either pre-processing or post-processing parameters is crucial. In Table 2 most recent examples of cereal-based products manufactured by 3D printing are presented.

Table 2. Recent examples of 3D-printed cereal-based products

Ingredients	Pre-processing	Investigated parameters	Post-processing	Final product	Main results	Literature
Wheat flour, olive oil, sodium chloride, water	- none	- the number and the position of the internal voids of various 3D virtual models	- cooking at 150°C for 18 min	- snack	- deformation during post-processing less than 8% - 3D-printed snacks exhibited a great increase in porosity fraction, from 5 to 25% - the pore's length reduced due to the crushing of dough's filament - printed snacks with controlled voids follow the rule of cellular material	Derossi et al., 2021
Wheat flour, distilled water, carrot puree	-thermo-mechanical treatment	- nozzle diameter: 2.5, 3.4, 4.3, 5.1, and 6 mm - filling rate: 40, 55, 70, 85, and 100% - baking time: 15, 20, 25, and 30 min - baking temperature: 145, 170, 195, and 220 °C	- baking	- cake	- higher nozzle diameter increased shape difference and impacted textural properties - higher filling rate lowered shape deformation - the optimum parameters: a nozzle diameter of 3.4 mm, a filling rate of 71% and a baking at 170 °C during 25 min	Guénard-Lampron et al., 2021
Wheat flour, grape pomace powder, powdered sugar, margarine, baking soda, water	- none	- formulation - nozzle diameter: - extruder motor speed: - printing speed:	- baking at 130 °C for 12 min	- cookie	- optimal conditions for desirable printability: nozzle diameter of 1.28 mm, extruder motor speed of 600 rpm, and print speed of 400 mm/min - increase in grape content gave higher viscosity and improved printability	Jagadiswaran et al., 2021
Wheat, rice, and tapioca flour, butter, shortening, powdered sugar,	- heating	- composition - internal structure - pre-processing	- baking at 177 °C for 10 or 12 min, depending on the printed shape	- cookie	- dough with added tapioca flour was not printable - pre-heating increased the yield stress and printing pressures	Pulatsu et al., 2021

non-fat milk powder					- reproducibility of dough with wheat flour and shortening was poor - higher layers with good visual appearance and resolution of printed structure were enabled with pre-heating	
Oat, rice, rye, and carob flour, butter, olive oil	- none	- butter substitution with olive oil - extrusion rate - nozzle speed	- baking at 180° C for 3 min	- cookie	- higher viscosity, lower baking loss, and stronger printing quality achieved with olive oil - cookies with olive oil and rye or carob flour printed close to the ideal 3D shape (accuracy ≥98%) - each subsequent 3D cookie is printed at lower extrusion rate (ratio of weight of printed sample and printing time)	Vukušić Pavičić et al., 2021
Wheat flour, white button mushroom, water	- blanching of mushrooms	- printing speed: 200, 400, 600, 800, and 1000 mm/min - nozzle diameter: 1.28 and 0.82 mm - mushrooms content: 5, 10, 15, 20, and 25% w/w - microwave power: 450, 540, 630, 720, 810, and 900 W - exposure time: 2, 4, 6, 8, 10, and 12 min	- microwave cooking	- snack	- good stability achieved at: 20% mushroom powder, 800 mm/min printing speed using a 1.28 mm diameter nozzle, 300 rpm extrusion motor speed - optimum post-processing conditions: 800 W for 10 min	Keerthana et al., 2020
Whole milk powder, wholegrain rye flour, water	- none	- ratio of milk powder and rye flour - dough storage time	- baking at 150 °C for 5 (1-layer samples) or 10, 12, 15, and 20	- cookie	- dough was printable after 4 h storage at room temperature - all samples retained shape during baking	Lille et al., 2020

			min (5-layer samples)		- rye flour lowered the porosity of cookies	
Wheat flour, *Arthrospira platensis*, *Chlorella vulgaris*, egg powder, crystal sugar, sunflower oil, baking powder, fat-free milk powder	- none	- addition of microalgal biomass: 1, 2, 3, and 4%	- freezing at -18°C for 90 min and baking at 105°C for 30 min	- snack	- high positive correlation between the concentration of microalgae and storage modulus or shear modulus - 3 and 4% of Spirulina gave the most accurately printed snacks - microalgal biomass lowered the shape deformation	Uribe-Wandurraga et al., 2020
Wheat flour, *Arthrospira platensis* biomass, butter, powdered sugar, milk, xanthan gum	- ultrasound-assisted extraction of antioxidants - encapsulation of freeze-dried *A. platensis* within alginate microbeads	- incorporation of dried biomass, freeze-dried antioxidant extract, and antioxidant extract encapsulated into alginate microbeads	- baking at 150°C for 25 min	- cookie	- all dough formulations showed high shape fidelity with the 3D model - encapsulation of extract gave stronger antioxidant activity	Vieira et al., 2020
Wheat flour, butter, powder sugar, milk	- none	- replacement of wheat dough with hydrocolloids (methylcellulose and xanthan gum) at 0.5, 1, 2, and 3 g/100 g dough basis	-baking at 170°C for 15 min	- cookie	- incorporated xanthan gum resulted in poor 3D printing performance but aided dimensional stability during baking - methylcellulose incorporation exhibited high printability but low dimensional stability	Kim et al., 2019
Green gram, fried gram, barnyard millet, ajwain seeds	- hot air drying of raw materials	- nozzle diameter: 0.36, 0.84, and 1.28 mm - nozzle height: 25, 50, 75, and 100% of its diameter	- deep frying at 165 °C - hot air drying at 100°C for 20	- snack	- desired resolution and the best geometry stability achieved at: nozzle diameter of 0.84 mm, nozzle height of 0.63 mm, printing speed of 2400	Krishnaraj et al., 2019

		- printing speed: 800, 1600, and 2400 mm/min - extruder motor speed: 75, 150, 225, and 300 rpm - microwave power: 280, 560, 840, 1120, and 1400 W	min, and deep frying - microwave drying		mm/min, extruder motor speed of 300 rpm - microwaved samples showed the lowest shape deformation	
Starch, milk powder, cellulose nanofiber, rye bran, oat or faba bean protein concentrate	- none	- post-processing	- freeze drying at -18 °C - oven drying at 100 °C for 20-30 min	- fibre-rich food	- printed structure was better preserved by freeze-drying - oven drying results in high shrinkage of samples	Lille et al., 2018
Wheat flour, larvae of Yellow mealworms (*Tenebrio molitor*)	- grinding of larvae to pass through 900 µm sieve	- baking time: 14, 18, and 22 min - baking temperature: 180, 200, and 220°C - enrichment with insect: 0, 10, and 20%	- baking at conditions according to experimental design	- snack	- addition of insects reduced shape deformation during baking - at optimal baking conditions (22 min at 200 °C) the addition of 10 or 20% of insects increased the total essential amino acid	Severini et al., 2018

5. Hypothesis, research objectives, and expected scientific contributions

This research hypothesizes the following:

1) processing with novel technologies (micronization, high-intensity ultrasound, vacuum microwave cooking, pulsed light) leads to the deactivation of undesirable enzymes without reducing the bioactive content of wheat bran,
2) bioprocessing of wheat bran with yeasts, lactic acid bacteria and inulinase reduces its fructans content,
3) extrusion-based 3D printing technology is applicable in the development of cereal products with the addition of pre-processed bran,
4) the addition of xylanase contributes to the rheological properties of the dough intended for 3D-printing and its stability in post-processing,
5) post-process baking leads to the desired sensory properties, structure and colour of 3D-printed products.

The general objective of this dissertation was to develop healthier 3D-printed snack products and breakfast cereals supplemented with pre-processed bran. Specifically, this dissertation aims to investigate and optimize the pre-processing of wheat bran, dough 3D printing process and post-processing method for 3D-printed snacks and breakfast cereals.

The research plan was divided into two parts:

The first part deals with the influence of wheat bran micronization, high-intensity ultrasound, pulsed light, and microwave treatment on its enzyme activity and bioactive compounds (*Publication No. 1* and *Publication No. 2*).

The second part includes the development of the formulation of 3D-printed snack and breakfast cereals supplemented with pre-processed wheat bran (*Publication No. 3, Publication No. 4, and unpublished data)*.

Throughout this dissertation the following questions were examined:

1) What are the optimal conditions of high-intensity ultrasound treatment and grinding of wheat bran? How do they affect wheat bran's polyphenol oxidase activity and pasting properties? (*Publication No. 1*)

2) Can high-intensity ultrasound-treated wheat bran be oxidatively stabile during ambient storage? (*Publication No. 2*)
3) How flour type, dough pH value and printing temperature determine the 3D-printing quality? Can microwaves and pulsed light inactivate polyphenol oxidase in wheat bran successful as high-intensity ultrasound with less water added? (*Publication No. 3*)
4) Can bioprocessing with yeast, lactic acid bacteria or the enzyme inulinase reduce fructans content in wheat bran? Is such pre-processed bran suitable for supplementing 3D-printed snacks? (*Publication No. 4*)
5) Can xylanase improve the 3D-printing quality of the bran-enriched snack of reduced fat content? (*unpublished data*)

This dissertation achieved following:

(i) a better understanding of the effects of micronization, high intensity ultrasound, pulsed light and microwaves on the bioactive compounds and activity of wheat bran enzymes,
(ii) guidelines for the reduction of fructans content in bran,
(iii) development of 3D-printed cereal products supplemented with wheat bran,
(iv) contribution to further research into extrusion-based 3D printing technology.

Chapter 2

Publication No 1: Influence of particle size reduction and high-intensity ultrasound on polyphenol oxidase, phenolics and technological properties of wheat bran

Journal of Food Processing and Preservation

Chapter 2

Publication No. 1

Habuš, M., Novotni, D., Gregov, M., Štifter, S., Čukelj Mustač, N.I, Voučko, B., Ćurić, D. (2021) Influence of particle size reduction and high-intensity ultrasound on polyphenol oxidase, phenolics and technological properties of wheat bran. *Journal of Food Processing and Preservation*, **45**(3), e15204. (**Q2**)
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Dubravka Novotni: Conceptualization, Methodology, Formal analysis, Supervision, Writing – original draft, Writing - Review & Editing.

Marija Gregov: Investigation, Methodology, Formal analysis.

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Bojana Voučko: Methodology, Project administration, Writing – review & editing.

Duška Ćurić: Resources, Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Chapter 3

Publication No 2: High-intensity ultrasound treatment for prolongation of wheat bran oxidative stability

LWT – Food Science and Technology

Publication No. 2

Habuš, M., Novotni, D., Gregov, M., Čukelj Mustač, N., Voučko, B., Ćurić, D. (2021) High-intensity ultrasound treatment for prolongation of wheat bran oxidative stability. *LWT – Food Science and Technology*, **151**, 112110. (**Q1**)
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Chapter 4

Publication No 3: Influence of flour type, dough acidity, printing temperature and bran pre-processing on browning and 3D-printing performance of snacks

Food and Bioprocess Technology

Chapter 4

Publication No. 3

Habuš, M., Golubić, P., Vukušić Pavičić, T, Čukelj Mustač, N., Voučko, B., Herceg, Z., Ćurić, D., Novotni, D. (2021) Influence of flour type, dough acidity, printing temperature and bran pre-processing on browning and 3D-printing performance of snacks. *Food and Bioprocess Technology*, **14**, 2365-2379. (**Q1**)
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Chapter 5

***Publication No 4*:** Bioprocessing of wheat and amaranth bran for the reduction of fructan levels and application in 3D-printed snacks

Foods

Chapter 5

Publication No. 4

Habuš, M., Mykolenko, S., Iveković, S., Pastor, K., Kojić, J., Drakula, S., Ćurić, D., Novotni, D. (2022) Bioprocessing of wheat and amaranth bran for the reduction of fructans levels and application in 3D-printed snacks. *Foods*, **11** (11), 1649.
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Article

Bioprocessing of Wheat and Amaranth Bran for the Reduction of Fructan Levels and Application in 3D-Printed Snacks

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Abstract: Bran can enrich snacks with dietary fibre but contains fructans that trigger symptoms in people with irritable bowel syndrome (IBS). This study aimed to investigate the bioprocessing of wheat and amaranth bran for degrading fructans and its application (at 20% flour-based) in 3D-printed snacks. Bran was bioprocessed with *Saccharomyces cerevisiae* alone or combined with inulinase, *Kluyveromyces marxianus*, *Limosilactobacillus fermentum*, or commercial starter LV1 for 24 h. Fructans, fructose, glucose, and mannitol in the bran were analysed enzymatically. Dough rheology, snack printing precision, shrinkage in baking, texture, colour, and sensory attributes were determined. The fructan content of wheat bran was 2.64% dry weight, and in amaranth bran, it was 0.96% dry weight. Bioprocessing reduced fructan content (up to 93%) depending on the bran type and bioprocessing agent, while fructose and mannitol remained below the cut-off value for IBS patients. Bran bioprocessing increased the complex viscosity and yield stress of dough (by up to 43 and 183%, respectively) in addition to printing precision (by up to 13%), while it lessened shrinkage in baking (by 20–69%) and the hardness of the snacks (by 20%). The intensity of snack sensory attributes depended on the bran type and bioprocessing agent, but the liking ("neither like nor dislike") was similar between samples. In conclusion, snacks can be enriched with fibre while remaining low in fructans by applying bioprocessed wheat or amaranth bran and 3D printing.

Keywords: bran fermentation; FODMAP; fructose; lactic acid bacteria; *Kluyveromyces marxianus*; *Saccharomyces cerevisiae*; inulinase; 3D-printability

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1. Introduction

Fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs) are not digestible in the human small intestine but are rapidly fermented by the gut microbiota. FODMAPs include fructans, galacto-oligosaccharides (GOS), lactose, fructose in excess to glucose, sorbitol, mannitol, and xylitol [1–3]. Fructans are considered prebiotics for healthy people and are an important source of sugar for yeast fermentation in bread making [4]. Nevertheless, they can trigger abdominal pain, swelling, constipation, and diarrhoea in patients with irritable bowel syndrome (IBS) and non-celiac gluten sensitivity (NCWS) [2,4,5]. For these patients, it is necessary to reduce the total intake of FODMAPs below the cut-off value of 0.5 g per serving [1,3]. At the same time, eliminating foods rich in fructans could result in insufficient intake of dietary fibre and micronutrients as well

as undesirable changes in the gut microbiota [4,6]. The market for low FODMAPs products is based on gluten-free products that are not sensory attractive and have low nutritional value [7]. So, there is a need to develop high-fibre but low-FODMAPs food.

Whole grain wheat, rye, and barley contain a high amount of dietary fibre, but also fructans and GOS [1,8,9], which are mainly concentrated in the bran [4,8,10,11]. Gluten-free grains such as oats, millet, rice, and maise contain small amounts of FODMAPs [9,12]. Although gluten-free, some pseudo-cereals such as amaranth have a dubious reputation for containing FODMAPs. Processed products made from amaranth grains, which are rich in micronutrients and bioactive compounds, can have high FODMAPs levels [13]. Still, the FODMAPs content of amaranth bran (AB) has not been reported yet.

Hydrolysis of cereal fructans and GOS can occur by germination, fermentation with microbial cultures, or with various enzymes, such as α-galactosidase, inulinase, or invertase [2,3,5,14]. Enzymatic degradation of fructans has already been achieved in whole wheat flour and lentils [1] as well as in agave juice [15]. For the degradation of wheat FODMAPs with fermentation, the activity of fructanase and invertase was proved to be crucial [14,16,17]. Bakery yeast (*Saccharomyces cerevisiae*) produces the enzyme invertase [3,4,18], while *Kluyveromyces marxianus* produces the enzyme fructanase [17,19]. They are often used as co-cultures in the production of whole wheat bread [4,5,11,17,19,20], rye bread, and chapatti [5,6] as *K. marxianus* lacks the ability to degrade maltose, the main fermentable sugar produced in starch hydrolysis [3,19]. In addition to the degradation of fructans, the *S. cerevisiae*-derived invertase also catalyses the hydrolysis of sucrose and raffinose oligosaccharides [4]. Longer fermentation with lactic acid bacteria could lead to more successful degradation of FODMAPs in bran [2,3,5]. The use of different *Lactobacillus* species results in fructan degradation in wheat steamed bread [2] and wheat bran (WB) [21] and has been suggested to reduce fructan content in malt [7]. However, some lactic acid bacteria, such as *Levilactobacillus brevis* or *Leuconostoc citreum,* are able to convert sugars into mannitol [22]. According to our knowledge, no study has investigated the effect of inulinase, *S. cerevisiae*, *K. marxianus,* or *L. fermentum* on the removal of fructan from wheat or amaranth bran.

Wholegrain products tailored to specific dietary needs, e.g., with reduced FODMAPs content, could be offered as healthy snacks. Healthy snacks are a rising popular food category, as consumers seek low-sugar and salt and high fibre snacks [23]. Three-dimensional (3D) extrusion-based printing represents a novel approach for producing nutritionally adapted and balanced cereal snacks [24,25]. It provides a possibility of using alternative ingredients, but it is necessary to understand their rheological properties, i.e., their ability to flow and support the given 3D structure [25–27], as well as the ability of the dough to resist deformation during post-processing [28]. In recent years, several studies reported success in the 3D printing of snacks [24,26,29]. To our knowledge, no study has investigated the impact of fructans removal techniques or fermentation in general on the 3D-printability of snacks. Thus, the aim of this study was to investigate the bioprocessing of WB and AB for the removal of fructans and their application in 3D-printed snacks. Bran was bioprocessed with bakery yeast *Saccharomyces cerevisiae* (BY) alone or combined with enzyme inulinase, yeast *Kluyveromyces marxianus*, or lactic acid bacteria (LAB) *Limosilactobacillus fermentum* or using a commercial starter of mixed yeast and LAB cultures.

2. Materials and Methods

2.1. Materials

WB was a gift from an industrial Farina mill (Granolio Inc., Zagreb, Croatia), while AB was kindly provided by RICHOIL (Lviv, Ukraine) and the Association of Amaranth Producers and Processors (Dnipro, Ukraine). WB contained 17.8% protein, 12.3% moisture, 25.6% carbohydrate, 36.1% dietary fibre (of which soluble 4.6%), 4.3% fat, and 3.6% ash [30]. AB consisted of 17.0% protein, 7.6% moisture, 57.5% carbohydrate, 11.4% fibre (of which soluble 1.7%), 4.2% fat, and 2.3% ash. WB and AB showed unimodal particle

size distribution with a median 50th percentile diameter of 177.00 ± 2.26 and 242.08 ± 0.46 μm, respectively, determined using the Mastersizer 2000 instrument equipped with the Scirocco 2000 dry dispersion unit (Malvern Instruments, Worcestershire, UK) [30]. Liven-do™ LV1 starter, containing *Levilactobacillus brevis*, *Lacticaseibacillus casei*, and *Saccharomyces chevalieri*, was donated by Lesaffre Adriatic Inc. (Prigorje Brdovečko, Croatia). *Limosilactobacillus fermentum* (DSM 20052) was provided by Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ, Braunschweig, Germany), while *Kluyveromyces marxianus* (NBRC 1777) was donated by the Laboratory for Biochemical Engineering, Industrial Microbiology and Malting and Brewing Technology, Faculty of Food Technology and Biotechnology University of Zagreb. Inulinase from *Aspergillus niger* (EC 3.2.1.26., 2000 U/g) was kindly provided by BIO-CAT (Troy, VA, USA). Dry BY (Lesaffre Adriatic Inc., Prigorje Brdovečko, Croatia), as well as the ingredients for the dough preparation (oat flour (9.5% proteins, 5.6% lipids, 2.15% fibre (Garden Ltd., Zagreb, Croatia)), rice proteins (83% proteins, 4.5% lipids (Biovega, Ltd., Zagreb, Croatia)), sunflower oil (Zvijezda Ltd., Zagreb, Croatia), salt (Solana Pag Inc., Pag, Croatia), and baking powder (Podravka Inc., Koprivnica, Croatia) were purchased at the local market.

2.2. Bioprocessing of Bran

A schematic representation of the experiments is shown in Figure 1. Unprocessed bran served as a control sample. Aqueous suspensions (15% *w*/*w*) of WB or AB were incubated with LV1 starter (0.6% *w*/*w* on bran) or BY *S. cerevisiae* (10^4 CFU/g or approx. 0.06% *w*/*w* on bran) alone or in co-culture with the following cultures: *K. marxianus* (10^4 CFU/g bran), *L. fermentum* (10^6 CFU/g bran), or inulinase (0.1% *w*/*w* on bran) at 37 °C for 24h. Bioprocessing time was defined in preliminary experiments. The pre-culture of *L. fermentum* was prepared in MRS broth (Biolife, Monza, Italy) containing 2% (*w*/*v*) glucose, while the broth medium for the propagation of *K. marxianus* contained yeast extract, peptone, and glucose (1, 2 and 2% *w*/*v*, respectively). Both pre-cultures were incubated at 37 °C. After centrifugation, the cultures were dissolved in sterile water. The inoculum was homogenized by vortexing for 1 min and immediately used for fermentation together with BY. Water addition was subtracted by the amount of water previously added with inoculum.

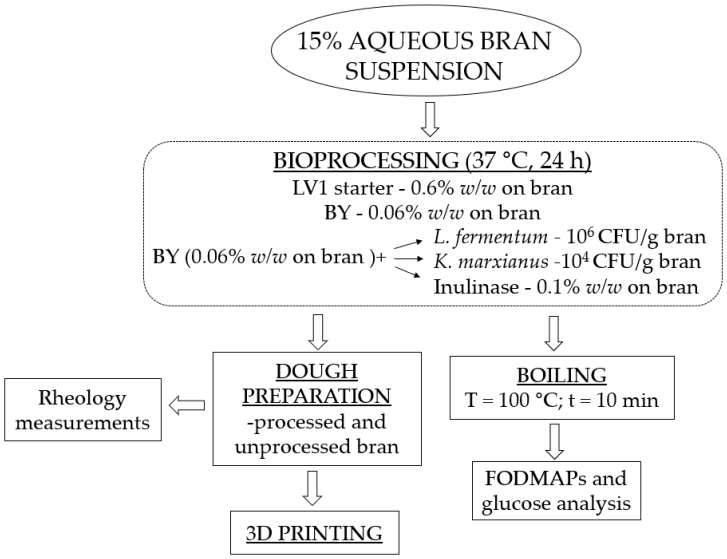

Figure 1. Schematic representation of bran bioprocessing and its use in dough preparation. BY, bakery yeast.

2.3. FODMAP Content

The content of fructans, fructose, glucose, and mannitol before and after bran bioprocessing was analysed enzymatically according to AOAC method 999.03 (with Fructan Assay Kit, Megazyme, Ireland), AOAC method 985.09 (with D-Fructose/D-Glucose Assay Kit, Megazyme, Ireland), and D-Mannitol Assay Kit (Megazyme, Ireland), respectively. Samples for the determination of fructose, glucose, and mannitol were freshly prepared on the day of analysis. Each sample suspension was heated at 80 °C for 10 min in a water bath. After cooling to room temperature and centrifugation at 4000 rpm for 10 min, 1.5 mL of the supernatant was centrifuged again at 14,700 rpm for 3 min [21]. The results for fructans represent the combined content of fructans and galactooligosaccharides, as the samples were not treated with α-galactosidase before analysis. A proximate total FODMAP content in 30 g of snack was calculated based on fructans, fructose, and mannitol content determined in the bran (3 g), oat flour (15 g), and rice protein (5 g) used for the preparation of the dough (Section 2.4.), considering the loss of water during baking (i.e., the baking loss). Baking loss was determined using the following formula [24]:

$$\text{Baking loss (\%)} = (m_{pd} - m_{bs}/m_{pd}) \times 100 \quad (1)$$

where m_{pd} represents the weight of dough before baking and m_{bs} the weight of baked snack.

2.4. Preparation of Dough

According to our previous study [24], the dough was prepared in three steps but with slight modifications. First, rice proteins, salt, baking powder, and sunflower oil were mixed with a hand mixer (M350LBW, Gorenje, Slovenia) (3 min at low speed), and then the suspension of unprocessed or bioprocessed WB or AB was added (mixing for 1 min at low and 1 min at medium speed), followed by the addition of oat flour (mixing for 2 min at low speed). The dough was immediately used for rheology analysis and 3D printing.

2.5. Rheological Properties

All oscillatory measurements were performed using a parallel plate geometry of 25 mm diameter with a 1 mm gap with an MCR 92 rheometer (Anton Paar, Graz, Austria). The amplitude sweep test (with the shear rate of 0.01–100 s^{-1} at a constant frequency of 1 Hz) was performed to determine the linear viscoelastic region (LVER) and the shear strain (0.05%) for the frequency sweep test, which was then employed in the range of 1–30 Hz at 20 °C [24]. Before each test, the dough was placed under the parallel plate and, after putting the plate down, trimmed if necessary. After duplicate measurements, the storage modulus (G′), loss modulus (G″), loss factor (tan δ = G″/G′), yield stress, flow point, and complex viscosity were calculated by the software Anton Paar RheoCompass (version 1.30.999, Graz, Austria).

2.6. Three-dimensional Printing and Post-Process Baking

The spiral shape (with 25 layers and a layer height of 0.4 mm) of the dough samples was 3D extruded using Createbot 3D Food Printer-Multi-Ingredient Support (Ningbo Createbot Electronic Technology Co., Ltd, Ningbo, China) and Cura 15.02.1 software. Printing was performed with a nozzle diameter of 1 mm, at a temperature of 20 °C, with a printing speed of 25 mm/s; thus, 407 s was needed to 3D print one sample.

Three-dimensional-printed samples were baked in a deck oven (EBO 64-320 IS 600, Wiesheu GmbH, Germany) for 18 min with the lower heater set at 140 °C and the upper heater at 160 °C [24]. Samples were cooled to room temperature before further analysis.

2.7. Three-dimensional Printability and Physical Properties of Baked Snacks

All physical properties were measured in 10 replicates, and the results are provided as mean values. The total height and line width at the top of the baked snack were determined at 4 positions (Figure 2a), while the snack diameter was determined at 2 positions (Figure 2b) using a calliper.

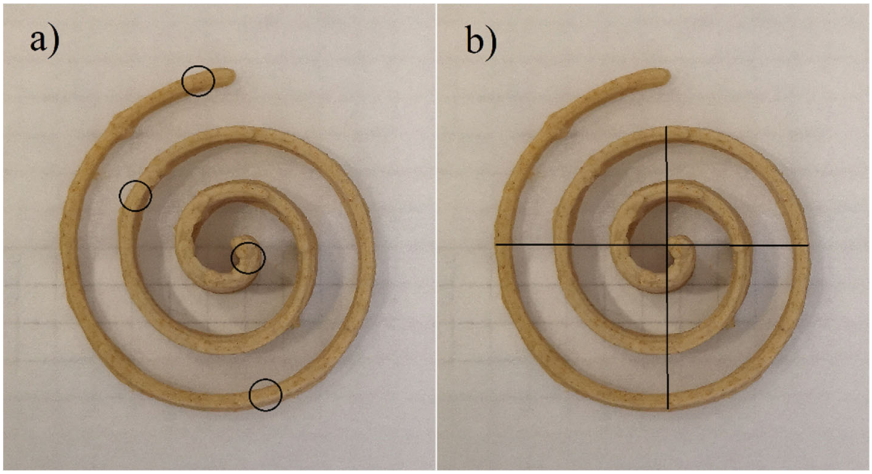

Figure 2. Measuring positions of: (**a**) snack height and line width; (**b**) diameter of baked snacks.

All samples were scanned at 600 dpi (CanoScan, LIDE 2020, Canon, Tokyo, Japan) after 3D printing and baking. The shape accuracy (%) of baked snacks was determined with digital image analysis (ImageJ, National Institutes of Health, Bethesda, MD, USA) as the proportion of black pixels, calculating the deviation of each printed sample from the one printed with the highest precision. In addition to the shape accuracy, printing quality was defined with 3D printing precision and shape shrinkage in baking, which was calculated as demonstrated previously [24]:

$$\text{Printing precision (\%)} = (D_L/D_n) \times 100 \quad (2)$$

where D_L is the width at the top of the 3D-printed dough (cm), and D_n is the diameter of the printing nozzle, both in cm.

$$\text{Shape shrinkage} = ((X_d - X_s)/X_d) \times 100 \quad (3)$$

where X_d and X_s are the total white pixels of the 3D-printed dough and baked snack, respectively.

The colour of the baked snacks, i.e., the lightness (L^*), redness (a^*), and yellowness (b^*), was measured with a colourimeter (Konica Minolta CM-700d, Tokyo, Japan). The browning index (BI) for each snack after baking was calculated using the following equation [24]:

$$BI = 100 \times ((((a^* + 1.75 \times L^*)/(5.645 \times L^* + a^* - 3.012 \times b^*)) - 0.31)/0.17) \quad (4)$$

The total colour change (ΔE^* value) between the first and last 3D-printed and baked snack was calculated [24]:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (5)$$

The hardness of baked snacks was analysed with a cutting test performed at a speed of 2 mm/s [29] using a texture analyser (Ametek Lloyd Instruments Ltd., West Sussex, UK) equipped with a 50 kg load cell and the Warner–Bratzler shear blade guillotine probe.

2.8. Sensory Analysis

The sensory evaluation of snacks with unprocessed and bioprocessed bran was carried out by a 13-member panel of semi-trained judges (average age of 35 years comprising 11 female and 2 male), employees from the Faculty of Food Technology and Biotechnology. Panellists had neither food allergies nor intolerances and were informed about the objectives of the study. Samples were randomly coded with three-digit numbers and served in random order in two sessions (containing either WB or AB) on two separate days. The descriptive sensory analysis included attributes related to the uniformity of surface colour, odour (bran, yeast), and taste/flavour (salty, bitter, oil, fermented) evaluated on a scale from not detectable (0) to attribute strongly expressed (5) [31]. Control samples with unprocessed bran were served as reference products with defined intensities of each attribute. A 5-point hedonic scale ranging from extremely dislike (1) to extremely like (5), where 2 = do not like it moderately, 3 = neither like it nor do not like it, and 4 = like it moderately, was used to assess the liking of snacks. Panellists were instructed to clean their palate between the samples with spring water.

2.9. Statistical Analysis

Factorial analysis of variance (ANOVA) was performed to establish the influence of the bran type and bioprocessing agents on the FODMAPs level of the bran, rheological properties, and 3D printability of the dough as well as physical parameters of the baked snacks. ANOVA and Tukey's post hoc test ($p < 0.05$) were carried out with Statistica 10 software (Stat Soft Inc., Tulsa, OK, USA). Principal component analysis (PCA) was performed using a PAST 4.09 software. The data matrix constructed of measured parameters was employed in unsupervised multivariate data processing in order to check the relationships between the investigated variables and 3D-printed snack samples [32].

3. Results and Discussion

3.1. FODMAP Content

Low fructan content was found in oat flour (0.15% dry weight), and rice proteins (0.14% dry weight) were used to prepare the dough. The fructan content of our unprocessed WB (Table 1) agrees with the previously reported fructans content of 2% (dry weight) in WB [8]. Unprocessed AB contained 2.5-fold fewer fructans compared to WB. The WB and AB were subjected to five different treatments to reduce their fructan content. All treatments resulted in a significantly lower content of fructans and GOS, depending on the interaction between bran type and bioprocessing agent ($p < 0.01$). Fermentation of WB with BY alone resulted in a 63% lower fructans content, while its combination with *L. fermentum* or *K. marxianus* resulted in a greater reduction in fructans and GOS, by 83 and 88%, respectively. In line with this, previous studies showed that the combined action of *S. cerevisiae* and *K. marxianus* leads to a 90% reduction in the fructans content of whole wheat bread, while treatment with bakery yeast alone leads to a 56–80% reduction, depending on BY concentration and fermentation time [3,4,17]. Lower degradation of wheat fructans by BY without *K. marxianus* may be due to a lower specificity of the invertase for higher polymerization oligosaccharides (fructans) as substrates or to the lower activity and amount of the synthesised enzyme from BY compared to the enzyme from *K. marxianus*. The most successful fermentation of WB was with the LV1 starter or the combination of BY with inulinase, both of which led to a 93% in fructans and GOS content. After the fermentation of AB, the fructan and GOS content were very low. Similar to WB, the greatest reduction of fructans and GOS by 92 and 95%, respectively, was also achieved in AB with BY alone or in combination with inulinase. Previously, the reduction of fructans content in WB after 18 h of incubation with different species of lactic acid bacteria ranged from 77% to almost complete degradation (99% reduction when using *Lactobacillus sanfranciscensis*) [21]. In addition, Atzler et al. [1] reported that fructans are not detectable after 2 h incubation of wholemeal wheat flour with inulinase (300 U/mL). Our study

shows that fructans can be degraded after long fermentation using yeast only, but in case of high concentrations such as in WB, bioprocessing means should be combined for bigger success.

Table 1. FODMAPs and glucose content in wheat and amaranth bran (% dry matter) and estimated total FODMAPs content in a serving of baked snacks (g/30 g).

Bioprocessing	Fructans	Fructose	Glucose	Mannitol	Total FODMAPs
Wheat bran					
None	2.64 ± 0.04 ^{a}	0.14 ± 0.05 ^{c}	0.47 ± 0.00 ^{efgh}	n.d.	0.12
LV1	0.18 ± 0.01 ^{de}	0.06 ± 0.00 ^{c}	0.03 ± 0.00 ^{i}	0.47 ± 0.01 ^{fgh}	0.06
BY	0.96 ± 0.02 ^{b}	0.09 ± 0.03 ^{c}	0.24 ± 0.02 ^{ghi}	0.65 ± 0.03 ^{bc}	0.08
BY + *L. fermentum*	0.33 ± 0.02 ^{c}	0.51 ± 0.06 ^{b}	0.09 ± 0.04 ^{i}	0.52 ± 0.01 ^{def}	0.08
BY + *K. marxianus*	0.30 ± 0.01 ^{c}	1.47 ± 0.16 ^{a}	0.52 ± 0.08 ^{cdef}	0.44 ± 0.01 ^{gh}	0.12
BY + inulinase	0.19 ± 0.02 ^{de}	n.d.	0.25 ± 0.02 ^{fghi}	0.74 ± 0.02 ^{a}	0.06
Amaranth bran					
None	0.96 ± 0.00 ^{b}	0.13 ± 0.02 ^{c}	0.17 ± 0.00 ^{i}	n.d.	0.07
LV1	0.11 ± 0.01 ^{ef}	n.d.	0.02 ± 0.00 ^{i}	0.49 ± 0.02 ^{efg}	0.05
BY	0.05 ± 0.00 ^{f}	0.30 ± 0.02 ^{c}	0.20 ± 0.01 ^{hi}	0.50 ± 0.01 ^{efg}	0.05
BY + *L. fermentum*	0.11 ± 0.00 ^{ef}	n.d.	2.34 ± 0.10 ^{b}	0.41 ± 0.00 ^{h}	0.05
BY + *K. marxianus*	0.19 ± 0.01 ^{de}	n.d.	5.10 ± 0.09 ^{a}	0.45 ± 0.01 ^{fgh}	0.06
BY + inulinase	0.08 ± 0.00 ^{f}	n.d.	0.05 ± 0.02 ^{defg}	0.58 ± 0.02 ^{c}	0.05

n.d. not detected. Different letters within the same column indicate significant differences ($p < 0.05$). BY, bakery yeast.

The hydrolysis of fructans releases glucose and fructose. When present in excess of glucose and exceeding the cut-off value of 0.5 g/100 g [17], fructose is also classified as a FODMAP [2,5]. Untreated, WB and AB contained fructose in a smaller amount compared to glucose (Table 1). Nevertheless, the fructose content increased during the fermentation of WB and even exceeded the glucose content after fermentation with BY in combination with *L. fermentum* or *K. marxianus*. After the 24h fermentation of AB, the fructose content increased only with BY. Co-culture of BY and *K. marxianus* resulted in 11 and 29% higher glucose content compared to the untreated WB and AB, respectively. Fermentation of AB with *S. cerevisiae* alone or in co-culture with *L. fermentum* resulted in 13% higher glucose content. Although fructose and glucose are released during the fermentation, their levels usually remain low as these sugars are consumed by yeasts and lactobacilli [17]. Heterofermentative lactic acid bacteria can further convert the released fructose into mannitol, which is also defined as FODMAP with the recommended cut-off value of 0.2 g per serving [3,14]. Here, mannitol was produced in each bran fermentation (Table 1) in an amount depending on the interaction between bran type and bioprocessing agent ($p < 0.01$). In both WB and AB, bioprocessing with BY and inulinase resulted in the highest mannitol contents. Therefore, it is necessary to follow the degradation products of fructans as they add up to the FODMAP content. However, considering the bran content in the snacks and the average baking loss (45%), the proximate total FODMAP content in 30 g of each snack would remain below the limit (Table 1).

3.2. Effect of Bran Bioprocessing on the Rheology and 3D-Printability of the Dough

The rheological properties investigated in this study were significantly influenced by the interaction between bran type and bioprocessing agent ($p < 0.01$). Higher G′ than G″ values for all samples (Figure 3a,b) indicated their solid-like behaviour, which is crucial for successful 3D printing, i.e., achieving dimensional stability after extrusion-based 3D printing [25]. Both the G′ and G″ of our oat-based dough were up to nine-fold higher compared to wholegrain rye dough with the milk powder, previously reported by Lille et al. [29]. Samples with either WB or AB bioprocessed with BY showed the highest loss factor (Figure 3c,d). This indicated that dough with added bran was bioprocessed with BY

demonstrated the most viscoelastic properties, i.e., had the highest ability to absorb energy and relieve stress.

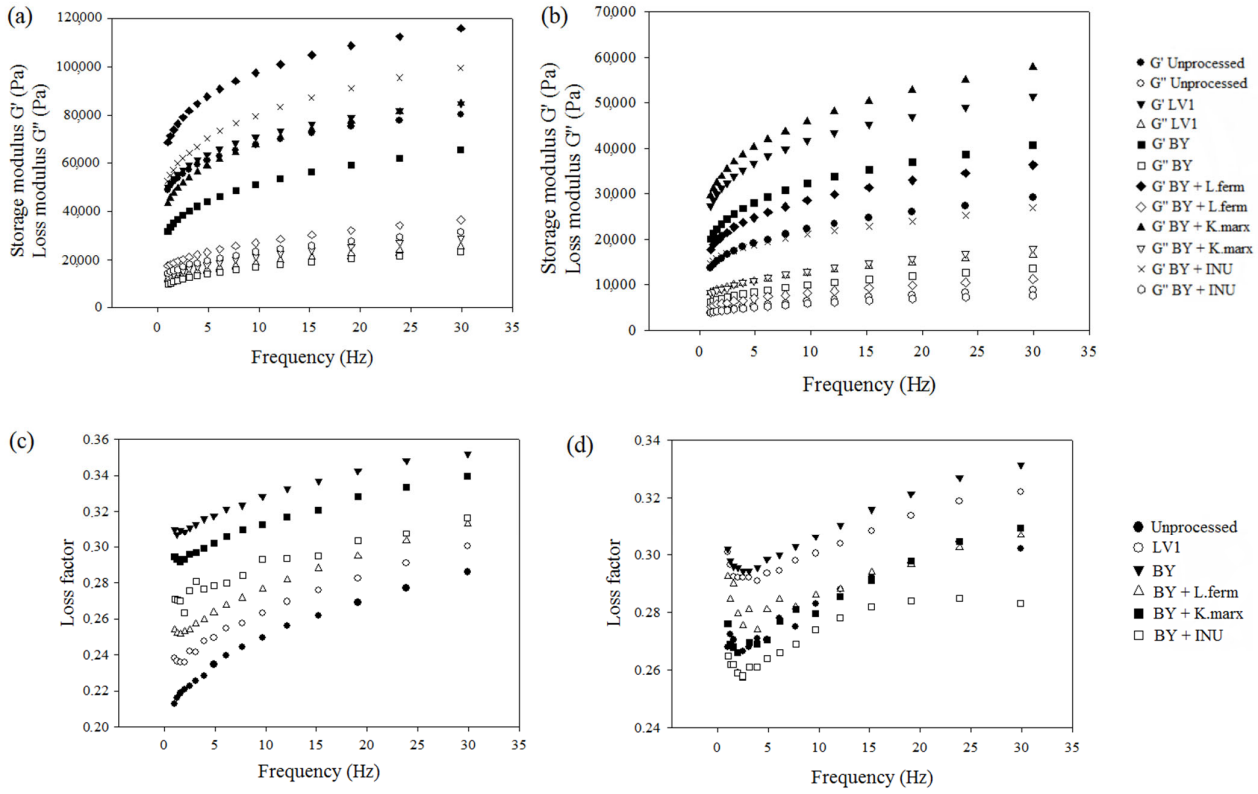

Figure 3. Storage and loss moduli of: (**a**) wheat bran; (**b**) amaranth bran, and loss factor of: (**c**) wheat bran; (**d**) amaranth bran. BY, bakery yeast; INU, inulinase.

The determined complex viscosity, yield stress, and flow point of the dough with added unprocessed WB (Table 2) did not significantly differ from those of the dough with added pea protein used in our previous study [24]. Moreover, the doughs with the addition of unprocessed WB showed higher complex viscosity, yield stress, and flow point compared to AB. This could be explained by the higher content of fibre, particularly soluble fibre, as well as the difference in fibre composition, i.e., arabinoxylans are highly present in WB [33]. The complex viscosity of the dough containing WB decreased by 9% only after fermentation with the co-culture of BY and *K. marxianus*, while BY alone and co-cultured with *L. fermentum* resulted in significantly higher complex viscosity. Furthermore, the complex viscosity of the dough with AB increased in all treatments, but significant were only bioprocessing with LV1, BY, and its co-culture with *K. marxianus*. AB contain pectin fibre as well as xyloglucans and galacturonans (galacturonic acid and galactose) [34], while *K. marxianus* possesses β-galactosidase, pectinase, and β-xylosidase [35], whose action at acidic pH (ranging between 4.2 and 4.3 in our samples at the end of fermentation) could lead to the solubilization of AB fibre and consequently increase dough viscosity. The addition of fermented WB increased the yield stress of the dough, with the co-culture of BY and L. *fermentum* being the most favourable (Table 2). A similar pattern was observed for AB-containing doughs, except that fermentation with BY and *L. fermentum* resulted in lower yield stress compared to doughs with unprocessed AB. Higher yield stress means that the dough has the ability to form self-supporting layers [36]. Similar to our results, Lille et al. [29] reported yield stress for rye dough ranging from 10 to 58 Pa. Regardless of the bran type, a higher flow point was observed in all bioprocessed bran-containing doughs, indicating that more extrusion should be applied for the dough to begin

to flow, which could also be linked with increased swelling and fibre solubility. Previously, the fermentation of amaranth flour [37,38], as well as WB [39,40] with various species of *Lactobacillus*, was found to improve the rheological properties of the dough and the quality of wheat composite bread. Additionally, oat flour provides improved viscosity compared to wheat flour [41]. Our study showed that the fermentation of both WB and AB with only BY or mixed cultures contributes to the dough rheology, unlike its combination with inulinase. Even though the rheology of dough with AB remains inferior to WB-containing dough. This could be due to the fact that WB contained more fibre than AB and additionally contains gluten proteins.

Table 2. Rheological properties and 3D printing precision of the dough.

Treatment	Complex Viscosity (Pa s)	Yield Stress (Pa)	Flow Point (Pa)	Printing Precision (%)
Wheat bran				
None	7893.2 ± 131.2 ^{de}	18.9 ± 0.3 ^{f}	254.8 ± 0.6 ^{ef}	84.3 ± 2.6 ^{defg}
LV1	8184.1 ± 185.0 ^{cd}	25.0 ± 1.0 ^{ef}	447.2 ± 0.8 ^{c}	86.3 ± 3.1 ^{cdef}
BY	10,466.2 ± 182.2 ^{b}	34.0 ± 1.0 ^{cde}	533.8 ± 0.5 ^{b}	93.1 ± 2.8 ^{a}
BY + *L. fermentum*	11,275.5 ± 27.5 ^{a}	51.4 ± 1.0 ^{a}	612.0 ± 1.6 ^{a}	94.9 ± 2.4 ^{a}
BY + *K. marxianus*	7182.2 ± 11.8 ^{e}	27.0 ± 1.0 ^{def}	467.4 ± 0.6 ^{c}	84.0 ± 2.7 ^{efg}
BY + inulinase	8632.3 ± 15.9 ^{cd}	26.4 ± 0.7 ^{def}	472.5 ± 8.9 ^{c}	88.4 ± 4.0 ^{b}
Amaranth bran				
None	2237.6 ± 24.1 ^{i}	5.1 ± 0.7 ^{g}	126.9 ± 1.0 ^{g}	83.3 ± 2.6 ^{fg}
LV1	4540.0 ± 139.5 ^{f}	37.4 ± 3.2 ^{bcd}	301.3 ± 4.4 ^{de}	82.8 ± 2.7 ^{fg}
BY	3326.3 ± 73.1 ^{gh}	27.9 ± 4.7 ^{def}	290.9 ± 2.2 ^{de}	86.1 ± 2.6 ^{cdef}
BY + *L. fermentum*	2931.7 ± 182.5 ^{hi}	2.2 ± 0.4 ^{g}	229.3 ± 2.4 ^{f}	80.1 ± 1.2 ^{g}
BY + *K. marxianus*	4879.6 ± 209.2 ^{f}	7.3 ± 0.1 ^{g}	290.9 ± 6.7 ^{de}	90.8 ± 2.0 ^{a}
BY + inulinase	2503.4 ± 125.3 ^{i}	20.8 ± 3.1 ^{f}	221.3 ± 2.5 ^{f}	84.0 ± 3.4 ^{efg}

Different letters within the same column indicate significant differences ($p < 0.05$). BY, bakery yeast.

The rheological properties of the dough have the greatest influence on 3D printing precision, which is equivalent to printing quality, as it is highly related to the reproducibility and consistency of 3D printing and the quality of the final product. The printing precision of dough containing unprocessed WB or AB was similarly satisfactory. Fibre-rich ingredients used for dough preparation have already been associated with high printing performance [24,27]. For a high printing precision, the dough needs to possess an appropriate viscosity, i.e., to be easily extruded while supporting the following deposited layers [42]. Indeed, we found a positive correlation ($r = 0.73$; $p < 0.01$) between the printing precision and the complex viscosity of our samples (Table 2). Bran bioprocessing had a positive or negligible effect on printing precision (Table 2), depending on the interaction between bran type and bioprocessing agent ($p < 0.01$). Dough containing WB fermented with BY or its co-cultures with *L. fermentum*, as well as dough with added AB fermented with BY and *K. marxianus*, were printed the most accurately. Thus, bran bioprocessing can contribute to dough printability, but the bioprocessing agent should be selected depending on the bran type.

3.3. Physical and Sensorial Attributes of Snack

No significant difference was found in average weight (1.1 ± 0.0 g), height (4.6 ± 0.1 mm), line width (1.6 ± 0.0 mm), or diameter (35.9 ± 1.6 mm) between the baked snacks as a function of bran nor bioprocessing type (data not shown). The dough was shrunk during baking depending on the bran type and bioprocessing agent (Table 3). On average, dough containing WB was less shrunk than dough with AB. Further on, samples with bioprocessed bran were significantly less shrunk by 20–69% compared to dough with unprocessed bran. This could be related to the difference in dough rheology since an inverse correlation was found between shape shrinkage and dough flow point ($r = -0.65$, $p = 0.02$). Bioprocessing of both WB and AB with co-culture of BY and *K. marxianus* resulted in the

lowest snack shrinkage, respectively. This behaviour might be linked with possibly the highest CO_2 production rate in the synergistic action of BY and *K. marxianus*, resulting in dough expansion [17].

Table 3. Physical properties of baked snacks.

Bioprocessing	Shape Shrinkage (%)	Lightness L^*	Redness a^*	Yellowness b^*	BI	Hardness (N)
Wheat bran						
None	28.6 ± 4.1 ^{de}	57.8 ± 2.0 ^{cd}	5.8 ± 1.3 ^{efg}	17.2 ± 0.4 ^{c}	42.3 ± 3.6 ^{ef}	12.0 ± 1.6 ^{bcd}
LV1	22.6 ± 5.3 ^{fgh}	60.3 ± 0.9 ^{b}	6.1 ± 0.4 ^{def}	18.8 ± 0.7 ^{defg}	44.2 ± 1.5 ^{cde}	11.1 ± 1.3 ^{defg}
BY	22.1 ± 3.4 ^{gh}	56.7 ± 1.0 ^{de}	7.6 ± 0.2 ^{bc}	17.0 ± 0.8 ^{bc}	44.8 ± 1.2 ^{cde}	10.2 ± 0.7 ^{efgh}
BY + *L. fermentum*	22.2 ± 2.9 ^{gh}	53.7 ± 1.8 ^{f}	5.6 ± 1.0 ^{fgh}	15.3 ± 1.1 ^{a}	40.6 ± 1.7 ^{fg}	12.3 ± 1.4 ^{abcd}
BY + *K. marxianus*	12.3 ± 2.3 ^{j}	55.0 ± 0.4 ^{ef}	6.9 ± 0.3 ^{cd}	16.4 ± 0.3 ^{bc}	44.0 ± 0.7 ^{cde}	10.1 ± 0.6 ^{efgh}
BY + inulinase	20.8 ± 3.2 ^{hi}	60.1 ± 0.3 ^{b}	7.7 ± 0.1 ^{ab}	20.0 ± 0.2 ^{fghi}	48.2 ± 0.3 ^{a}	11.7 ± 2.0 ^{cdef}
Amaranth bran						
None	48.2 ± 4.9 ^{a}	64.9 ± 0.8 ^{a}	3.3 ± 0.2 ^{i}	19.0 ± 0.6 ^{efgh}	37.8 ± 1.1 ^{h}	10.0 ± 1.4 ^{fgh}
LV1	31.6 ± 3.6 ^{bcd}	64.5 ± 0.5 ^{a}	3.5 ± 0.2 ^{i}	19.3 ± 0.3 ^{fghi}	39.0 ± 1.0 ^{gh}	9.7 ± 0.6 ^{gh}
BY	29.2 ± 4.5 ^{cde}	64.4 ± 1.5 ^{a}	5.0 ± 0.3 ^{h}	20.2 ± 0.4 ^{i}	42.7 ± 1.0 ^{def}	10.1 ± 0.9 ^{fgh}
BY + *L. fermentum*	30.3 ± 4.8 ^{cde}	63.8 ± 0.7 ^{a}	3.4 ± 0.3 ^{i}	18.6 ± 0.8 ^{def}	37.8 ± 2.0 ^{h}	9.1 ± 0.7 ^{h}
BY + *K. marxianus*	15.1 ± 3.1 ^{ij}	60.9 ± 0.8 ^{b}	5.3 ± 0.2 ^{gh}	20.0 ± 0.6 ^{ghi}	45.1 ± 1.3 ^{bcd}	9.6 ± 1.3 ^{gh}
BY + inulinase	25.2 ± 2.5 ^{efgh}	64.3 ± 2.0 ^{a}	5.3 ± 0.3 ^{gh}	20.1 ± 1.1 ^{hi}	42.7 ± 1.5 ^{def}	9.6 ± 1.0 ^{gh}

Different letters within the same column indicate significant differences ($p < 0.05$). BY, bakery yeast; BI, browning index.

It is known that bran has high polyphenol oxidase activity, causing undesirable browning during 3D printing due to the slowness of the process [24,30]. In this study, ΔE^* between the first and the last (10th) 3D-printed and baked snacks ranged from 0.12 to 0.87, which is defined as a difference in colour barely visible to the human eye [42]. Compared to matching WB, the addition of both unprocessed and bioprocessed AB resulted in significantly lighter, more yellow and less red snacks with a lower browning index (BI) (Table 3). BI is related to Maillard's reactions, the dextrinisation of starch, and the caramelisation of sugar during baking [42]. In this study, the BI of the snacks was significantly influenced by the interaction between bran and the type of the bioprocessing agent ($p < 0.01$). Compared to samples with unfermented bran, all WB and AB-containing snacks had a higher BI, except those fermented with BY and *L. fermentum*. The higher BI of snacks with WB bioprocessed with BY might be related to the bioavailability of amino acids during fermentation with BY [43], which are known to be involved in the formation of brown pigments [42]. In previous studies, acidic doughs were found to have higher L^* and b^* values compared to dough with added sodium bicarbonate, but there was no general rule for BI. A similar BI of around 42 was previously observed for 3D-printed snacks with added pre-processed WB [24].

The hardness of snacks was also significantly dependent on the interaction between bran and the type of pre-processing agent ($p < 0.01$). Snacks with WB were harder than snacks containing amaranth bran. The hardness of the snack was significantly correlated with the dough complex viscosity ($r = 0.748$; $p = 0.005$). Fermentation of WB and AB bran resulted in a lowering of snack hardness, except when WB was fermented with BY and *L. fermentum* (Table 3). Lille et al. [29] reported similar results for the hardness (11–20 N) of 3D-printed snacks as ours. Rani et al. [43] reported that rice-black gram snacks extruded after fermentation with BY had hardness in the range of 15–37 N, depending on barrel temperature, extruder screw speed, and die opening diameter. To the best of our knowledge, there are no studies that have investigated the effects of the bioprocessing agents used in this study on the textural properties of the snacks.

Results of the descriptive sensory analysis showed that there was a statistically significant difference between the samples in all evaluated attributes. A significant ($p \leq 0.02$)

dependence on the interaction between bran and bioprocessing agent type was observed for the bitter aftertaste, saltiness, and bran odour. Compared to wheat snacks, amaranth snacks, on average, had a more uniform colour, more pronounced bitter taste, yeast and bran odour, and less pronounced oil flavour ($p < 0.01$ for all attributes), which can be observed in Figure 4d. Although snacks with unprocessed WB had the least pronounced bitter aftertaste, the bioprocessing of WB with BY resulted in the least bitter snacks. As expected, the fermented flavour was significantly ($p < 0.01$) more expressed in both wheat and amaranth snacks after bran bioprocessing. Overall, bioprocessing attenuated the intensity of bran odour in amaranth snacks, whereby in wheat snacks, this was achieved only after treatments with LV1 and BY alone or combined with inulinase. ANOVA showed that the five-point hedonic scale did not detect any significant difference ($p \geq 0.09$) in liking amongst the samples. The hedonic score of wheat snacks ranged from 2.7 to 3.8, and for amaranth snacks, it was between 2.6 and 3.3. This indicated a need for further improvement of the snack formulation to meet consumers' expectations.

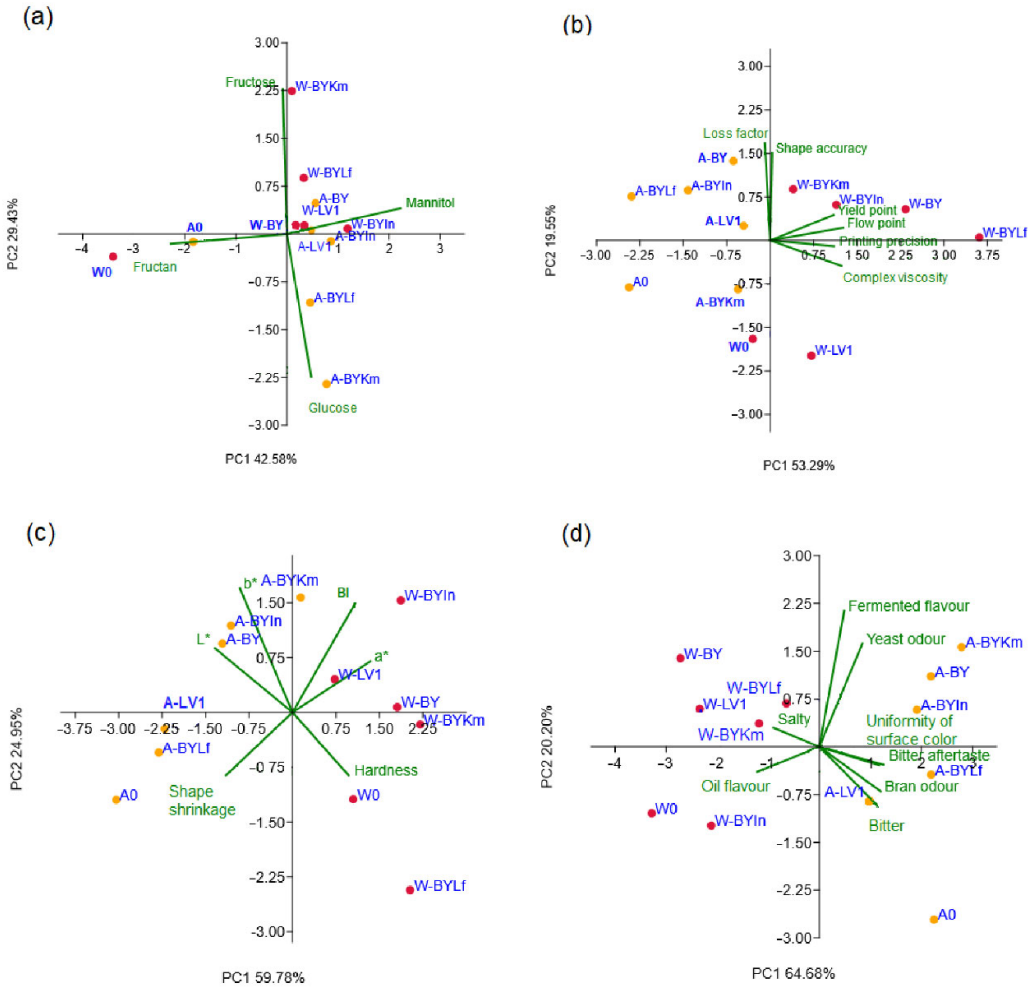

Figure 4. PCA bi-plots of investigated parameters: FODMAPs content (**a**), dough parameters (**b**), physical properties of snacks (**c**), and sensory attributes of snacks (**d**). W, wheat bran; A, amaranth bran; 0, control sample with unfermented bran; BY, bakery yeast; Lf, *Limosilactobacillus fermentum*; Km, *Kluyveromyces marxianus*; In, inulinase.

3.4. PCA

The results obtained using principal component analysis in the form of bi-plots correlating obtained snack products and analysing a set of variables are given in Figure 4a–d.

The negative correlation between fructans and mannitol content was obtained in bran samples ($r = -0.689$, $p \leq 0.05$). The PCA of the presented data explained that the first two components accounted for 72.0% of the total variance in the four variables factor space (sugar contents). Considering the map of the PCA performed on the data, the contents of mannitol (contributing 47.7% of the total variance, based on correlations) exhibited positive scores according to the first principal component (PC1), whereas fructans content (50.0%) showed negative score values according to PC1 (Figure 4a). The positive contribution to the second principal component (PC2) calculation was observed for fructose content (49.9%), while negative scores on PC2 calculation were observed for glucose content (48.3%). Figure 4a explicitly show the abundance of fructans in unprocessed samples W0 and A0, whereas bioprocessed samples were characterised either by fructose, glucose, or mannitol presence, as discussed in Section 3.1. Regarding FODMAPs, the map of PCA analysis of samples showed that the second principal component described the differentiation among WB and AB, while the first principal component described the variations in the bioprocessing between samples.

According to Figure 4b, loss factor and shape accuracy were expressed in the dough with added amaranth bran bioprocessed with BY or LV1 and dough with wheat bran bioprocessed with yeasts co-culture, while other 3D printing parameters were being expressed in fermented snack samples made of wheat bran. The complex viscosity was positively correlated with yield point ($r = -0.676$; $p \leq 0.05$), flow point ($r = -0.909$; $p \leq 0.01$), and printing precision ($r = -0.729$; $p \leq 0.01$). The PCA of the presented data explained that the first two components accounted for 72.84% of the total variance in the six variables factor space (3D printing parameters). The complex viscosity (contributing 27.4% of the total variance, based on correlations), yield point (21.7%), flow point (28.6%), and printing precision (22.1%) showed positive scores according to PC1 (Figure 4b). The positive contribution to PC2 calculation was observed for loss factor (50.8%) and shape accuracy (41.0%).

Figure 4c show that hardness was most expressed in WB-containing snacks either unfermented or fermented with BY and *L. fermentum*, while shrinkage characterised snacks containing AB unfermented or fermented with BY. The hardness was negatively correlated to diameter ($r = -0.699$; $p \leq 0.05$). The browning index was positively correlated with a^* colour coordinate ($r = 0.688$; $p \leq 0.05$). The PCA of the presented data (Figure 4c) explained that the first two components accounted for 84.73% of the total variance in the six variables factor space (baked snack parameters). The shape shrinkage (which contributed 8.4% of the total variance, based on correlations), L^* colour coordinate (15.5%), and b^* colour coordinate (8.0%) showed positive scores according to PC1 (Figure 4c), whereas a negative contribution to PC1 calculation was obtained by BI (10.0%), a^* colour coordinate (17.9%), and hardness (19.8%). A positive contribution to PC2 calculation was observed for BI (11.7%), while a negative influence on PC2 calculation was obtained for shape shrinkage (21.7%).

As shown in Figure 4d, fermented flavour and yeast odour were expressed in snacks with WB bioprocessed with BY and *L. fermentum* and AB bioprocessed with BY alone or in combination with *K. marxianus* or inulinase. Bitter taste and aftertaste, bran odour, and uniformity of surface colour were most expressed in amaranth snacks with unprocessed and LV1 or BY and *L. fermentum* co-culture-bioprocessed bran. Salty taste and oil flavour were characteristics of WB snack samples. The PCA of the presented data explained that the first two components accounted for 84.88% of the total variance in the eight variables factor space (sensory properties). The yeast odour (contributing 7.5% of the total variance, based on correlations), bran odour (14.5%), bitter taste (14.5%), and bitter aftertaste (16.9%) showed positive scores according to PC1, while a negative contribution to PC1 calculation was obtained by the uniformity of surface colour (13.7%), salty taste (9.1%), and oil flavour (14.5%). A positive contribution to PC2 calculation was observed for fermented flavour (50.7%) and yeast odour (29.4%), while a negative impact on PC2 calculation was observed for bitter taste (9.9%).

On average, wheat snacks were harder, darker, redder, saltier, had lower shrinkage and surface colour uniformity, and had more pronounced oil flavour while less pronounced bran flavour, bitter taste, and aftertaste than amaranth snacks.

4. Conclusions

In this study, we report for the first time the bioprocessing of wheat and amaranth bran with yeast, lactic acid bacteria, and inulinase, aimed at removing fructans, as well as improving the quality of 3D-printed snacks. Compared to amaranth bran, wheat bran contains a higher level of dietary fibre but also fructans. Bioprocessing lowers the fructans content in both brans, whereby the fructose released and the mannitol formed need to be followed. In addition, bioprocessing of the bran improves overall dough rheology, the precision of 3D printing, minimises shrinkage in baking, and contributes to the desired texture of the snacks. Bakery yeast successfully fermented wheat bran, assuring snack sensory and 3D printing quality. Overall, bioprocessed wheat bran at level 7% in the formulation could be used to produce low-FODMAPs snacks and labelled as a source of fibre. Amaranth bran has a further potential to enrich gluten-free snacks, particularly after bioprocessing with *K. marxianus*. Three-dimensional printing enables the fabrication of satisfactory snack products using milling by-products as an enriching ingredient intended for sensitive individuals and IBS patients. Future studies should investigate the shelf-life and cost efficiency of 3D-printed snacks with added bran to advance the sustainability of the food industry.

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Chapter 6

General discussion

1. Enzyme activity and bioactive compounds of pre-processed wheat bran

2. FODMAPs in bioprocessed wheat bran

3. Influence of flour type, pH value and temperature for successful 3D printing

4. Incorporation of pre-processed wheat bran into 3D-printed snacks

5. Snacks and breakfast cereals enriched with grain by-products

1. Enzyme activity and bioactive compounds of pre-processed wheat bran

The first part (*Publication No. 1* and *Publication No. 2)* comprises the processing of WB with grinding and HIU aiming at reducing its PPO activity, prolonging the storage-stability and preserving bioactive content. It also finds optimal conditions for WB particle size and HIU processing. A part of *Publication No. 3* additionally brings a comparison of HIU, vacuum microwave cooking and pulsed light processing of WB.

1.1. Effect of pre-processing on PPO, lipase and peroxidase

Incorporation of WB into cereal-based products represents a major challenge for food industry due to short storage-life of WB and undesirable changes it causes in the final product (Niu et al., 2014b; Yadav et al., 2010; Zhao et al., 2020). Therefore, the main goal is to reduce the activity of WB lipolytic and oxidative enzymes while preserving its content of bioactive compounds and AO. *Publication No. 1* for the first time combines the effect of HIU and micronization on PPO activity in WB. Previous studies (Liu et al., 2016; Niu et al., 2014a) show that the activity of cereal PPO enlarges as the particle size decreases (to 10 μm). After bran grinding in an ultracentrifugal mill to a median diameter of 50th percentile (d (50)) of 253 ± 7 μm, the activity of PPO was similar to that in coarse bran. However, ball grinding to d (50) of 10 ± 1 μm under liquid nitrogen cooling (cryo-grinding) significantly weakened PPO activity which could be explained by possible metal residues (Zn, Fe and Cr) normally found in traces after ball cryo-grinding and sieving (as here applied), whereby Zn^{2+} is an affective PPO inhibitor (Aydin et al., 2015).

Darkening or discoloration of dough and cereal-based products during storage is mainly related to PPO presence (Liu et al., 2016). In the paper of Niu et al. (2014b) HIU represented a successful tool for reducing activity of PPO and thus enzymatic darkening of wholemeal wheat flour. The flour contained lower levels of PPO activity after longer treatment time and the addition of ascorbic acid. It can be assumed that the HIU treatment would be more successful if it was applied directly on WB, since PPO is mostly located in WB itself (Zhao et al., 2020). In *Publication No. 1* it was observed how PPO activity in WB depends on treatment time, but also particle size of WB (*Publication No. 1* – Figure 2c). Ultrasonic inactivation of enzymes is mainly linked with the denaturation of protein, either by free radicals in sonolysis of water molecules or from the shear force resulting from the formation or collapse of cavitating bubbles (Niu et al., 2014b). Indeed, transmission electron images (*Publication No. 1* – Figure 3a) confirmed that proteins were denaturated in WB processed with HIU. As a rule, in temperatures

between 70 and 90 °C, PPO is almost completely inactivated (Yadav et al., 2010). As these temperatures were reached after HIU treatment (*Publication No. 1*), the question is whether the inactivation of PPO was caused by heating or other HIU affects also contributed. Therefore, after determining the optimal conditions for HIU treatment (60 – 100% amplitude for 10 – 15 min) and bran particle size (10 µm), this subject was thoroughly investigated by extending the research to the comparison of the HIU treatment to HIU with cooling and conventional heating. It was observed and confirmed that PPO is mostly heat sensitive. Finally, the strongest reduction of PPO activity (by 93%) was detected in fine-sized bran (d (50) of 10 ± 1 µm) processed with conventional heating as well as HIU.

Besides PPO, the presence of lipase, peroxidase, and lipoxygenase also makes bran unsuitable for most food applications due to rapid deterioration of its lipids. So far, WB has been processed with conventional heating, autoclaving, toasting, extrusion cooking, microwave and dielectric heating, superheated steam, and steam explosion aiming to inactivate its lipase and peroxidase, i.e. improve its storage properties (Hu et al., 2018; Kong et al., 2021; Sharma and Gujral, 2011; Sudha et al., 2011). In this purpose, the efficiency of HIU processing as well as the effect of particle size of WB was studied for the first time in *Publication No. 2*. In order to define the storage life of bran, it is necessary to monitor fatty acid content and certain indicators of deterioration, i.e. peroxide and anisidine value (Čukelj Mustač et al., 2020; Hu et al., 2018; Kong et al., 2021). The mechanism of deterioration consists of lipase initiating a release of polyunsaturated fatty acids from triglycerides, and then peroxidase promoting their oxidation (Hu et al., 2018; Piechowiak et al., 2018; Poudel and Rose, 2018). Thus, peroxide value is an indicator of the primary products of lipid oxidation, i.e. hydroperoxides, which can further be degraded by peroxidase (Hu et al., 2018) to secondary oxidation products, such as aldehydes and ketones represented by anisidine value (Hu et al., 2018; Icyer and Durak, 2018). A successful reduction of lipase (by 67%) and peroxidase (by 92%) activity in WB with HIU treatment can be observed in *Publication No. 2*. A reduction of particle size to d (50) of 177 µm was clearly insufficient to significantly impact enzyme activity. In contrast to results for PPO, HIU processing was more powerful in deactivating lipase and peroxidase than conventional heating. This obviously suggests that increase in temperature is not the only effect of HIU that can promote the enzyme inactivation. In the paper of Yu et al. (2020), rice bran showed 99.6% of lipase activity after even 90 min-ultrasonic processing of a 25% bran suspension. Therefore, less concentrated bran suspension (15%) prepared for HIU processing

in this dissertation further contributed to lipase deactivation, whereby, in order to achieve a desirable reduction in enzyme activity, it was sufficient to treat WB for only 15 min.

Initially, ground WB processed with HIU contained significantly higher free fatty acids and slightly lower peroxide value (*Publication No. 2*). The initial anisidine value of WB increased after HIU processing due to which it could be observed that hydroperoxides were degraded to secondary oxidation products. Similar results were given in the paper of Icyer and Durak (2018) on the ultrasonic treatment of rapeseed oil, while rice bran processed with ultrasound had higher PV (Mohammadi et al., 2021; Yu et al., 2020). Compared to previous studies, *Publication No. 2* was first to reveal changes in anisidine value during storage of unprocessed and WB processed with HIU. As expected, during storage, free fatty acids, peroxide and anisidine value increased (*Publication No. 2* – Figure 2a, b and c, respectively), whereby the deterioration was faster in ambient conditions (~21.5 °C) than in refrigerator (~7 °C). A product is considered acceptable when peroxide and anisidine values are less than 10 (Čukelj Mustač et al., 2020; He et al., 2020). Accordingly, HIU treatment was more efficient in prolonging the stability of WB than cold storage, whereas WB grinding showed a negative effect. So far, steam and dry heat treatment has prolonged the storage stability of WB from 15 to less than 30 days (Sudha et al., 2011). *Publication No. 2* has shown how HIU extends WB shelf life from 74 to 365 days. In conclusion, the unprocessed WB stored under ambient conditions, the unprocessed bran stored in the refrigerator, ground HIU-treated WB, and coarse HIU-treated WB would exceed the limit of PV or AV after 2 months, 200 days, 365 days, and 280 days of storage, respectively. Overall, HIU could be addressed as a very important strategy for extending the shelf life of WB.

The power of HIU to inactivate PPO was additionally compared to vacuum microwave cooking and pulsed light processing of bran. *Publication No. 3* brings the first research on the behaviour of PPO activity in WB after these processing techniques. In the papers of Pellicer et al. (2018) and Vollmer et al. (2020) the pulsed light processing successfully suppressed the activity of PPO of mushrooms and pineapple, respectively. The highest activity reduction was achieved after HIU processing (Publication No. 3), whereby HIU and pulsed light treatments ended with a similar temperature (~90 °C). Still, vacuum microwave cooking lowered the PPO activity to a comparable level as HIU, but at lower temperature and water addition, which indicates greater enzyme inactivation at partial vacuum. So far, PPO activity has been effectively reduced in barley after applying microwave cooking in the paper by Sharma and Gujral (2011), whereby lower oxygen availability during vacuum drying of cranberries was a major factor in their weak enzymatic browning (Zielinska et al., 2018). Overall, *Publication No. 3* defined HIU as the

most successful technique for reducing the PPO activity of WB and dough browning to slightly observable to human eye.

1.2. Effect of pre-processing on bioactive content and antioxidant activity

This section summarizes the results of WB processing on bioactive compounds, i.e. total phenolic content (TPC) and AO measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity or ferric reducing antioxidant power (FRAP) assay. *Publication No. 1* exhibited the positive effect of particle size reduction on TPC and DPPH AO of WB, which were dependent only on the bran particle size. Doubtless, reducing the particle size leads to increased phenolic compounds content due to their exposure after the destruction of the aleuronic layer and higher specific surface area (Brewer et al., 2014; Laddomada et al., 2015; Rosa et al., 2013). The vacuolization and fragmentation in fine-ground WB were observed in transmission electron images (Publication No. 1 – Figures 3b and c). Still, phenolic antioxidants are heat labile (Yadav et al., 2010). Therefore, mentioned temperature rise during HIU processing could have a detrimental effect on them. Indeed, HIU lowered both TPC and AO of WB. A decrease of phenolic content after ultrasonication has already been observed in papers by Čukelj Mustač et al. (2019) and Niu et al. (2014b). Although the grinding and HIU might lead to the release of phenolic compounds from WB matrix, the formation of excessive heat and free radicals can on contrary partly destroy them. This was further confirmed as cooling during HIU processing aided the preservation of TPC in WB of all particle sizes (d (50) = 510, 253, and 10 μm). Compared to unprocessed WB, TPC was even slightly higher when cooling was applied during HIU. Moreover, as expected, conventional heating resulted with the lowest TPC and AO.

Additionally, HIU processing of micronized WB was combined with the addition of XYL (10 UI), whereby the obtained results were published only via conference poster (Habuš et al., 2020). Wheat fibre is mainly insoluble, largely composed of arabinoxylans (pentosans) often esterified with phenolic acids, such as ferulic acid. The solubilisation of bran fibre can be achieved with XYL treatment or cryogrinding. A suspension of 15 g bran in 100 mL of water was incubated with xylanase from *Bacillus subtilis* (VERON® RL, 10 UI) in a shaking water bath at 55 °C for 16 h. The content of ferulic acid was determined with HPLC-DAD method. Unprocessed WB contained soluble fibre (4.6 g/100 g d.w.), pentosans (0.22 mg/g d.w.), TPC (0.68 mg GAE/g d.w.), and free ferulic acid (19.10 μg/mL). Regarding the bioactive profile, XYL treatment of both coarse and micronized WB resulted in significantly higher values for the ferulic acid content (around 65-fold). In the paper of Wu et al. (2017), XYL processing of WB resulted in 16.8% higher ferulic acid content, while combination of XYL and feruloyl esterase gave ferulic acid increased up to 70%. In our research, XYL treatment enhanced TPC,

ferulic acid, and AO depending on the interaction with cryogrinding ($p<0.01$). Overall, after the XYL treatment, the TPC nor soluble fibre content did not significantly differ between the coarse and micronized bran.

Along with the PPO activity, the effect of HIU processing was further compared to vacuum microwave cooking and pulsed light to establish which technique would be less harmful for the antioxidants in WB (*Publication No. 3*). Bran processed with vacuum microwave cooking or pulsed light showed even lower TPC than HIU-processed bran. According to the papers by Silva et al. (2019) and Zielinska et al. (2018) vacuum microwave cooking results in poorer TPC of pumpkin, although this reduction was still minor compared to boiling or microwave treatment. In *Publication No. 3* increased AO measured by FRAP assay was observed in bran processed with PL but not vacuum microwave cooking. This behaviour has been previously observed in various fruits, vegetables and fermented juice and explained with a photo-protective antioxidant defence response to oxidative stress which arose during processing with pulsed light (Denoya et al., 2020; Kwaw et al., 2018). On the other hand, these papers also revealed an increase in TPC and its significant correlation with AO, but at shorter processing time. Therefore, the most important is to optimize the treatment time, i.e. to control the temperature achieved at the end of the treatment.

1.3. Effect of pre-processing on flour-bran blend pasting properties

This dissertation also looked at the pasting properties of the WB-flour blend (*Publication No. 1* and *unpublished data*). The aggregation behaviour and breakdown of the gluten were analysed with a novel gluten peak tester (GlutoPeak), while starch-related physicochemical properties were investigated using MicroViscoAmylograph.

Generally, bran interrupts the formation of gluten network and affects starch pasting properties, and therefore is, as a rule, added up to a maximum level of 20% (Jin et al., 2020; Laddomada et al., 2015; Wang et al., 2018). In this dissertation, the addition of WB to white wheat flour (20% w/w of flour) resulted in reduced time before maximum torque falls off (PMT), regardless of its particle size (*Publication No. 1* - Table 4). Faster formation of strong gluten network can be detected with shorter peak maximum time and also higher maximum torque (Wang et al., 2018). According to Steglich et al. (2015), larger particles consist of more bran layers, making them stiffer and capable of damaging the gluten aggregation. Smaller ones are easier to embed in the gluten matrix because of their flexibility. WB is rich in glutathione which causes a decrease of bakery products volume through building disulphide bonds with the gluten network. Heating processes result in glutathione destruction and inactivation of proteases, thereby enhancing the gluten network (Anson et al., 2012; Demir & Elgün, 2013). Zhang et al. (2015) observed that HIU alters physical properties of wheat gluten by relaxing its network through tearing disulphide, hydrogen, covalent, and noncovalent bonds between gliadin and glutelin. In this dissertation, HIU treatment with cooling resulted with a significantly higher peak maximum time compared with HIU treated bran, i.e. showed no advantage.

Due to the high amylolytic activity, WB excessively reduces paste viscosity (Hidalgo et al., 2013). Indeed, in the paper of Lin et al. (2021) addition of WB resulted in a decreased peak viscosity, hot paste viscosity, final viscosity, breakdown value and setback value, whereby an increase in these properties was observed at lower particle size of bran. There are various contradictory studies on how bran particle size influences the dough and final products properties and quality. Some claim that smaller bran particles improve properties of Asian noodle, snacks, and flat bread, while others define particle size reduction as being unfavourable (Lin et al., 2021; Xu et al., 2018). Here, grinding bran to fine particle size resulted with the overall highest pasting values. Besides in the paper by Lin et al. (2021), Niu et al. (2017) also observed an enhancement of whole wheat flour pasting viscosities, when ground to fine particle size (24–107 μm) and attributed it to releasing of polysaccharides involved in gelatinization. Addition of HIU processed WB further enhanced paste viscosities of WB-flour blend

(*Publication No. 1*). This could be linked with potential inactivation of amylase after HIU treatment (Kadkhodaee and Povey, 2008), especially because cooling during treatment showed no advantage on pasting properties.

2. FODMAPs in bioprocessed wheat bran

FODMAPs include fructans, galacto-oligosaccharides, lactose, fructose when in excess to glucose, sorbitol, mannitol and xylitol, whereby WB are rich in fructans. An option to avoid gastrointestinal problems in people with functional bowel disorders which are related to the presence of FODMAPs is a dietary therapy based on avoiding foods with FODMAPs. The negative outcome of this could be insufficient intake of dietary fibre and phytochemicals (Nyyssölä et al., 2020). Fructans can be degraded with yeasts *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*, as well as sourdough fermentation (Nyyssölä et al., 2020). Besides fermentation, materials rich in FODMAPs are frequently enzymatically processed using inulinase, *α*-galactosidase or invertase. During fructans biodegradation, it is necessary to monitor a development of fructose and mannitol, which are also classified as FODMAPs. More precisely, fructose belongs to this group when present in excess of glucose and exceeding the cut-off value of 0.5 g/100 g (Fang et al., 2021; Schmidt and Sciurba, 2021; Struyf et al., 2018). Therefore, in *Publication No. 4*, WB and amaranth bran (AB) was processed for 24h at 37 °C with yeasts, lactic acid bacteria (LAB) and inulinase to bring the content of present fructans to a value that patients can tolerate.

The initial level of fructans has been 2.5-fold higher in WB compared to AB (both unprocessed). *Publication No. 4* revealed that bioprocessing reduced content of summed fructans and galactooligosaccharides (GOS) up to 93% depending on the interaction between bran type and bioprocessing agent. The hydrolysis action of *S. cerevisiae* alone was satisfactory but fortified when combined with *L. fermentum* or *K. marxianus*. The same pattern in whole wheat bread of stronger combined action of these yeasts has been shown in the paper by Struyf et al. (2018). This behaviour could be explained with lower specificity, activity or amount of the synthesized enzyme from *S. cerevisiae* (Laurent et al., 2020; Nyyssölä et al., 2020; Struyf et al., 2018). As the most successful option for reduction of fructans in WB was LV1 starter or the combination of *S. cerevisiae* with enzyme inulinase, which resulted in a reduction of fructans and GOS by 93%. Before *Publication No. 4* was published, WB was treated only with various species of LAB aiming to reduce fructan content (Prückler et al., 2015).

Fructose and glucose levels are expected to remain low during fermentation because they are consumed by yeasts and lactobacilli (Struyf et al., 2018). While following the level of fructose and glucose during WB fermentations, higher content of both sugars was observed (*Publication*

No. 4 – Table 1). Only treatment where fructose content exceeded the glucose content was in WB processed with *S. cerevisiae* in combination with *L. fermentum* or *K. marxianus*. Fructose is also problematic in the story about low-FODMAP food because it can further be converted to mannitol by heterofermentative LAB (Loponen and Gänzle, 2018; Nyyssölä et al., 2020). *Publication No. 4* showed the highest levels of mannitol in both WB and AB, after bioprocessing with *S. cerevisiae* and inulinase. Therefore, it is not enough to reduce the fructan content, but also to monitor the resulting content of fructose, glucose and mannitol to avoid a subsequent increase in total FODMAPs. At 20% addition level (flour basis) of WB, FODMAPs content would remain below the cut-off value (0.5 g per serving) in each investigated snack serving (30 g) (*Publication No. 4* – Table 1).

3. Influence of flour type, pH value and temperature for successful 3D printing

After collecting results on the efficiency of PPO inactivation with HIU, vacuum microwave cooking and pulsed light and their influence on WB bioactive compounds, as well as degradation of fructans in WB with fermentation and enzymatic treatment, research into the possibility of using such treated bran in 3D-printing may commence. 3D printing is a slow process. Printing 10 dough pieces of the chosen 3D-shape (*Publication No. 3* – Figure 1 a) requires 1 h during which 3D-printed dough easily darkens. Due to the mentioned darkening of the dough with added bran, main strategies for controlling the PPO activity are temperature, bran processing, and pH values regulation (Ghazal et al., 2019; Niu et al., 2014b). Between 70 and 90 °C a complete inactivation of PPO can be achieved, while the addition of reducing agents was effective in reducing the discolouration of barley dough (Quinde-Axtell et al., 2006). Therefore, in order to prevent the undesirable darkening of the dough as much as possible, a flour choice, addition of acidity regulators and the printing temperature were investigated in this dissertation.

In addition to usual information about colour, i.e. lightness (L^*), redness (a^*) and yellowness (b^*), for monitoring the darkening of the dough containing wholemeal flour or bran (Hidalgo et al., 2013; Niu et al., 2014b), total colour difference (ΔE^*) and browning index (BI) can be calculated (Pathare et al., 2013). As expected, dough became darker (*Publication No. 3* – Figure 2 a and b), redder and more yellow during 1 h period for both oat and barley flour, which gave a higher BI (*Publication No. 3* – Figure 2 c and d) and very distinct ΔE^* between first and last printed dough. Still, it was lower in case of using oat flour as well as the lower printing temperature (20°C). Lower pH value (5.4) resulted in higher initial L^* and b^*, but (ΔE^*) was lower at higher pH (7.0). All of that agrees with some of the previous studies. For example, in the paper by Zhao et al. (2021), the addition of oat or whole-wheat flour for the production of fresh wet noodle sheets led to a decrease in their lightness during storage. Ghazal et al. (2019) observed how increasing the pH moderates the colour change of 3D-printed anthocyanin-potato starch gel. Also, 3D-printed soy protein isolate, pumpkin and beetroot mixture exhibit stable colour at pH from 6 to 10 (Phuhongsung et al., 2020). Soysal and Söylemez (2004) reported the optimum pH range (between 5.0 and 6.0), while Yamasaki et al. (2008) defined 25 to 30°C as the optimum temperature conditions for PPO activity in WB. Results obtained in this dissertation followed these disclosures. When 3D-printing was performed at 20°C, dough darkened slower than dough printed at higher temperatures of 30 and 40°C.

The effect of flour choice, pH and printing temperature on printing precision was also investigated, whereby higher printing precision was observed when using oat flour, sodium bicarbonate and lower printing temperature. According to Varghese et al. (2020) dough for 3D-printed cookies is weaker and softer at higher temperature which offsets the precision of printing.

Overall, the use of oat flour, sodium bicarbonate, and printing temperature of 20 °C resulted in the lowest ΔE^* (3.25) while maintaining the highest printing precision (91%). The colour changes during 1 h of ambient storage can be seen in Publication No. 3 in Table 3. These conditions were further used for all experiments on the incorporation of pre-processed WB, except baking powder instead of sodium bicarbonate.

4. Incorporation of pre-processed wheat bran into 3D-printed snacks

With the results from the first part of the dissertation, pre-processed WB can be used for the preparation of the dough intended for 3D printing. *Publication No. 3* brings information on the application of WB pre-processed with HIU, vacuum microwave cooking and pulsed light in 3D-printed snack. Further, *Publication No. 4* revealed the possibility to 3D print dough with bioprocessed WB of reduced fructans content. In both studies, the printing precision was high (≥91%). In addition, investigation on the influence of the amount of oil (10, 20, and 30% on flour basis), XYL activity (17, 37, and 57 U/g on a flour-bran basis) and resting time (1, 2 and 3h) of the dough on the rheology of dough with added glucose oxidase (GOX), its 3D printing precision, shape shrinkage during baking, and textural and colour parameters of baked snacks was conducted. The central point was made in triplicate, giving 17 experiments (*Supplementary* - Table 1). The range of the independent variables was defined in preliminary experiments. The powder mixture of oat flour, pea protein and wheat bran (33 and 20% on flour basis, respectively) showed unimodal particle size distribution of median diameter of 50th percentile 64.54. ± 0.84 μm, which was determined with laser diffraction method according to AACC Method 55–40.01 using Mastersizer 2000 instrument equipped with Scirocco 2000 dry dispersion unit (Malvern Instruments, Worcestershire, UK), and 5.33 ± 0.02 % of the sample remaining in the supernatant after centrifugation, decantation and drying (solubility). Measuring the volume of a fixed weight of powder after it has been tapped for 10 times gave bulk density of 400.88 ± 9.94 kg/m^3, cohesion index of 21.57 ± 2.15, and Carr index of 25.59 ± 3.73. The dough was mixed in three stages as described in *Publication No. 3*, with slight modifications. In the first mixing stage, to the ingredients from the original formulation, GOX (0.1% on a flour-bran basis), XYL and sunflower oil (according to the experimental plan in Table 1) were added and mixed, followed by the addition of oat flour and 21 mL of distilled water. The dough was stored at 18.5 ± 1.5°C for 0.3 - 3.7 h, depending on the experimental run, before 3D printing and rheology analyses. Control samples without enzymes or containing only GOX without XYL and with 20% of oil were also prepared. Oscillatory measurements were undertaken as described in *Publication No. 3*, where additional temperature sweep test was conducted using a temperature-controlled device (P- PTD200/AIR, Anton Paar, Graz, Austria) in the range of 20 °C - 200 °C at a heating rate of 5 °C/min at an angular frequency of 1 Hz (Kim et al., 2019). Also, the maximum, cold paste, setback and breakdown viscosity were determined using a ViscoQuick (Brabender GmbH & Co. KG, Duisburg, Germany) (*Publication No. 1*). A suspension of dough mix (10 g at 14% moisture basis) and deionized

water (approximately 105 mL) was heated to 93°C (7.5 °C/min), held at 93 °C for 5 min and cooled to 50 °C (in duplicate). Torque's measuring range was 315 cmg. The fractured cross-sectional morphology of baked snacks (without added enzymes, with added GOX, and with added GOX and XYL at optimum conditions) was observed using a scanning electron microscope (Vega 3 LMH, Tescan, The Czech Republic) operating at an electron acceleration voltage of 10 kV. Before imaging, the fractured samples were coated with a thin layer of a gold-palladium alloy. Response surface methodology (RSM) was applied to evaluate the effect of the independent parameters on the dependent variables in terms of second-order polynomials (Eq. 1):

$$y_i=\beta_0+\sum\beta_i x_i+\sum\beta_{ii}x_i^2+\sum\beta_{ij}x_i x_j \quad (1)$$

where, y_i is the response, x_{ij} are the independent parameters and β_0, β_i, β_{ij} are the regression coefficients.

The adequacy and fitness of equations obtained for dependent parameters were tested by using analysis of variance (ANOVA). Desirability function was applied to numerically optimize the snack recipe using only significant variables after backward elimination procedure aiming at minimum shape shrinkage, hardness and fracturability, while maximum printing precision, a^* and b^*. After confirmation experiment, the prediction error (E, %) and root mean square error (RMSE) were calculated as:

$$E=((X_{ex}-X_{pr})/X_{pr})\times 100 \quad (2)$$

$$RMSE=\sqrt{\sum_{i=1}^{N}(x_{pr}-x_{ex})^2/N} \quad (3)$$

where X_{ex} is value obtained after the experiment, X_{pr} is the predicted value, and N is the sample size.

Partial least squares (PLS) modelling was performed using the Unscrambler X 10.4 software (CAMO Software, Oslo, Norway). Two types of PLS models were developed: i) first one for the analysis of the influence of independent variables used in the experiment design (XYL activity, oil content and resting time) on rheological and baked snack parameters and ii) second one for the analysis of the influence of dough rheology parameters on baked snack parameters. Applicability of the models was estimated based on the determination coefficients, the root mean squared errors and bias for calibration and validation.

Besides PLS models, which are oriented towards linear predictions, artificial neural networks (ANN) were also used to define possible nonlinear correlations. In this case, two types of ANNs were developed: i) first one linking the design of experiments' (DOE) parameters with all the responses for dough rheology and snack quality and ii) the second one linking dough rheology parameters with the quality of the final baked snack product. The data was divided into training (70%), validation (20%) and testing (10%) set randomly. Multiple layer perceptron (MLP) networks were developed, and their applicability was assessed based on the R^2 values for training, testing and validation, as well as based on the sum of squares error calculated according to eq. 4:

$$E_{SOS}=\frac{1}{2N}\sum_{i=1}^{N}\left(y_i-t_i\right)^2 \quad (4)$$

where N is the number of training cases, y_i is the prediction (network outputs) of training cases t_i and target values of the i^{th} datacase.

Furthermore, to estimate the impact of the selected inputs on the outputs of the developed ANN, global sensitivity analysis was performed. RSM, ANN and ANOVA were performed with Statistica v.14 software (Tibco Software, Palo Alto, USA).

4.1. Dough rheology and printing quality

Generally, 3D-printability refers to the ability of a viscoelastic material such as dough to be extruded smoothly through a nozzle and its ability to maintain the printed shape (Kim et al., 2019). The rheological properties of the materials to be used are crucial for a successful 3D printing process. The dough is a viscoelastic material that deforms under stress. The resistance to the viscous or elastic flow of a material in an oscillating motion is defined as complex viscosity. A high loss factor, defined as the ratio of loss modulus (G'') to storage modulus (G'), implies a predominantly viscous behaviour of the sample. Also, the extrudability of dough samples can be evaluated by determining the yield stress, a value that defines the resistance of the printed dough to breaking due to gravity and the accumulation of each new layer on it (Dankar et al., 2018; Derossi et al., 2020; Le-Bail et al., 2020; Pulatsu et al., 2020; Uribe-Wandurraga et al., 2020).

All dough samples showed larger G′ values than G″ in the linear viscoelastic region (*Publication No. 3 – Figure 4 a, Publication No. 4 – Figure 3 a and b*) which indicates a solid-like behaviour of the dough necessary for achieving dimensional stability and carrying its own weight after 3D printing by extrusion (Liu & Ciftci, 2020; Pulatsu et al., 2020). Investigation on xylanase addition detected G' (between 34270 and 96574 Pa) also being higher than G'' (between 10758 and 28332 Pa) (data not shown). Compared to the *Publication No. 3*, here the addition of GOX in the control dough resulted in higher G' (54761 Pa), G'' (13236 Pa) and complex viscosity (10007 Pa s). This agrees with the paper by Yang et al. (2014) where H_2O_2 released during GOX reactions was reported as responsible for the gelation of water-soluble pentosans and the oxidization of the free sulfhydryl groups of gluten proteins, leading to an increase in elasticity and a decrease in the extensibility of the dough. Among all techniques investigated in *Publication No. 3*, the addition of bran pre-processed with HIU resulted in the highest yield stress and complex viscosity of the dough. Further, besides xylanase treatment, the effect of oil content and resting time of the dough were also investigated. Higher complex viscosity, yield point, and flow point (*Supplementary* – Figure 1 a, b, and c, respectively) were measured for lower oil content, with linear effect being more significant than the quadratic one. This confirmed results from previous study where lower viscosity has been observed with increasing oil content (24-37%) in food paste intended for 3D printing (Liu & Ciftci, 2020). Both moduli, complex viscosity, yield point and flow point showed no significant dependence on the resting time of the dough (*Supplementary* – Table 2 and 3). So far, the effect of dough storage has been investigated only in the paper by Lille et al. (2020) who observed that rye

dough shows significant changes in rheological properties (G', G'', yield point) during storage at room temperature (1-4 h), which could be responsible for its negative impact on 3D printability. The enzymes added to our dough (XYL and GOX) are probably responsible for its stability. Hence, the maximum cold and setback viscosities (*Supplementary* - Figure 1 d) showed an inverse dependence on the resting time of the dough (p=0.06 and 0.03, respectively), but a significant positive effect of XYL content was observed for the breakdown viscosity (*Supplementary* - Table 2, Fig. 1 e), which regulates the shear and rupturing of the swollen starch granules. Higher breakdown viscosity after increasing the XYL content has already been reported in dough for whole grain crackers (Nikinmaa et al., 2019). The printing precision was high (*Supplementary* - Table 1) and significantly negatively affected by the XYL activity (*Supplementary* - Table 2, Fig. 1 f).

Further, all rheological properties investigated in the *Publication No. 4* were significantly influenced by the interaction between bran type and bioprocessing agent ($p < 0.01$). Usage of rice protein did not significantly change complex viscosity, yield stress and flow point of the dough with added unprocessed WB. Here, doughs with added WB showed higher complex viscosity, yield stress and flow point compared to AB (*Publication No. 4* – Table 2). As for fermented bran, complex viscosity of the dough containing WB decreased only after fermentation with the co-culture of *S. cerevisiae* and *K. marxianus*, while *S. cerevisiae* alone and co-cultured with *L. fermentum* resulted in significantly higher complex viscosity. On the other hand, all fermented AB resulted in higher complex viscosity. Yield stress increased in case of all pre-processed bran, with the AB fermented with *S. cerevisiae* and *L. fermentum* as an exception. This describes dough enriched with fermented WB and AB as being able to support upcoming layers during 3D printing process.

Printing precision, an equivalent to printing quality, is highly influenced by the rheological properties of the dough. Higher viscosity and loss factor are connected with improved shape fidelity of 3D-printed dough (Pulatsu et al., 2020; Sun et al., 2020; Vukušić Pavičić et al., 2021; Zhang et al., 2018). Therefore, bran pre-processing with HIU, vacuum microwave cooking and pulsed light resulted in higher shape accuracy of baked snacks due to enhanced viscosities of the dough observed in *Publication No. 3*. This was confirmed with PCA which clearly showed (*Publication No. 3* – Figure 6 a and b) how snacks with added pre-processed bran characterized by high viscosities, yield stress, printing precision, and shape accuracy were separated from unprocessed bran-containing dough. Also, improved printing precision of WB-containing

dough might be linked with the higher fibre content and presence of gluten proteins in WB compared to AB. XYL activity significantly negatively affected the printing precision (*Supplementary* – Table 2, Fig 1f). Dough containing WB fermented with *S. cerevisiae* or its co-cultures with *L. fermentum*, as well as dough with added AB fermented with *S. cerevisiae* and *K. marxianus*, were printed the most accurately. Previously, the fermentation of amaranth flour with various species of *Lactobacillus* was found to improve rheological properties of the dough and the quality of wheat composite bread (Carrizo et al., 2017; Gobbetti et al., 2019).

4.2. Snack properties

As a post-processing technique, baking may enhance the quality and flavour of 3D-printed dough. Still, to maintain the original printed shape, different additives and stabilizers with suitable thermomechanical properties can be used (Hussain et al., 2022; Kim et al., 2019; Lille et al., 2018; Vukušić Pavičić et al., 2021). All dough samples 3D-printed in this dissertation were shrunk during baking. This can be attributed to the dehydration process, the denaturation and contraction of proteins and the spread-inhibiting effect of starch gelatinization (Hussain et al., 2022; Lille et al., 2018; Pulatsu et al., 2020; Vieira et al., 2020). In *Publication No. 3*, shape deformation was reduced only for snacks with added bran pre-processed with HIU. Shrinkage of dough with WB pre-processed with vacuum microwave cooking or pulsed light was comparable to that with unprocessed bran (~ 32%). The stabilization effect of HIU can be connected with its application in making emulsion and gels (Nourbehesht et al., 2018). So far, Sun et al. (2020) investigated the properties of the 3D printing cookie dough pre-processed with a microwave, but no results for changes during baking were obtained. However, Keerthana et al. (2020) reported how microwave post-processing results in snack shrinkage of around 5%. Snacks to which only GOX was added showed a shrinkage of 16.7% after baking. Moreover, snack shrinkage was strongly inversely linear and positively quadratic dependent on the XYL activity (*Supplementary* - Table 2 and 3), while it was not affected by the amount of oil. Lower activity of XYL (17 U/g) resulted in higher shrinkage than only GOX, but higher activities of XYL reduced deformation. This behaviour might be linked with the synergistic effect of GOX and XYL. Products of lower activity of XYL participate in the reactions of GOX and thus promote protein aggregation, i.e. they strengthen the dough (Dai and Tyl, 2021; Yang et al., 2014), whereas at higher XYL concentrations, the solubilization of arabinoxylans probably predominates and promotes spreading (Nikinmaa et al., 2019). Indeed, it has already been reported that a clear level of xylanase (0.06 g/kg dough) is required to overcome the effect of GOX on dough rheology (Dai and Tyl, 2021) and its behaviour in baking. A synergistic action of GOX and xylanase was confirmed with scanning electron images (*Supplementary* – Figure 3 c-f). Snacks without added enzymes had relatively porous structure characterized by interconnected irregularly shaped voids about 50-100 μm in size (Fig. 3a). A denser and less porous structure was observed in snacks with added GOX and XYL (Fig. 3c and e). SEM micrographs at higher magnification (*Supplementary* - Fig. 3b, d and f) revealed that the protein network structure of snacks without the addition of GOX and XYL was relatively open and characterized by areas with starch granules only partially covered by the protein matrix

(*Supplementary* - Fig. 3b). The addition of GOX enhanced the protein network (*Supplementary* - Fig. 3d). Protein network of snacks was further strengthened by the addition of XYL at 37 U/g, which resulted in starch granules being fully embedded in a dense, closed and uninterrupted protein matrix (*Supplementary* - Fig. 3f). Thus, both GOX and XYL promoted protein aggregation, which agrees with previous study (Dai and Tyl, 2021), and might be related to less shrinkage in baking. Overall, when comparing all pre-processing methods of WB carried out in this dissertation, fermentation with co-culture of *S. cerevisiae* and *K. marxianus* was the most efficacious in maintaining the original 3D-printed shape.

Regardless of the bran pre-processing, the average weight, height, line width or diameter of snack did not differ between samples, whereby all baked snacks had a lower weight than raw dough, which is related to moisture loss during baking (Pulatsu et al., 2020; Vieira et al., 2020).

During the baking process, snacks became darker, redder and yellower. Snack browning could be linked with Maillard reactions and starch dextrinization (Jagadiswaran et al., 2021; Keerthana et al., 2020), non-enzymatic browning reactions (Pathare et al., 2013), which results from the carbohydrate degradation during heat treatments (Severini et al., 2018). Maillard products are perceived positively by consumers as they also contribute to the colour and flavour of the baked product (Pathare et al., 2013). Snacks enriched with pre-processed bran (HIU, vacuum microwave cooking and pulsed light) were significantly lighter and showed significantly smaller ΔE^* and browning values than snacks with unprocessed WB. Higher ΔE^* around 39 after microwave baking of snacks with added mushroom powder (Keerthana et al., 2020) were likely due to differences in recipe and baking process. In the paper by Krishnaraj et al. (2019) minor ΔE^* (1.78) of 3D-printed snacks was achieved with its microwave post-processing (280 W) which was linked with the prevention of browning reactions due to rapid moisture evaporation in the samples. The control snack with unprocessed WB had a ΔE^* of 3.25. Addition of GOX resulted in ΔE^* between the first and the last 3D-printed and baked snack of 0.90, barely visible to the human eye (Pathare et al., 2013). Therefore, GOX through degrading β-carotene, i.e. converting α-D-glucose into lactones and H_2O_2 (Yang et al., 2014) successfully minimized the enzymatic browning of the dough. BI is related to Maillard's reactions, dextrinization of starch and sugar caramelization during baking (Pathare et al., 2013). Reducing the oil content resulted in redder and yellower baked snacks with improved BI. As colour is a key quality parameter for consumers and influences their choice (Pathare et al., 2013), these results show that a desired reduction in the oil content can contribute to the

attractive colour of the snack. In line with this, in the paper by Colla & Gamlath (2015) lower fat content was associated with more browning of pea snack. Using unprocessed and pre-processed AB gave lighter, yellower and less red snacks. Higher BI was achieved with bran fermentation, except for bran fermented with *S. cerevisiae* and *L. fermentum.* Also, fermentation with *S. cerevisiae* causes higher BI of snacks possibly due to the bioavailability of amino acids known to be involved in the formation of brown pigments (Pathare et al., 2013).

Texture is of major importance, i.e. the most critical quality attribute of snack foods. Increasing the xylanase concentration and decreasing the oil content resulted in harder snacks after baking (*Supplementary* – Table 2 and 3). Fracturability of snacks showed no significant dependence on the oil content, while a higher amount of XYL and a longer resting time resulted in significantly lower fracturability (*Supplementary* – Tables 2 and 3). Consistent with this, whole grain crackers with added XYL exhibited improved textural properties (Nikinmaa et al., 2019). Previously, the addition of oil (3.5-24%) had shown no effect on the hardness of the expanded snacks (Menis-Henrique et al., 2020). A similar range (11-20 N) of fracture force of 3D-printed protein and fibre-rich rye snacks was reported by Lille et al. (2020). It can be concluded that 3D-printed snacks with moderate oil content but optimum XYL activity could have physical properties that would be acceptable to consumers. Further, snack with WB were harder than snacks containing AB. Fermentation of WB and AB bran resulted in lowering of snack hardness, except when WB was fermented with *S. cerevisiae* and *L. fermentum*. Rani et al. (2021) reported that rice-black gram snacks extruded after fermentation with *S. cerevisiae* had hardness ranging between 15 and 37 N, depending on barrel temperature, extruder screw speed and die opening diameter.

Sensory analysis results (Publication No. 4) for snack with added unprocessed and fermented WB and AB showed a statistically significant difference between the samples in all evaluated attributes. Unlike wheat snacks, snacks with added AB had a more uniform colour, more pronounced bitter taste, yeast and bran odour, and less pronounced oil flavour ($p < 0.01$ for all attributes). A significant dependence on the interaction between bran and bioprocessing agent type was observed for bitter aftertaste ($p < 0.01$), saltiness ($p = 0.02$), and bran odour ($p < 0.01$). Snacks with unprocessed WB had the least pronounced bitter aftertaste, while bioprocessing of WB with *S. cerevisiae* resulted in the least bitter snacks. As expected, fermented flavour was significantly more expressed in both wheat and amaranth snacks with added bioprocessed bran ($p < 0.01$). Overall, bioprocessing lightened the bran odour in amaranth snacks, whereby in

wheat snacks this was achieved only after treatments with LV1 and *S. cerevisiae* alone or in combination with inulinase.

RSM, PLS and ANN models were used to develop predictive models for determining dough rheology (flow point, yield point, complex, breakdown and maximum viscosity), 3D printing precision and snack quality (shape shrinkage during baking, hardness, fracturability, and colour). The conditions optimized by RSM, with a desirability coefficient of 0.75, considering the lowest shape shrinkage and the highest printing precision, were as follows: oil content 18-22%, XYL activity 37 U/g (on flour-bran basis) and dough resting time 1-3h. When lowest hardness and fracturability, while highest a^* and b^* were considered, the optimized conditions were similar (with a desirability of 0.63): oil content 18-21%, XYL content 31-42 U/g on flour-bran basis and resting time 1-3h. The prediction values and errors (in brackets) for these conditions (oil content 20%, XYL content 37 U/g on flour-bran basis and resting time 2 h) were: 98% printing precision (0.5%), 6% shape shrinkage (9%), hardness 24 N (2%), fracturability 1.5 mm (6%), a^* 8 (0.8%), and b^* value 15 (2%). Prediction errors of less than 10% implied the suitability of the models used and the success of the RSM optimization, despite the low R^2 for printing precision, a^*, hardness and fracturability (Table 4), as well as a significant ($p<0.05$) lack of fit for the models describing the effect of XYL on printing precision ($p=0.03$) and shape shrinkage ($p=0.01$). The fat content of the optimized snack formulation (Fig. 2) was 16.5%, of which 8.8% was saturated (palmitic and stearic acid).

Furthermore, twelve PLS models were developed linking XYL activity, oil content and dough resting time to breakdown, maximum hot and complex viscosity, yield point, flow point, printing precision, shape shrinkage, snack hardness, snack fracturability, a^*, b^* or BI. The highest R^2 values (for calibration and validation) were obtained for the complex viscosity and breakdown viscosity, while the lowest R^2 values were obtained for snack fracturability (*Supplementary* - Table 4). The RMSE values for all developed PLS models are low, while the bias values are equal or very close to 0, indicating an excellent fit of the developed models to the experimental data. A good-fitting model has R^2 values above 0.90, while values between 0.70 and 0.90 indicate that the model is fairly precise (Radoš et al., 2021). It can be concluded that the PLS models in this study correlate with XYL activity, oil content and resting time with relatively high precision and accuracy and can be used for quantitative and qualitative prediction of dough rheology parameters, as well as b^* and BI of snacks. In addition, different PLS models were developed to establish a possible correlation between dough rheology and

snack quality. In case of the PLS model that linked complex and breakdown viscosities with the 3D printing and snack quality, the highest R^2 values (0.783 for calibration and 0.731 for validation) were obtained for b^*. The use of PLS models for predicting a^*, hardness and fracturability based on dough rheology cannot be used for qualitative or quantitative purposes, while PLS models can be used for qualitative purposes in the case of printing precision, shape shrinkage, b^* and BI (*Supplementary* - Table 4).

The ANN models contained (i) 3 neurons (XYL activity, oil content, dough resting time) in the input layer, 10 neurons in the hidden layer and 12 neurons in the target layer (complex viscosity, flow point, yield point, breakdown viscosity, maximum viscosity, printing precision, shape shrinkage, hardness, fracturability, a^*, b^* and BI), or (ii) 5 neurons (complex viscosity, flow point, yield point, breakdown viscosity and maximum viscosity) in the input layer, 11 neurons in the hidden layer and 7 neurons in the target layer (printing precision, shape shrinkage, hardness, fracturability, a^*, b^* and BI) (*Supplementary* - Table 5). The R^2 values of the ANN model were higher than the PLS models (*Supplementary* - Table 4 and 5) and similar or higher than the RSM models (*Supplementary* - Table 4). Thus, ANN was successfully used to predict breakdown and complex viscosity, yield point, flow point, printing precision, shape shrinkage, a^*, b^* and BI based on the XYL activity, oil content and resting time of the dough, as well as to predict printing precision, shape shrinkage, a^*, b^* and BI based on dough rheology. A global sensitivity analysis of the developed ANNs was also performed to gain insight into the magnitude of the impact of the inputs on the targets used to develop the ANNs (*Supplementary* - Table 6). Oil content had the greatest impact on dough rheology and print quality, followed by XYL activity and rest time. For the ANN estimating the influence of dough rheology on printing quality, the greatest influence of flow point on the results was found, followed by complex viscosity, yield point, breakdown viscosity and maximum hot viscosity.

5. Snacks and breakfast cereals enriched with grain by-products

Based on the results provided in the previous sections, oat flour, wheat bran, sunflower oil or pea proteins were partially changed with amaranth bran, pumpkin seed cake and defatted flaxseed flour. Four snack recipes consisted of oat flour, sunflower oil, salt, baking soda, glucose oxidase, water, and either of the following: 1) wheat bran and pea protein (mixture WS), 2) pumpkin seed cake (WPFS), 3) defatted flaxseed flour (WFFS), 4) amaranth bran and rice protein (AS). Additionally, two breakfast cereal recipes consisted of oat flour, rice protein, wheat bran, baking soda, glucose oxidase, water, and sugar (WSBC) or honey (WHBC).

This part of the dissertation aimed to investigate dough rheology, printing quality and textural properties of 3D-printed snacks and breakfast cereals made with grain by-products. As in *Publication No. 3* and *Publication No. 4*, hearth and spiral 3D shapes were used for 3D printing of snacks, while breakfast cereals were 3D-printed in quadratic shape (*Supplementary* – Figure 3). All 3D shapes were extruded using the same printer throughout the research and baked at 180°C for 18 min (snack) or 10 min (breakfast cereal). 3D printing precision, shape accuracy and deformation were determined by digital image analysis. Amplitude and frequency sweep tests were performed on the dough to determine its rheological properties. Cutting tests were performed on final products with the texture analyser. All properties determined were significantly dependent on the dough mixture. The most viscous mixture (*Supplementary* – Figure 4) with the largest storage and loss modulus (*Supplementary* – Figure 5) was the snack mixture with only WB (WS). Dough for snacks, particularly with pumpkin seed cake flour, was printed with higher precision (between 95 and 98%) than dough for breakfast cereals (printing precision ranged between 68 and 82%) whose shape was deformed less during baking. Snack shapes shrank (between -12 and -48%), while breakfast cereals spread (by 75 and 80%) during baking. Except for amaranth mixture (AS), baked snacks were harder (from 1.68 to 2.84 g) than the breakfast cereals (from 0.63 to 0.78 g). The extent of shape deformation was inversely correlated (r= -0.85) to product hardness. Overall, the WPFS dough mixture was printed with the highest precision (98%) and showed the highest shape accuracy (98%) and the lowest deformation (12%). According to the given results, mixtures with grain by-products can be accurately 3D-printed in various shapes, but further modifications are required to avoid undesirable shape deformation in post-processing.

Chapter 7

Conclusions

Wheat bran particle size (from 500 to 10 μm) affects polyphenol oxidase activity, phenolic content, antioxidant activity, and water swelling, whereas ultrasonic treatment time affects only polyphenol oxidase activity. Bran suspension in water (15% w/v) by the end of the treatment reached the temperature from 57 to 94 °C and acoustic power (from 30 to 81 W) depending on the ultrasound amplitude. For the biggest reduction of polyphenol oxidase activity (by 93%), bran should be ground to fine particle size (10 μm) and its water suspension should be ultra-sonicated at 400 W, amplitude 60-100%, for 10 to 15 min. Bran grinding from coarse to fine particle and ultrasonic treatment have opposing effects on wheat bran's phenolic content, antioxidant activity, and water swelling. Cooling during ultrasonic treatment contributes to the preservation of phenolics and antioxidant activity, but limits the inactivation of polyphenol oxidase.

The combination of micronization and ultrasonic treatment without using additives can successfully improve different technological properties and enhance the content of free phenolic compounds of wheat bran. The optimum particle size of wheat bran should be addressed for each application.

Wheat bran oxidizes within 3 months of ambient storage. High-intensity ultrasound treatment with an amplitude of 80% for 15 min prolongs the oxidative stability at ambient condition of wheat bran by up to 12 months due to the reduction of lipase (64%) and peroxidase (90%) activity. When applied to a bran suspension of sufficiently low concentration (15% w/v), it can be imposed as a more efficient method of prolonging wheat bran oxidative stability than cold storage. The particle size reduction negatively affects wheat bran's stability and shelf life.

Dough made from oat or barley flour, enriched with wheat bran (20% on flour basis), with 20 layers and a printing speed of 25 mm/s can be successfully three-dimensionally-printed. Nevertheless, it shows very distinct colour changes over a printing time. Lower printing temperature (20°C) with a higher pH (5.4 vs 7.0) of the dough to some extent supresses the undesirable colour changes.

Wheat bran pre-processing with high-intensity ultrasound, vacuum microwave cooking or pulsed light stops the browning process within 50 min due to the reduction of polyphenol oxidase activity. It contributes to the snack's shape accuracy by enhancing the dough's viscosity. Pre-processing should be applied to other ingredients (e.g. flour) to achieve a greater effect on diminishing the colour changes of the dough.

The bioprocessing of wheat bran with yeast alone or combined with lactic acid bacteria or enzyme inulinase for 24h at 37 °C lowers its high content of fructans (2.64%), whereby the fructose released and the mannitol formed need to be followed. Bioprocessed wheat bran at level 7% in the formulation could be used in low-FODMAPs snack labelled as a source of fibre (>3 g per 100g).

Colour difference between first and last 3D printed snack with added pre-processed bran is still visible (ranging from 2.2 to 3.5), whereas the incorporation of GOX into the dough notably decelerates enzymatic browning resulting in small colour difference (<1.5).

Post-process baking results in shape shrinkage (32%) and additional browning of snacks enriched with unprocessed and pre-processed bran. Shrinkage can be reduced by pre-processing of bran with high-intensity ultrasound or bioprocessing, whereby the fermentation with co-culture of yeasts *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* is the most successful in preventing shrinkage (to 12%). In addition, fermentation of bran results in lower snack hardness, colour change and enhanced sensory properties of snack.

The snack formulation is highly sensitive to oil reduction but remains equally printable during 3h of dough rest. High printing precision and low shape shrinkage can be achieved with adding xylanase but its level must be carefully optimized for snack product. Dough with 20% oil and 37 U of xylanase per gram of flour-bran mixture, regardless of resting time, is least shrunk (only 5%) in baking, resulting in desirable structure, hardness and colour of snacks containing 16.5% of fat.

Response surface methodology and artificial neural networks are effective techniques for predicting dough rheology as a function of resting time, xylanase and oil amount, as well as the quality of 3D printing and snack. Artificial neural networks could be used to predict the quality of 3D-printed snack based on dough rheology.

Mixtures with grain by-products can be accurately 3D-printed in various shapes, including breakfast cereals.

Future investigations should focus on the oxidative stability of whole wheat grain treated with ultrasound before milling, as well as on the shelf life of 3D printed snacks and breakfast cereals with incorporated pre-processed wheat bran.

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Autobiography

Autobiography

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Supplementary

Table 1. The 3-factor central composite design and experimental data (responses).

Independent parameters				Responses					
Run	XYL (U/g)	Oil (%)	Time (h)	Printing precision (%)	Shape shrinkage (%)	a^*	b^*	Hardness (N)	Fracturability (mm)
1	17	10	1	97.7 ± 0.9	29.6 ± 4.2	5.7 ± 0.3	16.4 ± 0.6	22.6 ± 2.2	2.1 ± 0.2
2	17	30	3	98.7 ± 0.5	20.5 ± 3.1	7.0 ± 0.3	13.5 ± 0.6	21.4 ± 4.8	1.2 ± 0.3
3	57	10	3	97.8 ± 0.4	4.2 ± 0.5	8.7 ± 0.2	18.2 ± 0.6	27.2 ± 4.0	1.5 ± 0.3
4	57	30	1	95.4 ± 0.3	4.6 ± 0.8	6.4 ± 0.5	11.2 ± 0.6	25.1 ± 5.0	1.4 ± 0.2
5	37	20	2	98.0 ± 0.8	5.4 ± 1.1	7.9 ± 0.5	14.9 ± 0.7	22.1 ± 4.0	1.6 ± 0.2
6	17	10	3	98.2 ± 0.4	20.1 ± 2.4	8.2 ± 0.5	15.1 ± 0.4	18.6 ± 2.4	1.5 ± 0.2
7	17	30	1	98.8 ± 1.8	22.5 ± 2.1	6.9 ± 0.3	12.4 ± 0.4	18.2 ± 4.3	1.4 ± 0.3
8	57	10	1	96.3 ± 0.6	4.7 ± 0.5	8.4 ± 0.2	17.3 ± 0.4	25.4 ± 5.3	1.5 ± 0.2
9	57	30	3	97.6 ± 0.5	4.1 ± 0.7	6.9 ± 0.4	10.6 ± 3.1	20.4 ± 3.1	1.1 ± 0.4
10	37	20	2	97.9 ± 0.7	5.6 ± 1.1	8.1 ± 0.2	15.4 ± 0.3	21.4 ± 4.2	1.7 ± 0.3
11	3.7	20	2	99.0 ± 1.7	26.8 ± 3.2	7.4 ± 0.2	14.2 ± 0.5	17.3 ± 2.3	2.6 ± 0.3
12	70	20	2	96.0 ± 0.7	5.7 ± 1.8	8.1 ± 0.3	14.0 ± 0.4	25.4 ± 5.2	1.3 ± 0.2
13	37	3.3	2	97.7 ± 0.2	5.0 ± 0.9	7.9 ± 0.2	19.1 ± 0.6	30.4 ± 1.8	1.3 ± 0.4
14	37	36.7	2	96.5 ± 0.3	5.3 ± 0.8	6.3 ± 0.3	11.8 ± 0.6	16.5 ± 2.3	1.5 ± 0.3
15	37	20	0.3	97.2 ± 0.2	5.4 ± 0.7	8.1 ± 0.2	16.2 ± 0.6	21.9 ± 2.8	2.0 ± 0.2
16	37	20	3.7	96.2 ± 0.3	4.9 ± 1.0	7.7 ± 0.2	13.9 ± 0.5	26.8 ± 4.7	1.2 ± 0.3
17	37	20	2	98.2 ± 0.9	5.3 ± 0.9	7.8 ± 0.3	13.8 ± 0.5	26.9 ± 3.1	1.5 ± 0.3

XYL- xylanase*;* a^*- redness; b^*- yellowness.

Table 2. Analysis of variance and regression coefficients for response variables (real values).

Parameter	RSM							PLS ($p<0.05$)			
	Constant	XYL	Oil	Time	XYL^2	Oil^2	XYL*Oil	Constant	XYL	Oil	Time
CV	10242.7	n.s	-365.94^{***}	n.s.	n.s.	5.12^{***}	n.s.	9.02	-0.01	-0.16	6.33×10^{-4}
YP	32.94	n.s	-1.14^{***}	n.s.	n.s.	0.02^{**}	n.s.	29.79	-0.04	-0.52	1.97×10^{-3}
FP	760	n.s.	-30.05^{***}	n.s.	n.s.	0.40^{*}	n.s.	0.67	1.03×10^{-3}	-0.01	5.40×10^{-5}
BV	8.95	0.68^{***}	n.s.	n.s.	n.s.	n.s.	n.s.	9.88	0.12	-0.05	7.98×10^{-4}
MV	91.44	n.s.	-0.23^{*}	n.s.	n.s.	n.s.	n.s.	87.37	0.03	-0.09	5.21×10^{-4}
PP	99.01	-0.24^{***}	n.s.	n.s.	n.s.	n.s.	n.s.	99.14	-0.04	-0.01	1.60×10^{-4}
SS	37.67	-7.17^{***}	n.s.	n.s.	0.37^{***}	n.s.	n.s.	27.23	-0.41	-0.05	1.80×10^{-3}
a^*	5.20	0.34^{*}	0.16^{**}	n.s.	n.s.	$3.14 \times 10^{-3*}$	-0.01^{**}	7.86	0.01	-0.05	2.67×10^{-4}
b^*	15.48	n.s.	-0.04^{***}	n.s.	n.s.	n.s.	-0.03^{***}	19.26	-1.62×10^{-3}	-0.23	9.60×10^{-4}
BI	42.03	n.s.	-0.04^{***}	n.s.	n.s.	n.s.	-0.09^{***}	53.33	5.22×10^{-3}	-0.62	2.62×10^{-3}
H	16.83	2.17^{**}	-0.06^{**}	n.s.	n.s.	n.s.	n.s.	23.39	0.11	-0.23	1.54×10^{-3}
F	2.01	-0.06^{**}	n.s.	-0.17^{**}	n.s.	-0.14*	n.s.	2.11	-0.01	-8.25×10^{-3}	-1.80×10^{-5}

*, $p < 0.10$; **, $p \leq 0.05$; ***, $p \leq 0.01$; n.s., $p > 0.10$; XYL- xylanase; CV- complex viscosity; YP- yield point; FP- flow point; BV- breakdown viscosity; MV- maximum viscosity; PP- printing precision; SS- shape shrinkage; a^*- redness; b^*- yellowness; BI- browning index; H- hardness; F- fracturability.

Table 3. Comparison of RSM, PLS and ANN outcome with input variables as in the experiment design (oil content, xylanase content and resting time).

Variable	RSM			PLS						ANN		
	R^2	Adjusted R^2	RMSE	Validation			Calibration			R^2 - train	R^2 - test	R^2 - validation
				R^2	RMSE	Bias	R^2	RMSE	Bias			
Dough												
CV	0.937	0.928	0.432	0.852	0.623	-0.020	0.897	0.524	0	0.980	0.982	0.968
YP	0.880	0.863	1.979	0.815	2.507	-0.114	0.860	2.033	0	0.985	0.993	0.975
FP	0.893	0.878	0.050	0.814	0.067	-0.002	0.871	0.052	0	0.988	0.996	0.972
BV	0.780	0.786	1.141	0.828	1.118	0.050	0.859	0.942	0	0.912	0.919	0.924
MV	0.232	0.181	3.914	NA	5.104	-0.033	0.062	3.997	0	0.768	0.457	0.647
PP	0.522	0.490	0.759	0.406	0.887	-0.007	0.553	0.718	0	0.759	0.883	0.817
Baked snack												
SS	0.865	0.846	3.599	0.595	6.296	-0.229	0.705	5.016	0	0.954	0.952	0.978
a^*	0.643	0.524	0.572	0.159	0.821	0.019	0.391	0.652	0	0.928	0.902	0.914
b^*	0.899	0.876	0.836	0.752	1.296	0.009	0.819	1.035	0	0.903	0.964	0.946
BI	0.912	0.892	2.103	0.743	3.556	0.067	0.810	2.856	0	0.931	0.965	0.953
H	0.654	0.209	3.502	0.454	3.203	0.103	0.591	2.586	0	0.734	0.496	0.674
F	0.602	0.469	0.275	0.031	0.407	0.0009	0.321	0.318	0	0.803	0.899	0.609

RSM- response surface methodology; PLS- partial least squares; ANN- artificial neural network; RMSE- root mean square errors; CV- complex viscosity; YP- yield point; FP- flow point; BV- breakdown viscosity; MV- maximum viscosity; PP- printing precision; SS- shape shrinkage; a^*- redness; b^*- yellowness; BI- browning index; H- hardness; F- fracturability.

Table 4. Correlation coefficients for the developed ANN (MLP 5-11-7) containing parameters of the dough rheology in the input layer compared to PLS considering breakdown and complex viscosity.

Variable	ANN			PLS		
	R^2 - train	R^2 - test	R^2 - validation	Prediction error	R^2 Calibration	R^2 Validation
PP	0.808	0.725	0.749	0.009	0.755	0.699
SS	0.957	0.941	0.975	0.048	0.783	0.731
a^*	0.929	0.915	0.849	0.038	0.708	0.626
b^*	0.905	0.942	0.945	0.049	0.662	0.529
BI	0.932	0.945	0.941	0.126	0.190	0
H	0.787	0.522	0.733	0.199	0.712	0.542
F	0.819	0.864	0.553	0.021	0.282	0.061

PP- printing precision; SS- shape shrinkage; a^*- redness; b^*- yellowness; BI- browning index; H- hardness; F- fracturability.

Table 5. Structures, activation functions, R^2, and sum of squares error values of the developed ANNs for prediction of the dough viscosity, 3D printing quality and snacks quality.

Input	ANN structure	Training perf.	Test perf.	Validation perf.	Training error	Test error	Validation error	Hidden activation	Output activation
Oil content, xylanase content and resting time	MLP 3-10-12	0.887	0.867	0.865	251.874	118.979	431.960	Tanh	Logistic
Dough rheology	MLP 5-11-7	0.877	0.836	0.821	13.772	14.372	14.818	Tanh	Logistic

Table 6. Global sensitivity analysis for the developed ANNs.

Oil, xylanase content and resting time as inputs	ANN architecture/ Input	Oil	XYL	Time		
	MLP 3-10-12	39.454	22.235	8.496		
Dough rheology parameters as inputs	ANN architecture/ Input	FP	CV	YP	BV	MV
	MLP 5-11-7	18.000	14.800	13.168	10.476	4.014

XYL- xylanase; YP- yield point; FP- flow point; CV- complex viscosity; BV- breakdown viscosity; MV- maximum hot viscosity.

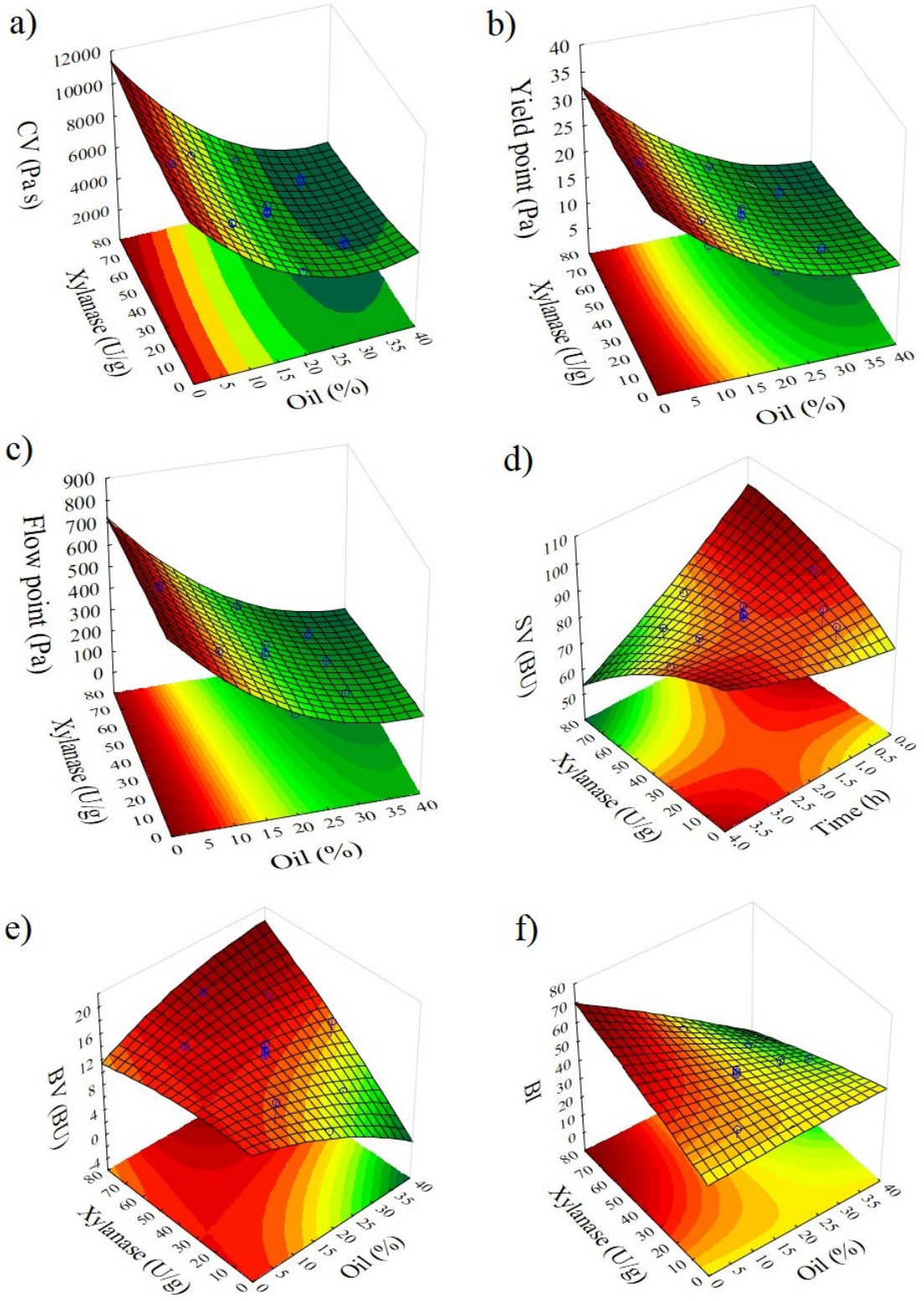

Figure 1. Response surface plots of effect of oil and XYL content or resting time of the dough on a) complex viscosity (CV), b) yield point, c) flow point, d) setback viscosity (SV), e) breakdown viscosity (BV), and f) browning index (BI).

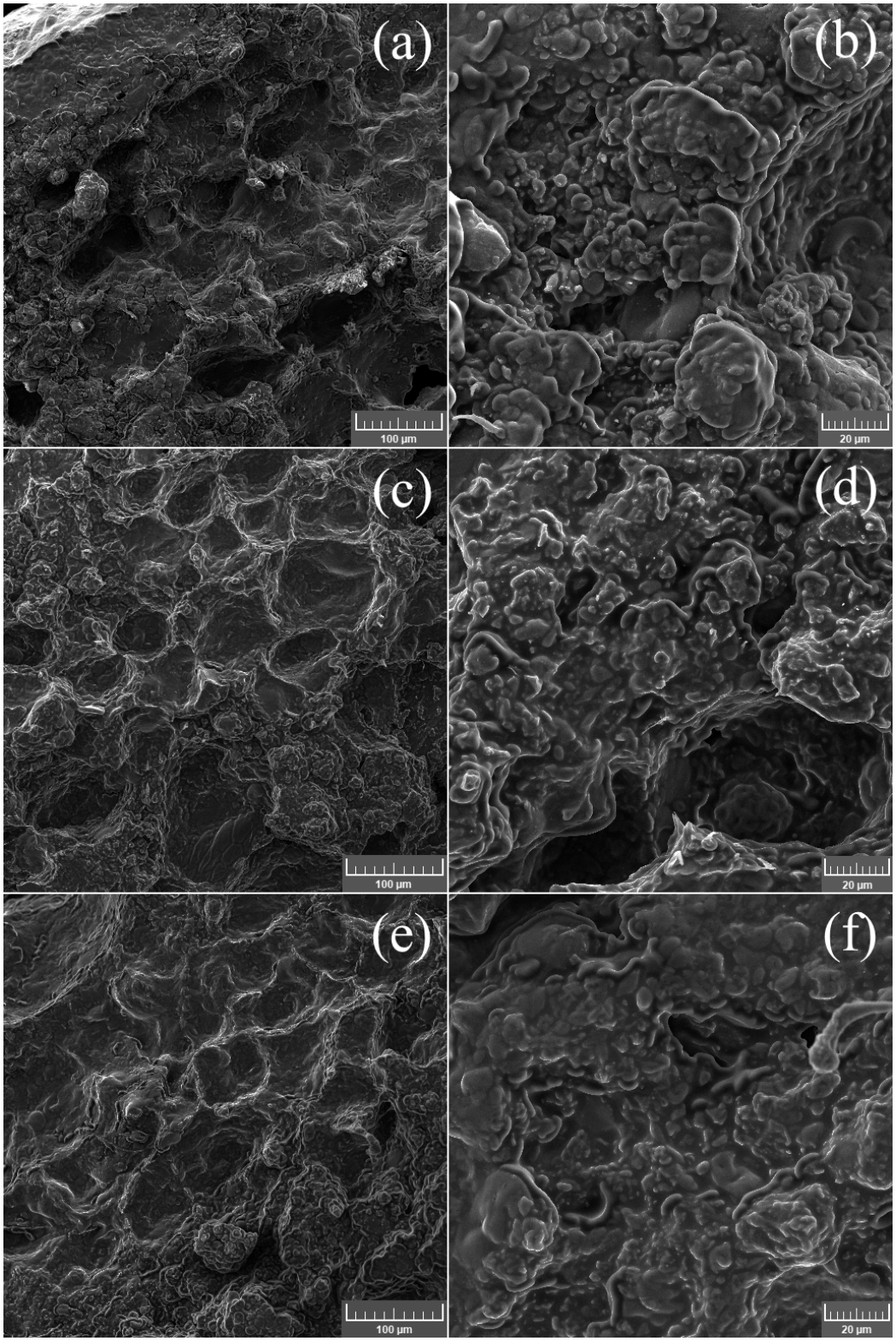

Figure 2. Fractured cross-sectional SEM micrographs of baked snacks a) and b) without added enzymes, c) and d) with added 37 U/g GOX, and e) and f) with added 37 U/g GOX and 37 U/g XYL. Scale bars represent 100 µm in figures a, c and e, and 20 µm in figures b, d and f.

Figure 3. Quadratic shape of 3D-printed breakfast cereals.

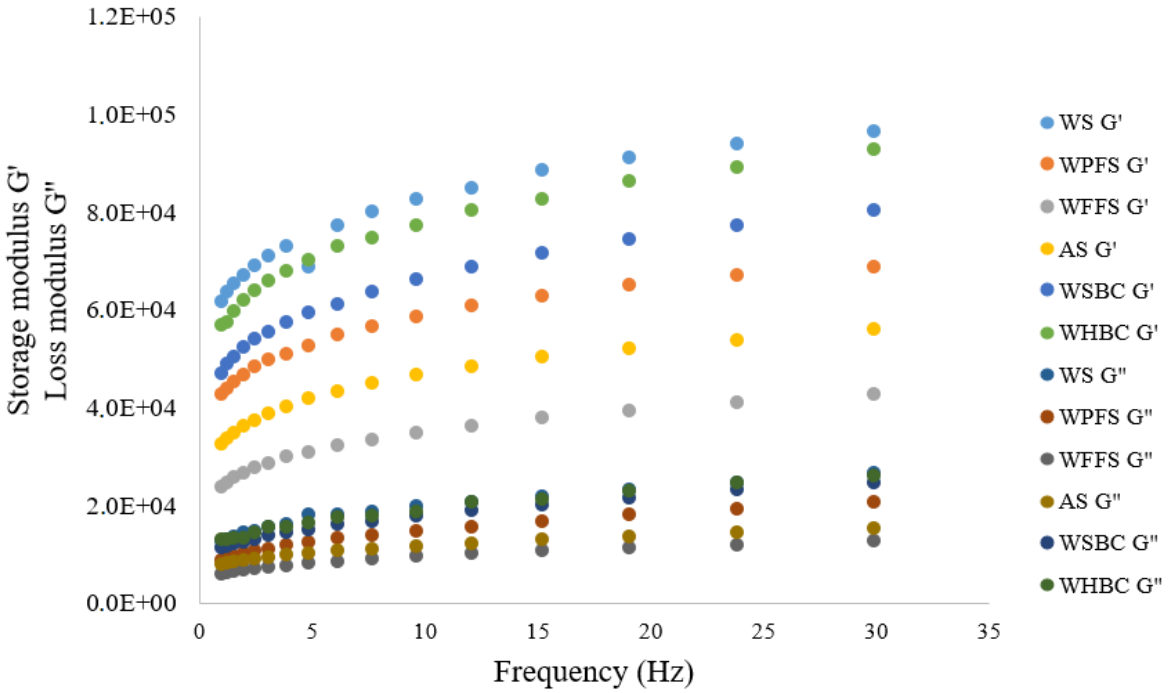

Figure 4. Storage modulus (G') and loss modulus (G'') of different dough mixtures for snacks and breakfast cereals. WS – snacks with wheat bran and pea protein; WPFS – snacks with wheat bran and pumpkin seed cake; WFFS - snacks with wheat bran and flaxseed flour; AS – snacks with amaranth bran and rice protein; WSBC – breakfast cereals with wheat bran, rice protein and sugar; WHBC – breakfast cereals with wheat bran, rice protein and honey.

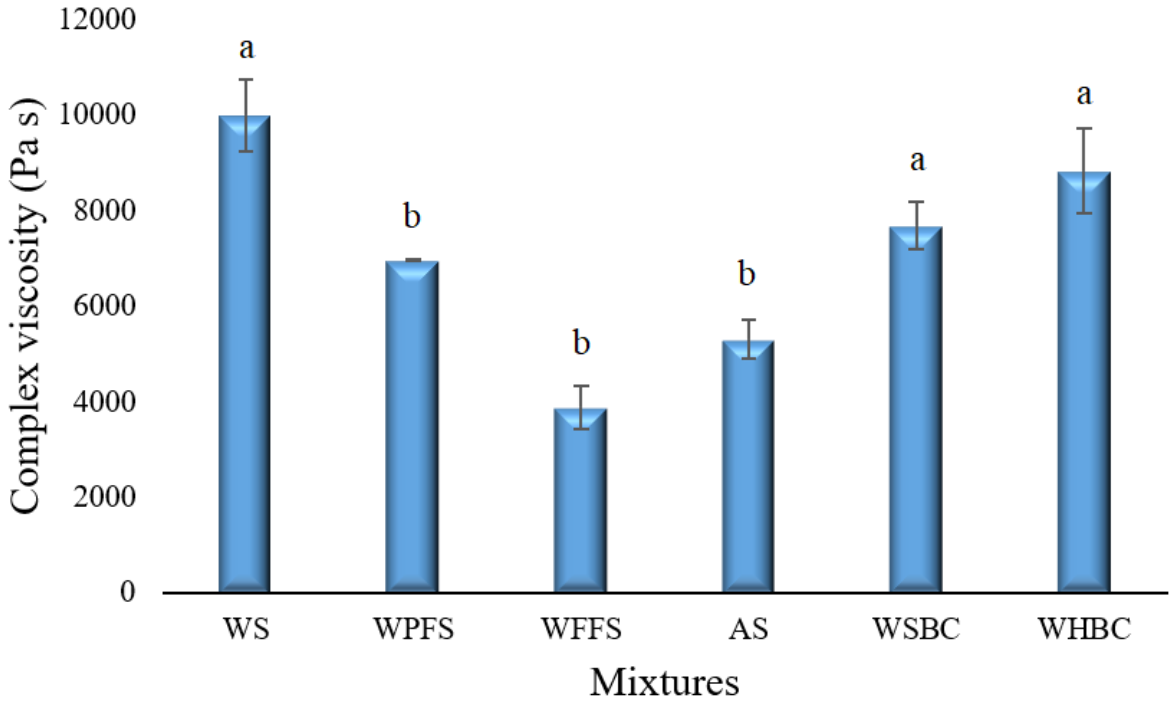

Figure 5. Complex viscosity of different dough mixtures. WS – snacks with wheat bran and pea protein; WPFS – snacks with wheat bran and pumpkin seed cake; WFFS - snacks with wheat bran and flaxseed flour; AS – snacks with amaranth bran and rice protein; WSBC – breakfast cereals with wheat bran, rice protein and sugar; WHBC – breakfast cereals with wheat bran, rice protein and honey.