Non-thermal technologies for nutritional and technological improvement of oat and barley in flat bread

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Non-thermal technologies for nutritional and technological improvement of oat and barley in flat bread

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Short abstract: Oats and barley milling products are a rich source of dietary fiber, especially β-glucans, but also possess high enzyme activity, contain antinutrients, and undesirably affect the technological and sensory properties of the bakery products. This dissertation investigated the pretreatment of oat and barley flour/bran using traditional (sourdough fermentation) and innovative techniques (high-intensity ultrasound, pulsed electric field) and the application of these pretreated raw materials in making flat bread. The addition of oat/barley bran improved the fermentation of flour to sourdough, which successfully slowed down the enzymatic browning of the retarded dough and reduced antinutrient content (32-38%) in flat bread. Ultrasonic pretreatment of oat and barley bran partially inactivated β-glucanase (by 82% and 55%), reduced antinutrients (by 17% and 39%) and increased the β-glucans content (25-65%) in the flat bread. Processing of oat and barley flour with a pulsed electric field also reduced its β-glucanase activity (by 77% and 40%), improved the solubility of non-starch polysaccharides, and increased the β-glucans content in flat bread (21-32%). These innovative and traditional techniques, in combination or individually, improved the techno-functional properties of flour/bran by enabling fiber enrichment, improving texture profile, while maintaining consumer acceptance of flat bread.

Keywords: *barley, bran, flat bread, high-intensity ultrasound, non-starch polysaccharides, oat, pulsed electric field, sourdough fermentation, β-glucanase*

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Tomislava Grgić, 448/PT

Sažetak: Zob i ječam bogat su izvor prehrambenih vlakana, posebice β-glukana, međutim posjeduju visoku enzimsku aktivnost, sadrže antinutrijente te nepoželjno utječu na tehnološka i senzorska svojstva pekarskih proizvoda. U ovoj disertaciji istražena je prethodna obrada zobenih i ječmenih posija te brašna tradicionalnim (fermentacija kiselog tijesta) te inovativnim tehnikama (ultrazvuk visokog intenziteta, pulsirajuće električno polje) te primjena tako obrađenih sirovina u izradi tankog kruha. Dodatak zobenih/ječmenih posija poboljšao je fermentaciju brašna u kiselo tijesto, što je uspješno usporilo enzimsko posmeđivanje tijesta tijekom procesa odgođene fermentacije i smanjilo sadržaj antinutrijenata (32-38%). Prethodna obrada zobenih i ječmenih posija ultrazvukom visokog intenziteta rezultirala je djelomičnom inaktivacijom endogene β-glukanaze (za 82% i 55%), smanjenim udjelom antinutrijenata (za 17% i 39%) i povećanim udjelom β-glukana (25-65%) u tankom kruhu. Obrada zobenog i ječmenog brašna pulsirajućim električnim poljem rezultirala je smanjenjem β-glukanazne aktivnosti (za 77% i 40%), poboljšanom topivosti neškrobnih polisaharida te povećanim udjelom β-glukana tankog kruha (21-32%). Ove inovativne i tradicionalne tehnike, u kombinaciji ili zasebno, poboljšale su tehno-funkcionalna svojstva brašna i posija, omogućujući obogaćivanje vlaknima, poboljšanje teksture tankog kruha, uz zadržavanje senzorske prihvatljivosti potrošača.

Ključne riječi: *fermentacija kiselog tijesta, ječam, neškrobni polisaharidi, posije, pulsirajuće električno polje, tanak kruh, ultrazvuk visokog intenziteta, zob, β-glukanaza*
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Extended abstract

Flat bread is the oldest type of bread consumed worldwide and its popularity is increasing. However, flat breads have a low fiber content because the main ingredient is refined wheat flour. Whole grain flour and bran are rich in fiber and other bioactive compounds, with oats and barley being the main sources of β-glucans among the cereals. At the same time, the high fiber content of these raw materials but also high activity of endogenous enzymes reduces their techno-functional properties why they are rarely used in bread making. The aim of this dissertation was to investigate the market and technological potential of oat and barley flour and bran in making flat bread, with a focus on techno-functional properties and nutritive improvement after pretreatment using non-thermal technologies or traditional sourdough fermentation. The effects of adding oat and barley bran on the acidification kinetics of oat and barley flour during sourdough fermentation and the effects of sourdough addition (30 and 50% of dough weight) on enzymatic browning during the retardation process (24 and 48 hours) were investigated. Furthermore, the influence of innovative technologies (high-intensity ultrasound and pulsed electric field) on the activity of endogenous β-glucanase and phytase, β-glucans contents and their structure, bioactive compounds, antioxidant activity, phytic acid content as well as the influence on the functional properties of these raw materials were investigated. The influence of application of treated oat and barley bran and flour on nutritive value, physical properties and consumers acceptance of flat bread was investigated. Among Croatian flat breads, single-layer flat bread predominates, and various types of *pogacha* are the most common. In the production of all Croatian flat breads, refined wheat flour is used as the main ingredient, and yeast is the main leavening agent. Whole grain wheat, corn or rye flour are seldom used, whereas oat- or barley- containing flat breads do not exist on the Croatian market. Oat and barley bran contained 2-3 times more dietary fiber, β-glucans, phenolic compounds and even 2-5 times more phytates than their flour counterparts. The mineral content was almost the same in barley flour and bran, but 2 times higher in oat bran than in flour. Compared to oat flour, barley flour was characterized by a higher content of minerals and β-glucans, a 2 times higher content of dietary fiber, total phenols and phytates, while the opposite was the case when comparing oat bran and barley bran. The addition of oat and barley bran to oat and barley flour (1:3) positively affected sourdough fermentation (with LIVENDO® LV1 starter, for 24 h, at 30°C). The obtained sourdough successfully slowed down the enzymatic browning of the bread dough during retardation (at 2 ± 1°C) and enriched breads with β-glucans. Bread from retarded

dough had reduced content of phytic acid (27-38%) but also β-glucans (4-28%) compared to flat bread from no-time method. The bread crumb with 50% sourdough was slightly less cohesive and elastic than the flat bread with 30% sourdough. The specific volume of flat bread with 50% sourdough was lower than that of bread with 30% sourdough. The oat flat bread had a harder crumb than the barley flat bread, and the increase in the darkness of the crust was more pronounced in oat bread than in barley bread. Pretreatment of oat and barley bran with high-intensity ultrasound (24 kHz, 400 W 217.5 kJ kg^{-1} and 348 kJ kg^{-1}-P, respectively) significantly reduced endogenous β-glucanase activity (by 82% and 55%), increased extractability (by 12%) and solubility (by 31-40%) but decreased the molecular weight (7% and 22%) of β-glucans. Meanwhile, it increased the activity of endogenous phytase (by 40% and 44%) and decreased the amount of phytic acid (by 17% and 39%). Furtheron, the degradation of β-glucans of ultrasonicated bran during sourdough fermentation and bread making was prevented. Flat breads with sourdough made from ultrasound pretreated oat bran had 27% and 20% lower crumb hardness and chewiness than the control oat flat breads, respectively, whereas these differences were not present in the barley flat breads. Treatment of oat and barley flour with a pulsed electric field (150 Hz, 12 kV cm^{-1}, 162 ms) also resulted in a decrease in endogenous β-glucanase activity (by 77% and 40%), enhanced β-glucans extractability (by 33.5%), with a minimal effect on their molecular weight, as well as increased levels of water-soluble arabinoxylans (by 56% and 68%). Hence, oat- and barley-containing flat breads were high in fiber and contained64-148% more β-glucans than the wheat control bread. Flat bread made from PEF-treated oat and barley flour had a smaller volume, similar crumb texture (hardness and chewiness), a slower staling rate and similar consumers' acceptability compared to the control oat and barley flat bread. Due to the improved functional properties, pretreated oat and barley flour and bran can be successfully applied for improvement of nutritive value and prolongation of shelf life while maintaining some of the physical properties and consumers' acceptance of flat bread.

Keywords: *β-glucanase*, *β-glucan molecular weight, bran, consumers, flat bread, high-intensity ultrasound, non-starch polysaccharides, pulsed electric field, sourdough fermentation*

Proširenisažetak

Tanak kruh je najstarija vrsta kruha koja se konzumira širom svijeta i čija je popularnost u porastu. Obzirom da se kao glavni sastojak koristi rafinirano pšenično brašno, tanak kruh ima nizak udio prehrambenih vlakana. Brašno od cjelovitog zrna i posije bogate su prehrambenih vlakana i drugih bioaktivnih spojeva, a zob i ječam glavni su izvori β-glukana među žitaricama. Istovremeno, visok sadržaj prehrambenih vlakana u ovim sirovinama, ali i visoka aktivnost endogenih enzima umanjuje njihova tehno-funkcionalna svojstva zbog čega se rijetko koriste u proizvodnji kruha. Cilj ove disertacije bio je istražiti tržišni i tehnološki potencijal zobenog i ječmenog brašna i posija u proizvodnji tankog kruha, s fokusom na tehno-funkcionalna svojstva i poboljšanje nutritivne vrijednosti nakon predobrade netoplinskim tehnologijama ili tradicionalnom fermentacijom kiselog tijesta. Istraživani su učinci dodavanja zobenih i ječmenih posija na kinetiku kiseljenja zobenog i ječmenog brašna tijekom fermentacije kiselog tijesta i učinci dodavanja kiselog tijesta (30 i 50% težine tijesta) na enzimsko posmeđivanje tijekom procesa odgođene fermentacije (24 i 48 sati). Nadalje, istražen je utjecaj inovativnih tehnologija (ultrazvuk visokog intenziteta i pulsirajuće električno polje) na aktivnost endogene β-glukanaze i fitaze, sadržaj β-glukana i njihovu strukturu, bioaktivne komponente, antioksidacijsku aktivnost, sadržaj fitinske kiseline kao i utjecaj na funkcionalna svojstva ovih sirovina. Ispitan je primjena tretiranih zobenih i ječmenih posija te brašna na hranjivu vrijednost, fizikalna svojstva i senzorsku prihvatljivost potrošača tankog kruha. Među hrvatskim tankim kruhovima prevladava jednoslojni tip, a najzastupljenije su razne vrste pogače. U proizvodnji svih hrvatskih tankih kruhova kao glavni sastojak koristi se rafinirano pšenično brašno te kvasac kao glavno sredstvo za dizanje. Brašno cjelovitog zrna pšenice, kukuruzno ili raženo brašno od cjelovitog zrna rijetko se koriste, dok tanki kruh koji sadrži zob ili ječam nije prisutan na hrvatskom tržištu. Analiza kemijskog sastava zobenog i ječmenog brašna i posija pokazala je da posije sadrže 2-3 puta više prehrambenih vlakana, β-glukana, fenolnih spojeva i čak 2-5 puta više fitata od brašna. Sadržaj minerala bio je gotovo isti u ječmenom brašnu i posijama, ali 2 puta veći u zobenim posijama nego u brašnu. U usporedbi sa zobenim brašnom, ječmeno brašno odlikuje se većim udjelom minerala i β-glukana, 2 puta većim udjelom prehrambenih vlakana, ukupnih fenola i fitata, dok je kod usporedbe zobenih i ječmenih posija bio obrnut slučaj. Dodavanje zobenih i ječmenih posija u zobeno i ječmeno brašno (1:3) pozitivno je utjecalo na fermentaciju kiselog tijesta (sa LIVENDO® LV1 starterom, 24 h, na 30°C). Dobiveno kiselo tijesto uspješno je usporilo enzimsko posmeđivanje krušnog tijesta tijekom procesa odgođene fermentacije (na 2 ± 1°C) i obogatilo kruhove β-glukanima. Kruh dobiven metodom odgođene fermentacije imao je smanjen sadržaj fitinske

kiseline (27-38%), ali i β-glukana (4-28%) u odnosu na tanak kruh dobiven brzim postupkom.Tanak kruh s 50% kiselog tijesta imao je manju tvrdoću i žvakljivost od kruha s 30% kiselog tijesta. Specifični volumen tankog kruha s 50% kiselog tijesta manji je od kruha s 30% kiselog tijesta. Zobeni tanak kruh imao je veću tvrdoću te je tamnjenje krušne korice bilo izraženije nego li kod ječmenog kruha. Predobrada zobenih i ječmenih posija ultrazvukom visokog intenziteta (24 kHz, 400 W 217,5 kJ kg^{-1} odnosno 348 kJ kg^{-1}-P) značajno je smanjila aktivnost endogene β-glukanaze (za 82% i 55%), poboljšala ekstrakciju (za 12%) i topljivost (za 31-40%), ali smanjila molekularnu težinu (7% i 22%) β-glukana. Istovremeno, povećala je aktivnost endogene fitaze (za 40% i 44%) i smanjila sadržaj fitinske kiseline (za 17% i 39%). Nadalje, spriječena je razgradnja β-glukana ultrazvučno obrađenih posija tijekom fermentacije kiselog tijesta i izrade kruha. Tanki kruhovi s kiselim tijestom od zobenih posija prethodno tretiranih ultrazvukom imali su 27% odnosno 20% nižu tvrdoću i žvakljivost od ječmenih tankih kruhova, dok razlike nisu bile prisutne kod ječmenog tankog kruha. Tretiranje zobenog i ječmenog brašna pulsirajućim električnim poljem (150 Hz, 12 kV cm^{-1}, 162 ms) također je rezultiralo smanjenjem aktivnosti endogene β-glukanaze (za 77% i 40%), poboljšanom ekstrakcijom β-glukana (za 33,5%) s minimalnim učinkom na njihovu molekulsku masu, kao i povećanom razinom arabinoksilana topivih u vodi (za 56% i 68%). Dakle, tanki kruhovi koji sadrže zob i ječam bili su bogati vlaknima i sadržavali su 64-148% više β-glukana nego kontrolni kruh od pšenice. Tanak kruh od zobi i ječma tretiranih PEF-om imao je manji volumen, sličnu teksturu (tvrdoću i žvakljivost), sporiju stopu starenja i sličnu prihvatljivost potrošača u usporedbi s kontrolnim zobenim i ječmenim tankim kruhom. Zbog poboljšanih funkcionalnih svojstava, predobrađeno zobeno i ječmeno brašno i posije mogu se uspješno primijeniti za poboljšanje nutritivne vrijednosti i produljenje roka trajanja tankog kruha uz istovremeno zadržavanje određenih fizikalnih svojstava te prihvatljivosti potrošača.

Ključne riječi: *fermentacija kiselog tijesta, ječam, neškrobni polisaharidi, posije, potrošači, pulsirajuće električno polje, tanak kruh, ultrazvuk visokog intenziteta, zob, β-glukanaza*

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Information about the Supervisor – Prof Dubravka Novotni, Ph.D.

DUBRAVKA NOVOTNI is a full professor at the University of Zagreb Faculty of Food Technology and Biotechnology since 2023. In 2011 she obtained a Ph.D. in Nutrition from the same faculty. She is 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 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. 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), as well as the principal investigator of the Croatian partner in „Flat bread of Mediterranean area; innovation & emerging process & technology“, Partnership for Research and Innovation in the Mediterranean Area (PRIMA, 2031). Dubravka Novotni has published over 50 peer reviewed journal articles with more than 500 citations (h-index 13) and authored several book chapters with recognized scientific publishers like Elsevier, Springer and CRC Press. She presents her work 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, 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).

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List of abbreviations

OB – oat bran

BB – barely bran

OF – oat flour

BF – barley flour

DY – dough yield

US – high-intensity ultrasound

PEF – pulsed electric field

NSPs – non-starch polysaccharides

AXs – arabinoxylans

WE-AX – water-extractable arabinoxylans

WU-AX – water-unextractable arabinoxylans

TCD – total color difference

DPPH – 2,2-diphenyl-1-picrylhydrazyl

FRAP – ferric reducing antioxidant power

TPC – total phenolic content

WS – water swelling

WRC – water retention capacity

PA – phytic acid

FTIR – fourier-transform infrared spectroscopy

Ws – specific energy input

Mw – molecular weight

τ_{max} – maximum stress tolerated by the sample

G' – storage modulus

G' '– loss modulus

PPO – polyphenol oxidase

AGGEN – aggregation energy

PMT – peak maximum time

General introduction

Flat bread is the oldest type of bread, which is consumed worldwide (Boukid, 2022). Although flat breads originated in a rural society, their adaptability and ease of use have made them very popular in their areas of origin and increasingly beyond (Pasqualone, 2018). There are many variations of this type of bread, but thinnest is their common characteristic. The basic raw material for making this bread is refined wheat flour, which makes it low in nutrients, especially dietary fiber. With the increasing popularity of flat breads among consumers, but also with the growing consumer awareness of the health benefits of dietary fiber, the demand for innovative products that combine convenience with increased nutritional value is growing (Garzon et al., 2022). A good source of dietary fiber are whole grain flours and cereal bran, of which oats and barley are the main source of β-glucans among the cereals (Nirmala Prasadi and Joye, 2020).

Oats and barley are underutilized in food production and are mainly used as animal feed, for malt and beer production. The rich nutritional composition of flour and even more bran, which is characterized by a high dietary fiber and protein content, fats, vitamins, minerals, and phenolic compounds, makes these cereals increasingly interesting alternative raw materials that will partially replace wheat flour in bread making. The antioxidant, anti-inflammatory and anti-proliferative effect is attributed to secondary plant substances, i.e. phenolic compounds, sterols and phytic acid (PA). However, in addition to antioxidants, PA also acts as an antinutrient that binds minerals and hinders their bioavailability (Valoppi et al., 2021).

This health promoting effects of oat and barley are primarily attributed to the high dietary fiber content, mainly β-glucans (Grundy et al., 2018; Hussain et al., 2021). According to the European Food Safety Agency (EFSA, 2011), the consumption of barley or oat fiber in foods in a minimum amount of 6 g per 100 g kcal of product can contribute to lowering plasma cholesterol levels and a reduction in the postprandial glycemic response. However, the most important change that occurs in β-glucan during the processing of oats/barley and oat/barley-based products is depolymerization. Oat (OF) and barley flour (BF) and even more oat (OB) and barley bran (BB) possess endogenous β-glucanase which depolymerizes β-glucans and thus reduce their molecular weight and viscosity (Pérez-Quirce et al., 2016; Pérez-Quirce et al. 2017). The β-glucanases are activated when the flour is hydrated, making fermentation the most critical step in bread making (Johansson et al., 2018). Research on methods for inactivating endogenous β-glucanase and preserving β-glucans in bread making is limited. Commonly used techniques include thermal (autoclaving, scalding and oven heating) or chemical methods (ethanol refluxing or the use of organic acid salts) (Lazaridou et al., 2014; Moriartey et al.,

2010; Rieder et al., 2015a; Rieder et al., 2015b; Tosh et al., 2012), which have a negative impact on the technological properties of flour and on consumer acceptability (Rieder, Balance et al., 2015b). In general, the effectiveness of innovative technologies on the activity of endogenous β-glucanase of cereals has been insufficiently studied.

Furthermore, in addition to β-glucans, the second most important non-starch polysaccharides (NSPs) are arabinoxylans (AXs), which are mostly insoluble fiber component in cereals. Numerous health benefits are attributed to the AXs, such as strengthening the immune system, reducing the risk of type 2 diabetes, heart disease (Rosicka-Kaczmarek et al., 2016). However, β-glucans and AXs, their content, their water solubility, and their structure (molecular weight (Mw)) have a significant influence on the technological properties of the flour or bran, the rheology of the dough and the physical properties of the bread (Cao et al., 2023; Holtekjølen et al., 2008). Studies have shown that the addition of OF or BF or bran negatively affects the physical properties of bread in terms of reduced volume, increased hardness, and chewiness of the bread crumb (Blandino et al., 2015; Chauhan et al., 2018; Flander et al., 2007; Krochmal-Marczak et al., 2020; Wenjun et al., 2018). These defects are the result of NSPs that limit the proper development of the gluten network and negatively affect the structure of the dough (Courtin and Delcour, 2002; Ma et al., 2021).

Most research is aimed at improving the technological and sensory quality of whole grain bread by modifying the recipe i.e., adding additives as structuring agents (Dapčević-Hadnađev et al., 2022). On the other hand, there is less focus on technological solutions that could influence the structural changes of whole grain biopolymers and thus increase their potential for bread making. The potential of traditional (sourdough fermentation) and innovative techniques such as high-intensity ultrasound (US) and pulsed electric field (PEF) to improve the performance of BF and OF or BB and OB in bread making has hardly been explored.

Traditional sourdough fermentation has gained renewed interest as a means of making better use of non-wheat grains in bread production. It improves the nutritional value of bread (reduction of PA and increase in mineral content, higher phenolic content, and antioxidant activity), techno-functional properties of flour/bran and the overall quality of bread (Dapčević-Hadnađev et al., 2022; Ramos et al., 2021). Furthermore, due to its low pH value, sourdough has a suppressive effect on the activity of endogenous flour enzymes, which can slow down undesirable enzymatic reactions during bread baking. However, there is a large knowledge gap about the suppressive effect of sourdough on enzymatic browning during the retardation

process. Since oat flour has a weak acidification power, little is known about the influence of bran addition on the kinetics of oat and barley flour acidification.

In addition to the traditional technique, the US proved to be a successful technology for reducing antinutrients from millet and rice bran (Mohammadi et al., 2021; Yadav et al., 2021) and for reducing enzyme activity (polyphenol oxidase (PPO), peroxidase (POD), lipase) in whole wheat flour, wheat bran, various vegetables, and fruits (Habuš et al., 2021b; Habuš, et al., 2021c; Safwa et al., 2023). Still, the influence of US technology on the activity of endogenous β-glucanase and phytase activity and on the PA content of oat and barley bran has not yet been investigated. Ultrasonication also increases the solubility of the dietary fiber and changes the starch structure, which leads to better technological properties of corn and rice flour and ultimately to improved physical properties of bread (texture and specific volume) (Jalali et al., 2020; Ma et al., 2022; Vela et al., 2023). However, the effects of US on the structure of NSPs and the techno-functional properties of oat and barley bran are still unexplored.

In addition, PEF treatment leads to changes in the structural properties of polysaccharides such as starch, pectin, and chitosan and thus to their physicochemical properties (Han et al., 2012; Ma et al., 2012; Maniglia et al., 2021; Rivero-Ramos et al., 2023). PEF-induced changes in the structure of biomacromolecules also led to an increased activity of wheat α- and β-amylase (Carregari Polachini et al., 2023), but also to a decrease in the enzymatic activity of soybean and tomato lipoxygenase (Li et al., 2008; Min et al., 2003). As previous studies have mainly focused on the effects of PEF on individual cereal molecules, the literature lacks comprehensive information on how PEF treatment affects the physical and functional properties of starchy foods, especially oat and barley flour. Due to the different mechanisms of action, both US and PEF are successful techniques for the modifying enzyme activity and improving the extraction efficiency of β-glucans and other biomolecules (Benito-Román et al., 2013; Duque et al., 2020b).

The aim of this study was to explore the innovative techniques i.e., US and PEF on enzyme activity, antinutrient reduction and β-glucans preservation in OB, BB, OF and BF, while preserving the acidification power of the treated raw materials, and their application in flat bread with the aim of improving the nutritional value and extending shelf life while maintaining technological quality and consumer acceptability. The traditional sourdough fermentation of oats and barley flour was investigated depending on the adding bran and used for extending the shelf-life of dough using the retardation process and for improving the nutritional properties of wheat-oat and wheat-barley composite flat breads. Hence, the aim was

to improve nutritive value and shelf-life of flat bread while preserving their physical and sensory properties through the enhancement of techno-functional properties of OB, BB, OF and BF.

This dissertation, in the form of published papers and a final comprehensive review, aims to provide new insights into the effects of US and PEF treatment on enzyme activity, the structure of NSPs (β-glucans and AXs) and the functional properties of OB, BB, OF and BF. In addition, it provides an insight into the kinetics of sourdough fermentation of oats and barley and the use of the sourdough obtained to suppress browning of flat bread doughs during retarded dough method.

The findings of the US and PEF treatments on the enzyme activity and β-glucans content, solubility and structure of oats and barley will contribute to further research on the application of these technologies with the aim of modifying the biopolymers of cereals and improving the functional properties of flour and bran. In addition, the information obtained on the positive influence of the addition of BB/OB on the acidification kinetics of BF and OF will sufficiently expand knowledge of the value and potential of this by-product of cereal processing. The use of innovative and traditional techniques or their combination opens up new possibilities to improve the nutritional and physical properties of flat breads.

The general discussion of this dissertation provides valuable information on the pre-processing of oat and barley flour and bran, from enzyme activity, NSPs structure and techno-functional properties to their final incorporation into flat breads through the sourdough fermentation process.

Chapter 1

Theoretical background

1. Flat bread – the oldest form of bread
2. Oats and barley – source of non-starch polysaccharides
3. Pre-processing of oat and barley – traditional and innovative techniques
4. Hypothesis, research objectives, and expected scientific contributions

1. Flat bread – the oldest form of bread

Bread continues to be a global staple, adapting to the changing needs of today's society. Flat breads attract consumer attention for its adaptability and ease of use. These ancient breads, which have been found in Mesopotamia, ancient Egypt, and the Indus civilization, probably represent mankind's earliest attempts to food processing (Pasqualone, 2018). They are traditionally eaten in regions such as the Middle East, Africa, India, Central America, and Europe, where this type of bread symbolizes not only nutrition, but also the unity of cultures and the common history of mankind (Boukid, 2022). The popularity of flat breads is increasing worldwide with the transition from traditional to commercial production (Boukid, 2022). There are a variety of this bread whose common characteristic is a thickness of a few millimeters to a few centimeters, which distinguishes them from the more voluminous breads (Pasqualone, 2018). Furthermore, their large surface area presents a challenge due to the dough browning during its storage at room temperature or during storage in the refrigerator to extend its shelf life (Cauvain, 2007; Banerji et al., 2019).

Depending on the composition and methods of preparation, flat breads are divided into two different categories, namely single-layer and double-layer (Kumar, 2016). Single-layer flat bread is divided into unleavened and leavened. Leavened flat bread is made from semi-liquid dough or batter. For semi-liquid doughs, gluten-free raw materials such as rice, sorghum, corn, and oats are usually used, which are fermented twice before baking (Boukid, 2022). Flat breads made from dough are leavened, such as the Croatian flat bread *pogacha*, which is allowed to rise twice before baking at high temperatures. Single-layer, unleavened flat breads are 12-25 cm in diameter and 1.3-3 mm in thickness and are traditionally baked on a clay grill (naan), an iron pan (roti), baked flat in a pan with oil (paratha) or deep-fried (poories) (Boukid, 2022; Diddana et al., 2021). Double-layer flat breads are usually 4-20 mm thick and have a diameter of 20 cm, such as Arabic flat bread (*pita*) or Croatian flat bread (*lepinja*). They are baked at very high temperatures (350-600 °C), causing them to expand twice and take on the shape of a balloon with separate top and bottom sides (Boukid, 2022). The expansion depends on the viscoelastic properties of the flour, with flours with a high gluten content being preferred (Boukid, 2022). After baking, the resulting bubble is deflated by cooling, resulting in a pliable flat bread that is an ideal base for various fillings (meat, vegetables, etc.).

The main ingredients of flat breads are water, flour, salt, and yeast, while the main production steps are kneading of ingredients, leavening (optional), shaping and baking (Pasqualone, 2018). Flat breads, usually consist of wheat, maize, and rice flour as the main raw materials (Serka et al., 2019). However, in regions such as Africa and India, other cereals i.e., barley, oats, millet, rye, and sorghum are also used, while they are used less frequently in Europe (Boers et al., 2017; Mehfooz et al., 2018). Flat breads usually have a low dietary fiber content, mainly because refined wheat flour dominates as the main ingredient (Garzon et al., 2022). However, to increase the fiber, protein, and mineral content of wheat-based flat breads, alternative flours such as oats, barley, millet, rye, sorghum, or legumes have been used (Bhavya and Prakash, 2021; Boers et al., 2017; Boukid, 2022; Garzon et al., 2022; Kahlon et al., 2019; Kumar et al., 2021; Mehfooz et al., 2018). By using different enriching ingredients and bread making techniques, it's possible to produce flat breads with improved nutritional profiles that meet specific nutritional needs of consumers while maintaining their overall quality.

As consumer interest in flat breads increases, there is an increased need for innovation to create products that balance convenience with improved nutritional profiles (Garzon et al., 2022). At the same time, growing awareness of the health benefits of dietary fiber is driving demand for high-fiber flat breads. It is known that the fiber component of cereals reduces the risk of diseases such as high blood pressure, diabetes, and colon cancer, which underlines the importance of this nutritional enrichment (Wei et al., 2022). The addition of high-fiber ingredients such as millet flour, wheat bran, barley bran, oat bran, and chickpea flour can improve the fiber content but also other nutritional aspects of flat breads. Studies have shown that an increase in fiber, especially β-glucan, in flat breads leads to a higher content of slowly digestible starch and resistant starch. This in turn helps to lower the glycemic response and index of flat breads, contributing to better glycemic control (Boers et al., 2017; Gujral et al., 2018; Robert et al., 2016; Sharma & Kotari, 2017). In the development of Balzam flat bread with a high β-glucan content and a relatively low glycemic index, wheat flour was supplemented with barley flour (Koksel et al., 2024). Partial replacement of wheat flour with barley flour also significantly lowered the glycemic index of roti flat bread (Mansoor et al., 2019). Barley flour was used together with whole wheat flour to improve the quality and nutritional value of barbari bread (Naji-Tabasi et al., 2022). Partial replacement of wheat flour with oat flour resulted in chapatti flat bread with an increased content of bioactive compounds (Gujral et al., 2013).

Although the addition of oat or barley flour or bran to bread significantly increases the nutritional value and contributes to the improvement of the modern diet. There are also certain limitations related to undesirable levels of antinutrients and possible technological and sensory deficiencies. Wheat bread enriched with oat or barley flour, especially with bran, shows a deterioration in physical properties, such as a reduction in specific volume, an increase in hardness and chewiness of the crumbs and their progressive increase during 48-72 h of storage at room temperature (El-Taib et al., 2018; Koksel et al., 2024). To extend the shelf life of the dough, the dough retardation method at refrigerator temperature is usually used, which is a challenge in the world of flat bread due to its large surface area and enzymatic browning catalyzed by PPO, an oxidative enzyme.

2. Oats and barley – source of non-starch polysaccharides

Growing concern about rising rates of chronic disease and obesity, as well as scientific evidence of the health benefits of dietary fiber consumption, have attracted considerable attention. This has raised consumer awareness of nutrition and sparked greater interest in high-fiber foods and ingredients such as whole grain (Ramezani et al., 2024). Oats and barley are cereals mainly used as animal feed, for malt and brewing and are underutilized in food production. According to a report by the Food and Agriculture Organization of the United Nations (FAOSTAT, 2023), 1.55 billion tons of barley and 26.4 million tons of oats were produced worldwide in 2022. The health benefits of these cereals are linked to their richness in compounds such as dietary fiber (mainly β-glucans), proteins, phenols, vitamins, bioactive peptides, and lipid components (sterols and fatty acids) (Gangopadhyay et al., 2015). Due to these high-value nutritional properties, oats and barley have great potential for use in a wide range of cereal-based foods as partial or complete substitutes for the cereals currently in use.

2.1. Oat (Avena sativa L.)

Oat (*Avena sativa*) is a cereal originating in the Mediterranean area and the Middle East that has long been used as a fodder crop throughout history (Capillas and Herrero, 2024). The consumption and global production of oats is much lower compared to wheat, corn, rice, and barley, due to the insufficient number of commercially available oat-based products (Grundy et al., 2018). In contrast to other cereals, oats have a special nutritional profile. It is

rich in soluble dietary fiber, carbohydrates, lipids, proteins, vitamins, minerals, and phenolic compounds (Joyce et al., 2019). With the growing awareness of the physiological effects of consuming oat-containing foods, the cultivation and use of oats for human consumption has greatly increased.

Oat grains consist of a protective hull and a groat (caryopsis), which is made up of bran, germ, and starchy endosperm (Miller and Fulcher, 2011). The bran, a nutrient rich by-product of milling, surrounds the endosperm, on which the aleurone and subaleurone layers are connected (Grundy et al., 2018). Endosperm and bran differ in their nutrient composition, and processing methods can further alter the ratio of nutrients (Grundy et al., 2018). The bran is richer in minerals, vitamins (mainly vitamin E), PA and NSPs such as AXs and β-glucans compared to the endosperm (Grundy et al., 2018). Within the endosperm, protein and lipid concentrations increase toward the periphery, while starch concentrations increase toward the center (Miller and Fulcher, 2011).

Oats' health benefits stem mainly from their rich dietary fiber content, ranging from 10% to 38% depending on variety and cultivation (Prasadi et al., 2020). Of particular note is that soluble fiber accounts for 50%, which distinguishes oats from other cereals, which generally contain more insoluble fiber (Abrahamsson, 2020). The most important soluble fiber component, β-glucans, make up about 70% of this fraction (Abrahamsson, 2020). Research indicates that consuming oat-based foods can reduce postprandial glycemic responses, regulate blood pressure, and lower the risk of cardiovascular diseases. Additionally, the bioactive nature of β-glucans contributes to the anti-inflammatory and anti-tumor properties of oats (FDA, 1997; EFSA, 2010; Zou et al., 2015).

However, the positive effects of oat consumption are also associated with other bioactive compounds. Oats have a special nutritional profile compared to other cereals, which is characterized by its high protein content (15-20% of the grain weight) (Ma et al., 2021) About 30% of the proteins are present in the embryo (Paudel et al., 2021). The main storage proteins in most cereals (wheat, barley, rye) are prolamins which are the main components of gluten (Alemayehu et al., 2023). Oats is an exception, where globulins play the main role with 55% of the total protein content (Broeck et al., 2016). The next more abundant are avenins, which account for 10-13% of the total protein content of oats, and prolamins which make up a minor percentage (Broeck et al., 2016; Klose and Arendt, 2012). Another reason that distinguishes oats from other cereals is its special protein composition, which consists of essential amino acids such as lysine and threonine (Klose and Arendt, 2012). Therefore, oat proteins have a

higher biological value than other prolamin-rich cereals and can contribute to lowering total serum and LDL cholesterol levels (Paudel et al., 2021; Tong et al., 2016). Nevertheless, The European Commission (EC 41/2009) reports that oats can be included in the diet of people with gluten intolerance (celiac disease). However, oat cultivar used for a gluten-free diet should be carefully selected, produced, and processed to avoid contamination with other gluten-containing cereals (Silano et al., 2014). Furtheron, oats have the highest fat content of all cereals with a lipid content of 5 to 9% in oat groats (Paudel et al., 2021), with monounsaturated fatty acids (MUFA, C18:1) and polyunsaturated fatty acids (PUFA, C18:2) being the most abundant and unsaturated fatty acids (C16:0) the least (Broeck et al., 2016). This fatty acid profile and high lipid content makes oats a valuable food with health benefits such as reducing the risk of cardiovascular disease (Kouřimská et al., 2018).

The health benefits of oats are also associated with other minor components such as tocols (tocopherols and tocotrienols), phenolic compounds and sterols (Martínez-Villaluenga and Peñas, 2017). Several phenolic compounds have been identified in oats, act as strong free radical scavengers, with the highest content in bran (Martínez-Villaluenga and Peñas, 2017. The most abundant is ferulic acid, followed by caffeic and sinapic acid (Soycan et al., 2019). Other phenolic compounds include avenanthramides (AVAs), *p*-hydroxybenoic acid, vanillic acid, tricin, protocatechuic acid, syringic acid, *p*-coumaric acid, tricin, apigenin, luteolin, kaempferol and quercetin (Paudel et al., 2021). Phenolic compounds can be present in free form, as soluble conjugates or bound to cell wall constituents, which determines their potential health benefits (Grundy et al., 2018). Those bound to cell wall components, such as arabinoxylan, have limited bioavailability compared to those in free form.

Avenanthramides are the most abundant phenolic alkaloids and are not found in other cereal grains. Most AVAs are found in bran (20-90 μg/g), with AVA-A (2p), AVA-B (2f) and AVA-C (2c) being the most abundant (Gangopadhyay et al., 2015; Paudel et al., 2021; Yang et al., 2014). The ability to scavenge reactive oxygen species (ROS) is crucial for their strong antioxidant activity, but anti-inflammatory and anti-proliferative activities are also associated with AVAs (Fu et al., 2015). In vitro studies have proved the attenuating effect of AVAs on the proliferation of colon cancer cells (Wu et al., 2018), as well as on the regulation of intestinal microflora and the reduction of harmful microbes (Zhang et al., 2020).

However, phenolic compounds and the PPO and POD, which are mainly found in bran, represent a challenge for the food industry to participate in the enzymatic browning of dough, fruit, and vegetables (Brütsch et al., 2018; Moon et al., 2020). These enzymes catalyze two reactions, namely the hydroxylation reaction of monophenols to o-diphenols and the

oxidation reaction of o-diphenols to o-quinones in the presence of molecular oxygen, which can then react with the phenolic groups of phenolic acids (Brütsch et al., 2018; Niu et al., 2014; Quinde-Axtell et al., 2006). The resulting quinones can further non-enzymatically react with other functional groups, such as amines, thiols, and phenolics, and form complex-colored products i.e. relatively insoluble brown pigment known as melanin (Brütsch et al., 2018; Moon et al., 2020; Niu et al., 2014). This browning has a negative impact on the sensory properties of the product, including the taste, texture, and appearance of cereal-based products such as dough for Indian multigrain flat bread (chapatti) and noodles (Banerji et al., 2019; Ma et al., 2023; Yadav et al., 2010). Considering that the rate of enzymatic browning is determined by the enzymatic activity of PPO, its inactivation by novel (thermal or non-thermal) or traditional (fermentation) processing methods can slow down these undesirable reactions (Banerji et al., 2019; Niu et al., 2014; Yadav et al., 2010).

Oats also contain other antioxidant phytochemicals such as sterols (0.45 mg/g) and PA (5.6–8.7 mg/g) (Martínez-Villaluenga and Peñas, 2017. The PA has antioxidant activity due to its ability to chelate metal ions, rendering them catalytically inactive and inhibiting the production of free radicals by metals. However, this chelating effect reduces the bioavailability of essential minerals and phytic acid acts as a main antinutrient in oats (Baumgartner et al., 2018; Valoppi et al., 2021). In addition, the endogenous phytase, which hydrolyzes PA, has a low activity that is 20 times lower compared to the wheat phytase (Brinch-Pedersen et al., 2014). In recent years, studies have shown that the utilization of processes such as fermentation and hydrothermal treatment, soaking, germination, extrusion, or ultrasonic treatment can effectively decrease the concentration of PA in oat flour or bran, rice bran, BF, and finger millet (Baumgartner et al., 2018; Mohammadi et al., 2021; Rogers et al., 2017; Yadav et al., 2021).

The high fiber content and the absence of gluten limit the bread-making possibilities of OB or OF (Popa and Berehoiu, 2021). Previous studies have shown that increasing the proportion of oats in bakery products such as bread was associated with a reduction in the specific volume of wheat-oat composite bread and a deterioration in sensory properties (Astiz et al., 2023; Ivanišová et al., 2023; Tamba-Berehoiu et al., 2019).

2.2. Barley (Hordeum vulgare L.)

Barley (*Hordeum vulgare* L.) is the fourth most cultivated cereal after wheat, rice, and maize (Boukid, 2024). This cereal has a high functional value as it is a good source of nutrients, i.e. dietary fiber, proteins, minerals, and phytochemicals.

As well as oat grain, barley grain is composed from hull and caryopsis which is composed of bran, germ, and a large endosperm (Geng et al., 2022; Perera et al., 2022). The bran makes up for 7-12%, while the endosperm makes up 80% of the grain (Perera et al., 2022; Sharma & Kotari, 2017). The endosperm consists of the aleurone and subaleurone layer, the starchy endosperm, the embryo-surrounding region, and the endosperm cell walls (Perera et al., 2022). The aleurone layer is rich in soluble protein and is a source of endogenous enzymes, lipids, and vitamins (Perera et al., 2022). The endosperm cell walls are mainly composed of β-glucans (70%) and a smaller amount of AXs (20%) (Andriotis et al., 2016). In contrast, the aleurone cell walls consist mainly of AXs (67-71%) and smaller amounts of β-glucans (26%) (Izydorczyk and Dexter, 2008).

The main nutritional component of barley is dietary fiber, which accounts for 11-34%, with a smaller proportion (3-20%) consisting of soluble dietary fiber (Djurle et al., 2016; T. Guo et al., 2020a; Nirmala Prasadi & Joye, 2020). The most important soluble fiber are β-glucans (4-9%), which, like oats, have an EFSA-approved health claim for their contributing to maintaining normal blood cholesterol levels (EFSA, 2011; Hussain et al., 2021). However, interest in barley is also growing due to the higher proportion of biologically important insoluble fibers, namely AXs, which are also beneficial to human health (Geng et al., 2022).

Barley is not only rich in dietary fiber, but also in proteins (10-20%), which are mainly concentrated in the endosperm (Huang et al., 2020). Barley proteins consist of 75% gluten (50% prolamins and 25% glutenins) (Farag et al., 2020), and are an excellent source of essential amino acids such as threonine, valine, lysine, and phenylalanine with potential health benefits (Sullivan et al., 2013). In addition, compared to oats, barley has a lower lipid content (2-3%), which is particularly abundant in the endosperm (Raj et al., 2023). The most important fatty acids in barley are linoleic acid (50–60%), palmitic acid (20–30%), oleic acid (10–15%) and linolenic acid (4–9%) (Farag et al., 2020).

Barley contains a variety of different phytochemicals with antioxidant and antiproliferative effects, including lignans, tocols, phytosterols, flavonoids, phenolic acids, and folates (Fogarasi et al., 2015). Phenolic acids (0.6-1.3 mg/g) make up the majority of

phytochemicals in barley and are mainly concentrated in the outer layers of the grain (Holtekjølen et al., 2006; Raj et al., 2023). The most abundant phenolic acid is ferulic acid (68%), followed by coumaric, vanillic, syringic and sinapic acids (Raj et al., 2023). Most phenolic acids are present in bound form, esterified in cell wall components such as hemicellulose and arabinoxylans (Raj et al., 2023).

The browning that occurs in barley products limits its use by the food industry and consumers. Phenolic compounds and PPO, which are most concentrated in the bran, are responsible for the color change of the dough. Therefore, bran removal, heat treatment, exclusion of oxygen and the use of browning inhibitors (ascorbic acid, 4-hexylresorcinol, sodium bisulfite, EDTA, benzoyl peroxide) are effective in slowing down the discoloration of barley flour dough due to the inactivation of PPO (Baik et al., 2008; Quinde-Axtell et al., 2006). Similarly, superheated steam treatment, ultrasound treatment, pulsed light and vacuum microwave treatment were also used, and different pH values were tested, on the activity of PPO whole wheat flour and bran, as well as on the browning of semi-dried whole wheat noodles, wheat 3D-printed snacks, and wheat noodle sheets (Guo et al., 2020b; Habuš et al., 2021a; Zhao et al., 2020). However, heat treatments lead to denaturation of the proteins and gelatinization of the starch, which alters the physical and functional properties of barley flour, while the antibrowning agents are declared as additives, which reduces consumer preference for products containing them (Baik et al., 2008).

Three main types of flavonoids, namely anthocyanins, flavanols and proantocyanidin polymers, which have a protective effect against cancer and coronary heart disease, have been found in barley (Raj et al., 2023). Their concentration varies between 0.6 and 3.1 mg/g and correlates with the color of barley grain, flour, or bran (Kim et al., 2007; Z. Liu et al., 2013). In contrast to other cereals, barley contains measurable amounts of the flavonoid catechin, which has anti-carcinogenic, anti-allergic and anti-inflammatory properties (Raj et al., 2023). Furtheron, barley contains tocols (tocopherols and tocotrienols) and is one of the largest cereal sources of tocotrienols with great antioxidant effects that stimulate the immune system (Raj et al., 2023).

As in oats, the main antinutrient of barley is PA (3.85-9.85 mg/g) (Dai et al., 2007), and an additional challenge is the low phytase activity, which is 2-fold lower compared to wheat (Brinch-Pedersen et al., 2014). In bread production, the process steps are important as the prolonged fermentation (especially when using sourdough) significantly reduces (50-95%) the

PA content in whole grain foods and leads to improved bioavailability of minerals (Buddrick et al., 2014; Fang et al., 2023).

Recent studies have shown that the addition of barley flour or bran to noodles, chapatti, taftaan flat bread, wheat bread significantly improves the nutritional profile by increasing the fiber content and antioxidant properties (Ding et al., 2024; Gujral et al., 2018; Mansoor et al., 2021; Mariotti et al., 2014; Sharma & Kotari, 2017 Zheng et al., 2023). However, the use of BF or BB in bread making is limited as they affect the sensory properties of the bread, i.e., lower loaf volume, harder and chewier crumb, bitter and darker bread (Mansoor et al., 2022; Mariotti et al., 2014; Pejcz et al., 2017; Robles-Ramírez et al., 2020).

Table 1 shows the research available to date on the topic of enriching flat bread with oats and barley, and it evident that this topic has not yet been sufficiently researched, particularly in the case of oats.

Table 1. Report on the effects of the addition of oats or barley on the nutritional and sensory properties of wheat flat bread.

Bread type	Added alternative raw material	Flour addition/substitution (%)	Effects on the nutritional properties of flat bread	Effects on the physical/sensory properties of flat bread	Reference
Wheat-barley composite pan bread	barley flour	10, 15 and 20% substitution of wheat flour	increased dietary fiber, β-glucans, minerals, and antioxidant level	increased crumb hardness, chewiness, and springiness; reduced bread specific volume	El-Taib et al., 2018
Wheat-oat composite flat bread (*chapatti)*	oat flour	25 and 50% addition to wheat flour	increased total phenolic and flavonoid content; increased antioxidant potential and higher reducing power		Gujral et al., 2013
Wheat-barley composite flat bread (*chapatti)*	barley bran	34% addition to refined wheat flour	higher β-glucans, total phenolic and flavonoid content	more pliable and softer after 48 h of storage compared to wheat control (according to sensory analysis)	Gujral et al., 2018
Wheat-barley composite flat bread (*bazlama*)	hull-less barley flour	15, 30, 45 and 60% addition to wheat flour	higher β-glucnas, Mg, K, Mn, Fe, Zn content; increased total phenolic content and antioxidant activity;	higher bread crust yellowness value; increased crumb hardness	Koksel et al., 2024

			lower glycemic index		
Chickpea flour-based flat bread	barley flour	10-40% substitution of chickpea flour	higher total dietary fiber content; decline in protein and fat content; lower glycemic index	improved textural properties (lower crumb hardness and increased elasticity); sensory acceptable addition of up to 30%	Mansoor et al., 2021
Wheat-barley composite flat bread (*roti*)	barley flour	5, 10, 20, 25 and 50% substitution of wheat flour	lower glycemic index (50% substitution)	increased crumb hardness (50% substitution); lower crumb resilience; no significant difference in sensory properties between flat breads with 5-25% barley flour and wheat control bread	Mansoor et al., 2019

2.3. Non-starch polysaccharides in oat and barley

Oat and barley are characterized by their NSPs, of which the primary soluble form, β-glucans predominate, as well as insoluble AXs in much lower proportion (Valoppi et al., 2021).

The β-glucans content in barley and oats ranges from 2.5% to 11.3% and 2.2% to 7.8%, respectively (Lazaridou et al., 2007). Cereal mixed-linkage (1 → 3), (1 → 4)-β-D-glucans are linear unbranched polysaccharides that are built up of β-D-glucopyranosyl monomers connected through glycosidic bonds (Figure 1a) (Lazaridou et al., 2007). Their fine structure consists mainly of β-D-glucopyranosyl (β-D-Glcp) units, with about 30% linked via 1,3-glycosidic bonds and 70% via 1,4-glycosidic bonds (Biliaderis, 2007; Zhang et al., 2019a). Considering that the structure of oat and barley β-glucans differs, with barley β-glucans containing a higher number of long cellotriosyl sequences, their solubility in water is not the same (Mikkelsen et al., 2013). The proportion of soluble β-glucans in oats varies between 27-51% and is higher than that of barley, which contains 18-39% soluble fraction (Gajdošová et al., 2007). β-glucans with higher Mw show higher viscosity in aqueous solutions and have strong gelling properties, which increases their physiological benefits (Goudar et al., 2020; Zhang et al., 2019a).

The second most abundant NSP in oat and barley are AXs which are found in the cell walls of aleurone cells and starchy endosperm and are responsible for maintaining cell integrity (Izydorczyk and Biliaderis, 2007; Mio et al., 2022; Zambrano et al., 2023). Compared to other grains, the amount of AXs in barley is similar to that in wheat (5.8%), lower than in rye (7.6–12%), but higher than in oats (2.7– 3.5%), sorghum (1.8%) or rice (2.6%) (Izydorczyk and Biliaderis 2007). AXs features a linear xylose backbone with attached arabinose side chains branching from it. This heteroglycan compound comprises β-(1→ 4) linked D-xylopyranosyl residues (*Xylp*) and α-L- arabinofuranosyl units (*Araf*), forming its distinct structure (Figure 1b) (Zambrano et al., 2023). Their abilities to dissolve in water, depends on the degree of the substitution on the AX molecule, and they can be classified as soluble and insoluble AXs (Rosicka-Kaczmarek et al., 2016). Where the higher degree of *Araf* substitution to the *Xylp* backbone result in higher solubility (Zambrano et al., 2023). The soluble AXs are referred to as water-extractable AX (WE-AX), while the insoluble ones are referred to as water-unextractable AX (WU-AX) (Rosicka-Kaczmarek et al., 2016). AX is mainly insoluble dietary fiber (Fadel et al., 2018), which possess a biological activity manifested in their action as prebiotic dietary fiber and suppresses constipation (Kellow & Walker, 2018; Wang et al., 2016).

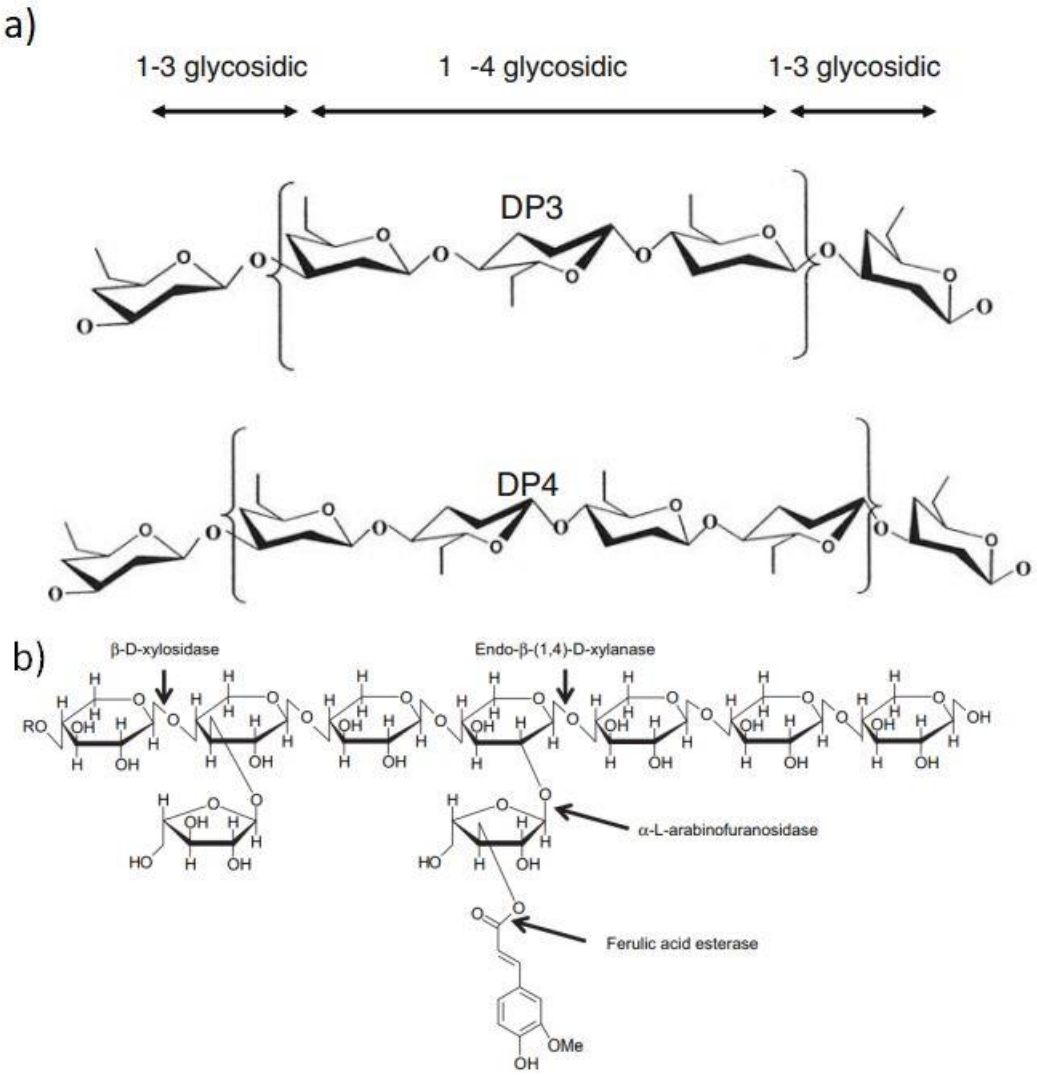

Figure 1. Molecular structure of cereal a) β-glucans (Vasanthan and Temelli, 2008) and b) arabinoxylans (Dornez et al., 2009).

Additionally, their impact extends to various physiological functions, such as strengthening the immune system, reducing the risk of type 2 diabetes, heart disease and certain types of cancer (Rosicka-Kaczmarek et al., 2016). Despite these health benefits of β-glucans and AXs, they limit the use of these cereals and significantly influence the properties of the dough and the final baked product (Cao et al., 2023). Their influence on bread making depends not only on their content and water solubility, but also on their size, *i.e.,* Mw (Holtekjølen et al., 2008).

There are several research on the effect of BF or OF, and BB or OB addition in bread making of wheat-oat/barley composite breads (Blandino et al., 2015; Chauhan et al., 2018; Flander et al., 2007; Krochmal-Marczak et al., 2020; Pejcz et al., 2017; Robles-Ramírez et al.,

2020; Tiwari et al., 2013; Wenjun et al., 2018). The general conclusion is that the inclusion of these raw materials in bread making leads to a reduced bread volume, harder and denser crumb structure. These deteriorated physical properties of bread are a consequence of the weakening of the gluten network, as NSP is a physical barrier to the formation of the gluten network during dough development, resulting in a weaker dough structure (Courtin and Delcour, 2002).

However, the most important change that occurs in β-glucan during the processing of oats/barley and oat/barley-based products is depolymerization. Oat and barley flour and even more bran have activated endogenous β-glucanase which depolymerizes β-glucans and thus reduce their Mw and viscosity (Pérez-Quirce et al., 2016; Pérez-Quirce et al. 2017). In the production of oat/barley-based bakery products, there is a decrease in the Mw of β-glucans as β-glucanases are activated when the flour is hydrated, making fermentation the most critical step in bread making (Johansson et al., 2018). Factors such as water content, β-glucanase level, flour/bran particle size and incubation time are critical in controlling the degradation of β-glucan during food production (Johansson et al., 2018; Vatandoust et al., 2012). Therefore, the impact of β-glucan on health depends not only on its Mw, concentration, structure, and behaviorin solution, but also on the processing of the raw materials rich in this fiber or incorporation of it into food products (Henrion et al., 2019). Knowledge of the β-glucanase activity of oats or barley is valuable from a technological point of view for improving the quality of dough and bread (Li et al., 2020). However, the physiological effects of β-glucans remain uncertain. Various thermal and chemical approaches have been used to inactivate β-glucanase to preserve β-glucans content and structure during bread making, but their influence on the structural properties of preserved β-glucans and the general technological properties of the material is questionable. This opens up scope for research into innovative techniques in cereal processing with the aim of inactivating β-glucanase and preserving β-glucan structure and content, as well as to provide flour or bran with improved bread making properties and bread with acceptable physical and sensory attributes.

Due to their nutritional value, oats and barley are increasingly used in the bakery industry. However, there is an increasing need for a solution that allows a reduction in the concentration of antinutrients (PA), technological improvements of the flour or bran and a further improvement of the nutritional value by preserving biologically valuable compounds such as β-glucans, as well as improving the physical and sensory properties of the bread.

3. Pre-processing of oat and barley – traditional and innovative techniques

Whole-grain alternative cereals exploitation in bread making is reduced due to the lower technological quality compared to refined wheat (Dapčević-Hadnađev et al., 2022). The modification of their physico-chemical properties before dough preparation is important to improve the quality of the bread (Vela et al., 2023). The modification methods can be genetic, mechanical, chemical, enzymatic, or physical (Zheng et al., 2013; Zhu et al., 2015). In most cases, overcoming these shortcomings of whole grain flour or bran is solved by changing and improving the recipe by adding certain additives (Dapčević-Hadnađev et al., 2022). Physical modifications are stand out as a solution as they involve the use of environmentally friendly technologies and reduce the use of chemicals and processing time (Amini et al., 2015).

3.1. Traditional technique – sourdough fermentation

In addition to the above-mentioned modification methods, sourdough fermentation as a traditional biochemical process is a popular technology for improving the functional, nutritional, and sensory properties of baked goods (Ebrahimi et al., 2022). Sourdough fermentation is based on the synergistic action of lactic acid bacteria (LAB) and the endogenous microflora of the cereals. There are two types of sourdough, namely type 1, which is fermented spontaneously with naturally occurring microorganisms, and type 2, which is fermented with added LAB and yeast (Weckx et al., 2019). Sourdough is not only an alternative to baker's yeast but also one of the best natural ingredients for delaying bread staling, preventing its microbial spoilage, and improving its overall quality (Arora et al., 2021; Ebrahimi et al., 2022). The use of sourdough as a natural leavening agent is classified as a clean label ingredient (Vargas and Simsek, 2021).

Due to the activity of microbial phytase, but also endogenous cereal phytase, which is activated at lower pH values during sourdough fermentation, an effective reduction of PA of fermented raw materials was reported (Leenhardt et al., 2005; Özkaya et al., 2018; Yildirim and Arici, 2019). In sourdough-containing doughs and breads, the lowering of the pH during the formation of organic acids leads to the inhibition of enzyme activities such as α-amylase (Dapčević-Hadnađev et al., 2022). However, there is a lack of research on the influence of adding sourdough on the browning rate of doughs during a longer storage period, for example during delayed fermentation. A retarded dough method is a delayed fermentation at 0-10°C for 14-24 h, which is generally used to improve the flavor of sourdough bread (Banerji et al., 2019). A high insoluble dietary fiber content, especially WU-AXs from whole grain flour and bran, as

well as their particle size, have a detrimental effect on the dough and the end product through various mechanisms: dilution of gluten, high water absorption of fiber that leads to insufficient hydration of gluten protein, NSPs that act as a physical barrier for the formation of gluten networks are some of them (Ma et al., 2021). Sourdough fermentation improves the solubility of dietary fiber, especially AXs. The positive effects of this biochemical process on bread made from whole grain barley or oats are also due to its ability to soften the bran particles during fermentation. This softening effect reduces the mechanical disruption of the gluten network and the gas cells in the dough (Rieder et al., 2012). Numerous studies have shown a higher soluble dietary content, improved dough rheology and consequently better physical properties (lower crumb hardness and greater specific volume) of bread made with sourdough (Cera et al., 2024; Gidari-Gounaridou et al., 2023; Olojede et al., 2020; Pejcz et al., 2017; Rieder et al., 2012; Tomić et al., 2023; Özkaya et al., 2018).

Recent studies have shown that breads containing sourdough have a richer volatile profile and a more pronounced flavor than breads without sourdough due to microbial and yeast metabolism, the Maillard reaction and the enzymatic or autoxidation of flour lipids during the fermentation process (Cera et al., 2024; Pétel et al., 2017; Warburton et al., 2022). During sourdough fermentation, organic acids (mainly acetic and lactic acid) are produced, which have an inhibitory effect on the growth of pathogens. This antimicrobial component of sourdough is the reason why sourdough breads have longer shelf life than sweet breads (Gidari-Gounaridou et al., 2023; (Ma et al., 2021; Sun et al., 2020). In addition, sourdough fermentation is an effective approach to reduce the bread ageing rate as it prevents the recrystallization of amylopectin in bread (Katina et al., 2006; A. Liu et al., 2024).

To overcome detrimental effects of some cereal endogenous enzymes, previous research has mainly focused on the inactivation of endogenous flour or bran enzymes such as PPO, POD, lipase, or lipoxygenase to extend the shelf life of the raw material but also of the final product (Habuš et al., 2021b; Habuš et al., 2021c; Li et al, 2022; Mohammadi et al., 2021; Zheng et al., 2023). However, there are only a limited number of studies on methods for inactivating endogenous β-glucanase and preserving β-glucans in bread production. Most used are thermal methods such as autoclaving, scalding and oven heating (Lazaridou et al., 2014; Rieder et al., 2015a; Rieder et al., 2015b) and chemical methods such as ethanol refluxing and the use of salts of organic acids (calcium propionate, potassium sorbate and sodium benzoate) (Moriartey et al., 2010; Tosh et al., 2012). These thermal and chemical processes are an obstacle as they can have a negative impact on the technological properties of the flour and consumer acceptance

(Rieder et al., 2015a). One of the emerging technologies used to inactivate β-glucanase in rice flour is microwave irradiation (Pérez-Quirce et al., 2016; Pérez-Quirce et al. 2017), but there are no studies on the effects of other related technologies such as US and PEF. Table 2 provides a brief overview of the research to date on the use of US and PEF with the aim of modifying enzyme activity.

3.2. High-intensity ultrasound

The US is emerging non-thermal, non-toxic, and environmentally friendly food processing technology. The advantages of this technology are low energy and time consumption, and preserved food nutrients (Estivi et al., 2022; Safwa et al., 2023). The mechanism of action is characterized by the induction of acoustic cavitation caused by the production, subsequent growth and collapse of larger bubbles that release a high amount of energy, resulting in cell disruption and increased mass transfer (Bhargava et al., 2021; Safwa et al., 2023). The impact of US cavitation effect is multifaceted, capable of either hindering or facilitating cell activity for purposes of sterilization or microbial growth promotion (Zhang et al., 2023). Ultrasound is beneficial in extraction process of different phenolic compounds from seeds, cereal bran, fruits, and other plant materials (Gueffai et al., 2022; Milićević et al., 2021; Safwa et al., 2023). It has also been successfully used for the extraction of NSPs, i.e. β-glucans from barley flour (Benito-Román et al., 2013). The reduction of PA from cereals such as rice bran and finger millet can be facilitated by US technology (Mohammadi et al., 2021; Yadav et al., 2021). Treatment with US can inhibit various enzymes, but it has attracted attention primarily because of the inhibition of browning enzymes (Safwa et al., 2023). The combined effect of US treatment and ascorbic acid successfully reduced the PPO activity of whole wheat flour (Niu et al., 2014). The PPO, POD and lipase activity of wheat bran was also significantly reduced (Habuš et al., 2021a; Habuš et al., 2021c). In addition, US treatment proved to be successful in reducing PPO activity of various fruits and vegetables (Safwa et al., 2023). However, treatment of millet bran with US resulted in increased PPO activity (Čukelj Mustač et al., 2019). The US-assisted physical modification of flour mainly refers to the main components, namely starch and fiber (Vela et al., 2023). The US treatment of whole grain flour has been shown to significantly improve the water solubility, water absorption, and swelling power of quinoa, buckwheat, and rice flour (Harasym et al., 2020; Vela et al., 2021; Zhu and Li, 2019). Previous research has shown that the use of US pregelatinized starch can improve the textural properties of bread (hardness and chewiness) (Jalali et al., 2020; Ma et al., 2022;

Vela et al., 2023). In addition, it has been shown to increase the solubility of polysaccharides from rice bran, making them a suitable substrate for fermentation in the production of nutraceuticals (Vaitkeviciene et al., 2022).

The effectiveness of US treatment on enzyme activity, the extractability of phenolic compounds and the change in functional properties of the sample depends on the sample matrix and the process equipment and parameters (Estivi et al., 2022; Čukelj Mustač et al., 2019; Vela et al., 2021).

3.3. Pulsed electric field

The pulsed electric field is a non-thermal technology capable of improving extractability and increasing the functionality of nutritionally valuable components (Arshad et al., 2020). The fundamental operating principle is based on the application of short pulses (μs-ms) of strong electric fields to the food placed between two electrodes (Figure 2) (Arshad et al., 2020; Duque et al., 2020a). PEF treatment increases the transmembrane potential and initiates the formation of pores in the membrane of a plant, animal, or microbial cell (Arshad et al., 2020). Electroporation can be categorized as reversible (release of the cell membrane) or irreversible (collapse or lysis of the cell membrane) depending on the electric field intensity (EFI). Both types are used in various food processing applications and can be controlled depending on the intended function (Safwa et al., 2023). As it contributes to the permeabilization of cell membranes, PEF treatment led to an improved extraction efficiency by increasing the mass transfer rate (Kumari et al., 2018). Various bioactive molecules (β-glucans, phenols, pigments, etc.) from cereal grains, fruits, vegetables, herbs, etc. were extracted by PEF (Duque et al., 2020a; El Kantar et al., 2018; Pataro et al., 2018; Tzima et al., 2021).

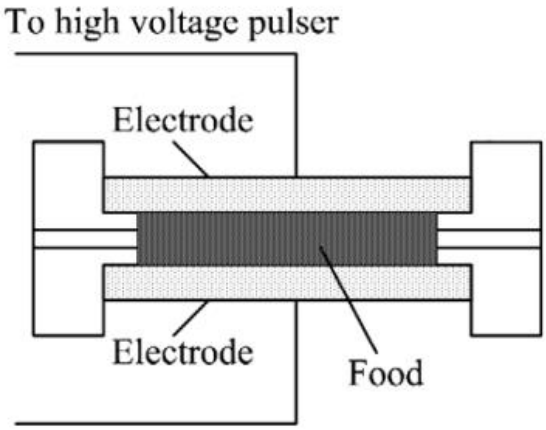

Figure 2. Scheme of PEF static treatment chamber (Qin et al., 1994).

Moreover, the application of PEF treatment has been shown to induce alterations in the physicochemical properties of biomacromolecules (Duque et al., 2020a, 2020b; Jaeger et al., 2010; Maniglia et al., 2021). Without chemical residues and requiring low energy and time consumption, PEF has emerged as a promising technique for modulating the activity and stability of numerous enzymes (Jaeger et al., 2010; Li et al., 2022; Ohshima et al., 2007; Zhang et al., 2017). PEF treatment increased α- and β-amylase activity in wheat grain and consequently improved the malting process (Carregari Polachini et al., 2023), inactivated orange juice peroxidase (Elez-Martínez et al., 2006), lipoxygenase of soybean and tomato juice (Li et al., 2008; Min et al., 2003) and alkaline phosphatase in bovine milk (Sharma et al., 2014). Currently, most research is focused on the application of PEF technology with the aim of improving germination and the malting process (Table 2).

Table 2. Overview of the available literature on processing with US and PEF technology for the purpose of modifying the enzymatic activity of cereals.

Material	Processing technique	Conditions	Purpose	Results	Reference
Wheat seeds	PEF	25% seeds-water suspension pulse width 100 µs EFI 2, 4, 6 kV cm^{-1} Frequency 1 Hz	improvement of germination	increased amylase and protease activity	Ahmed et al., 2020
Wheat grains	PEF	EFI 3 kV cm^{-1} Ws 9.9 and 19.8 kJ kg^{-1}	improvement of germination and hydration	increased α- and β-amylase activity	Carregari Polacchini et al., 2023
Millet bran	US	15% bran-water suspension Power 400 W frequency 24 kHz time 5, 12.5, 20 min amplitude 60, 80, 100 %	inhibition of the activity of the oxidative enzyme; improvement of physical and nutritional quality	increase in PPO activity	Čukelj Mustač et al., 2019

Wheat bran	US	14% bran-water suspension power 400 W frequency 24kHz amplitude 100% time 2 min temperature 95 ± 0.5°C	inhibition of enzymatic browning of 3D printed snacks	decrease in PPO activity	Habuš et al., 2021a
Wheat bran	US	15 mL bran-water suspension power 400 W frequency 24 kHz amplitude 80% time 15 min	prolongation of wheat bran oxidative stability	decrease in POD and lipase activity	Habuš et al., 2021b
Whole wheat flour	US in combination with ascorbic acid	10% flour-water suspension power 750 W, frequency 20 kHz pulse mode 25s on/5 s off temperature < 50°C	inhibition of enzymatic darkening of whole wheat raw noodles	decrease in POD activity	Niu et al., 2014
Barley grain	PEF	pulse width 6 µs EFI 0.5, 1, 3 kV cm^{-1} frequency 20 Hz Ws 0.5, 1, 5 kJ kg^{-1}	improvement of barley germination for malting process	increase in α-amylase activity	Saxton et al., 2024
Barley seeds	PEF	pulse width 4, 6, 8 µs frequency 300, 500, 700 Hz voltage 6, 9, 12 kV time 6, 10, 14 min	elevation of α-amylase activity; improvement of malting process	increase in α-amylase activity	Zhang et al., 2019b

However, different PEF treatment parameters can have different effects on enzyme activity, which can either activate or deactivate them (Ohshima et al., 2007). In addition, the effects of PEF technology on the physicochemical properties of polysaccharides such as corn, wheat and cassava starch, sugar beet pectin and chitosan have been investigated (Han et al., 2012; Ma et al., 2012; Maniglia et al., 2021; Rivero-Ramos et al., 2023). Previous research was mainly based on the effects of PEF technology on individual cereal molecules, mostly wheat starch and proteins (Li et al., 2019; Zhang et al., 2021). So far, the effectiveness of PEF treatment has been tested on wheat, oat, and cassava flour (Achayuthakan et al., 2023; Conde et al., 2022; Duque et al., 2020b, 2020b, 2022). Still, current literature does not adequately describe how PEF treatment affects the physical and functional properties of starchy foods, with a notable gap in understanding its effects on oat and barley flour. The effects of PEF technology on cereals in general, especially on polysaccharides and the activity of their endogenous enzymes, have only been studied to a limited extent.

4. Hypothesis, research objectives, and expected scientific contributions

This research hypothesizes the following:

1. High-intensity ultrasound or pulsed-electric field treatment reduces the activity of endogenous cereal β-glucanases and the concentration of antinutrients
2. Non-thermally treated raw materials are convenient for sourdough process
3. Flat bread with added oat or barley sourdough has improved nutritional value, longer shelf life and higher consumer acceptance

The general objective of this dissertation was to gain knowledge about the influence of US and PEF on the properties of oat and barley bran and flour, respectively, the possibilities of sourdough process of treated materials. Furthermore, the goal is their application in a single-layer flat bread to improve the nutritional value and extend the shelf life while maintaining the technological quality and consumer acceptance.

Given its complex nature and large scope, this study is divided into four interconnected parts:

In the first part of the research, a database of different types of flat breads was established to obtain an overview of different recipe, production process and nutritional value (***Publication No. 1***).

In the second part of the research, the influence of bran addition on the kinetics of sourdough fermentation of oat and barley flour was investigated. The use of sourdough in the retardation of dough for flat bread was addressed (***Publication No. 2***).

In the third research part the influence of ultrasound treatment on β-glucanase activity, phytic acid and total phenolic content, and functional properties of oat and barley bran were examined (***Publication No. 3***). The effects of ultrasound treatment on the structure of the non-starch polysaccharides and the ability of the treated material to ferment into sourdough were investigated, as well as the use of the sourdough obtained for the making of nutritionally improved flat breads (***Unpublished data***).

The fourth part of the research investigated the effects of pulsed electric field technology on the inactivation of β-glucanase, the structure of non-starch polysaccharides of oat and barley flour,

the rheological properties of dough and the application of the treated materials in flat bread (***Publication No. 4***).

Throughout this dissertation the following questions were examined:

1) What type of flat bread is widely available on the Croatian market and what are the main ingredients? Are Croatian consumers interested in flat bread with increased β-glucan content, enriched with oats and barley or their sourdough? Are Croatian bakery manufacturers interested in increasing their production capacity for the production of flat bread?
2) What are the main ingredients, production processes and main quality characteristics of flat breads from the Mediterranean region? (*Publication No. 1*)
3) Can the addition of oat and barley bran accelerate the kinetics of sourdough fermentation of oat and barley flour? Can the addition of oat and barley sourdough contribute to the shelf-life by slowing down the enzymatic browning of bread doughs during dough retardation? What are the effects of dough retardation on glucan and phytates content? (*Publication No. 2*)
4) Can high-intensity ultrasound simultaneously reduce β-glucanase activity and the concentration of antinutrients in oat and barley bran? (*Publication No. 3*) Are the treated brans convenient for sourdough process? Does the US treatment have a positive effect on the retaining of β-glucans in flat bread? (*Unpublished data*)
5) How pulsed electric field treatment of oat and barley flour affects the β-glucanase activity, the concentration and the molecular weight of β-glucans and the functional properties of flour? Can the flour pretreatment yield in breads of increased dietary fiber and β-glucans content while preserved quality features? (*Publication No. 4*)

Throughout this dissertation next was achieved:

1) detailed insight into the flat breads produced in the Mediterranean region (8 countries), information on flat bread production steps, main ingredients, and end-product characteristics.
2) better understanding of the effects of the addition of bran on the acidification power of oat and barley flour and the influence of sourdough on the browning of flat bread dough during retardation

3) better understanding of the effects of high-intensity ultrasound and pulsed electric field on the enzyme's activities, especially β-glucanase, β-glucans concentration and molecular weight, structure of non-starch polysaccharides, and techno-functional properties of oat and barley flour
4) development of nutritionally improved wheat-oat and wheat-barley composite flat breads and sourdough flat breads made from US- or PEF-pretreated oat and barley flour

Chapter 2

Scientific papers

1. *Publication No. 1:* The large and diverse family of Mediterranean flat breads: A database
2. *Publication No. 2:* Sourdough fermentation of oat and barley flour with bran and its application in flat bread made with no-time and dough retardation methods
3. *Publication No. 3:* Ultrasound-assisted modification of enzymatic and antioxidant activities, functional and rheological properties of oat and barley bran
4. *Publication No. 4:* Pulsed electric field of oat and barley flour: Influence on enzymes, non-starch polysaccharides, dough rheology properties, and application in flat bread

Chapter 2

Publication No. 1

Pasqualone, A., Vurro, F., Summo, C., Abd-El-Khalek, M. H., Al-Dmoor, H. H., **Grgić, T.,** Ruiz, M., Magro, C., Deligeorgakis, C., Helou, C., Le-Bail, P. (2022) The large and diverse family of Mediterranean flat breads: A database. Foods, **11**, 2326. **(Q1)**

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MDPI

Article

The Large and Diverse Family of Mediterranean Flat Breads: A Database

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Abstract: An in-depth survey was conducted by collecting information from web sources, supplemented by interviews with experts and/or bakers, to identify all the flat breads (FBs) produced in the nine Mediterranean countries involved in the FlatBreadMine Project (Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta and Spain), and to have an insight into their technical and cultural features. A database with information on 143 FB types (51 single-layered, 15 double-layered, 66 garnished, 11 fried) was established. Flours were from soft wheat (67.4%), durum wheat (13.7%), corn (8.6%), rye, sorghum, chickpea, and chestnut (together 5.2%). The raising agents were compressed yeast (55.8%), sourdough (16.7%), baking powder (9.0%), but 18.6% of FBs were unleavened. Sixteen old-style baking systems were recorded, classified into baking plates and vertical ovens (*tannur* and *tabun*). Artisanal FBs accounted for 82%, while the industrial ones for 7%. Quality schemes (national, European or global) applied to 91 FBs. Fifteen FBs were rare, prepared only for family consumption: changes in lifestyle and increasing urbanization may cause their disappearance. Actions are needed to prevent the reduction of biodiversity related to FBs. Information in the database will be useful for the selection of FBs suitable to promotional activities and technical or nutritional improvement.

Keywords: flat bread; flour quality; traditional bread; ethnic food; baking system; vertical oven; bread culture; food heritage; food diversity; quality schemes

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1. Introduction

Bread is one of the basic components of the daily diet, and its history is linked to human history. The "flat" breads include a multitude of bread types different from each other but always relatively thin, ranging from a few millimeters to a few centimeters in thickness. These breads, whose origin is very ancient [1], are produced all over the world. Those spread from the Fertile Crescent reached the Mediterranean area (North Africa, Southern Europe, Middle East and Anatolian peninsula), the Indian subcontinent, and the Caucasian region, up to Xinjiang [2], as well as the Arabian peninsula, with interlinks with

the Horn of Africa [2,3]. Flat breads are also produced in the American continent, mostly in Central and South America [4], but also in the North, owed to Native Americans [5].

Flat breads meet the need of increasing the sustainability of food system for several reasons: (i) Can be obtained from cereals other than wheat, as well as pseudocereals or pulses, allowing the use of local productions from marginal lands; (ii) require short baking times, eventually even without using an oven (under hot ashes); (iii) can wrap around food or serve as a spoon, reducing tableware use and water consumption; (iv) are transported with little encumbrance and reduced energy impact; (v) if baked to dryness, have a quite long shelf life, reducing bread waste [2].

These strong points made flat breads very popular so that, though having an ancient origin, they have survived until today. Nowadays, these highly versatile breads can be produced either in the same way as they were made thousands of years ago or in modern fully automatic industrial lines. In addition, they can be seasoned or stuffed with a variety of ingredients becoming cheap, convenient, and palatable street foods. Renowned examples of these are the *döner kebab* and the related *shawarma* and *gyro*, i.e., finely sliced roasted meat rolled, or stuffed, in a pocket-type flatbread (typically named "*pita*") with salad and various sauces [6,7]. Other examples are the Italian *pizza* and *focaccia*, or the French *fougasse*, prepared by seasoning the surface of the flattened dough disc with several ingredients, before baking [8]. The fast pace of the modern lifestyle has led to a growing demand for ready-to-eat foods and a concomitant increase in the consumption of flat breads. The global market for these products accounted for $81,796.6 million in 2018 and is expected to grow to $145,180.9 million by 2027 [9].

Although flat breads are an ancient and consolidated product, there is still much room for technical and nutritional improvement. The baking step is typically very fast, being carried out at high temperature with direct heating. This process may cause quality and safety issues such as burned edges, due to non-uniform heat distribution, and the formation of combustion contaminants such as benzopyrenes and polycyclic aromatic hydrocarbons (PAHs) [7,10]. New baking systems have been recently proposed, such as an indirect heating plant with a rotary baking tray [11]. Moreover, the Bake Off Technology (BOT), consisting of producing bread from industrial refrigerated, frozen or non-frozen bakery goods (partially-baked bread or "part-baked" bread) to be sold for domestic baking, has increased its market share indicating a growing interest by consumers [12], and could be applied to flat breads. Flat breads, which are a staple food of high nutritional importance in many countries, are also suitable for reformulation with a variety of fortifying ingredients of animal or plant origin, able to raise the content of proteins and micronutrients [13,14].

In this context, an international research project, namely "Flat Bread of Mediterranean area: Innovation & Emerging process & technology" (FlatBreadMine) has been recently financed by the European Union H2020-PRIMA, with the main aim of valorizing and innovating flat breads. However, to propose technical innovations (such as low-pressure baking and part-baking) and nutritional improvement (by incorporating flour of legumes, acorns, or carobs), a precise picture of the existing flat breads is needed, in order to select the most suitable ones.

The aim of this work was, therefore, giving an insight into the technical and cultural features of the flat bread types produced in each one of the Mediterranean countries involved in the FlatBreadMine project (namely Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta and Spain). The steps to achieve this goal are: (1) To identify all the flat bread types produced in the selected countries; (2) to collect information on their main technical characteristics, from the starting ingredients to the end-product, including the cultural features; (3) to set up a database containing all the information; (4) to examine and interpret the information collected [15] in order to highlight the diversity of flat breads across the considered countries and to define the selection criteria.

2. Materials and Methods

2.1. Surveyed Area

Nine countries of the Mediterranean area, involved in the FlatBreadMine project, were objects of study: Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta, and Spain.

2.2. Subject of the Survey

A survey was carried out to collect information on flat breads, including traditional and artisanal ones. The surveyed flat breads had to be original and native of each surveyed country. Therefore *pizza*, for example, which has an Italian origin, was listed as an Italian flat bread and surveyed only in Italy, although prepared also in the other countries object of the study.

For each flat bread, the following data were collected: (i) The regional area or town of origin, and the area of marketing and diffusion; (ii) the ingredients used in bread preparation (flour, yeast, additional ingredients, and their ratio); (iii) the raw material characteristics (type of flour and its quality features; type of yeast; information on any additional ingredient); (iv) the production process, step by step (kneading time and temperature; conditions of the first leavening step; shaping specifications in terms of average diameter and thickness; conditions of the second leavening step; time and temperature of baking; oven type); (v) the characteristics of bread (type and size; optimal quality features; artisanal or 35-industrial); (vi) the main references and sources of information.

2.3. Data Collection

Data were collected between October 2021 and May 2022. The first step was the identification of all flat breads produced in each country, which was done by accessing the websites of bakers' associations, food blogs, and scientific literature; browsing the official lists of traditional food products uploaded onto the websites of the EU, the Italian Ministry of Agriculture, and Slow Food; consulting local experts. The latter were scholars involved in the protection and rediscovery of traditional food products including bread, who helped to uncover rare breads not regularly available in the market. They were selected via convenience sampling, based on direct knowledge with the researchers involved in the study, and were contacted by phone for advice.

The second step consisted in collecting the information referred to in Section 2.2. for each flat bread. Information was primarily retrieved from web sources: official technical datasheets of breads awarded of quality marks, scientific literature, websites of bakers' associations, and food blogs. Missing information in web sources was sought from the experts and/or from bakery managers/owners through structured face-to-face/phone interviews. The recruitment of respondents (experts and/or bakers), according to convenience sampling, was based on direct knowledge with the researchers involved in the study, or supported by the bakers' associations, who introduced the researchers. Interviews were based on a questionnaire (Supplementary Table S1) composed by qualitative and quantitative open-ended questions, which was pre-tested with the president of the consortium of bakers specialized in the production of *Focaccia barese* flat bread (Bari, Italy), who was asked to answer the questions and comment on their feasibility, to avoid excessively generic, or too specific and technical questions, difficult to understand. After pre-testing, technical questions regarding bread packaging, modified atmosphere and storage conditions were deleted. To reduce work, considering the great number of surveyed breads (n = 143), only the questions necessary to fill the information gaps with respect to web sources were asked. In addition, experts who knew more than one type of flat bread, as well as bakers who produced more than one type of flat bread, were asked to provide information on all of them. The first contact was by telephone, to present the study and to make an appointment for the face-to-face or phone interview, if the participant agreed. The choice between face-to-face and phone interview was made according to the interviewee's preference. The interviewers were the researchers involved in the study. They facilitated the comprehension, also linguistic, of the questionnaire, which was written in English.

The interviewers let the conversation flow naturally and took notes of the answers to each question. Data were anonymized and treated in an aggregated way.

2.4. Database Structure

A database was set up by the Excel software (Microsoft Office, Version 2018 for Windows, Microsoft Corporation, Redmond, Washington, DC, USA) to gather information on the flat breads of each surveyed country. The database structure included one row per each bread type and 27 columns for the above reported information. In addition, a representative picture was included for each type of bread.

The database was uploaded onto the FlatBreadMine project website and is publicly accessible at the link: https://flatbreadmine.eu/resources/ (accessed on 7 July 2022).

2.5. Data Analysis

The distribution of data was analyzed as percent frequency by Excel software (Microsoft Office, Version 2018 for Windows, Microsoft Corporation, Redmond, Washington, DC, USA).

3. Results and Discussion

3.1. Flat Bread Diversity in the Surveyed Area

A total of 143 different flat bread types were found to be produced in the surveyed countries, distributed as follows: 14 from Croatia, 8 from Egypt, 3 from France, 23 from Greece, 75 from Italy, 6 from Jordan, 7 from Lebanon, 2 from Malta, and 5 from Spain (Figure 1) [16].

Figure 1. Geographical distribution of flat breads in the surveyed area (Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta, and Spain).

The high number of flat breads recorded in Italy was probably due to the existence of strong regional gastronomic differences within the Italian territory. Furthermore, the consolidated tendency to rediscover and keep alive the memory of small-scale, local, and traditional food products, included flat breads, finds its maximum expression in Italy, home of the Slow Food Foundation for Biodiversity [17]. This trend, appreciated by modern consumers [18,19], aligns with the European policies for promoting traditional foods and protecting their origin [20], and will be discussed more in depth in Section 3.9.

Another important factor is the different meaning that flat breads assume in different areas. In Italy these products are considered as a delicacy, admitting many variations on a regional basis, while in the areas where flat breads originated in the antiquity, i.e., Middle East [1,2] (Jordan and Lebanon, for this survey), or in closer countries, such as Egypt, they represent staple foods that are consumed daily, therefore are less affected by variations.

Flat breads can be classified into plain (further categorized into single- or double-layered), garnished (seasoned or stuffed), and fried. Table 1 shows the occurrence of flat breads in the different categories through the surveyed countries. Garnished flat breads accounted for 46.2%, with a 27.3% contribution by Italy. These flat breads, prepared as specialties to be consumed occasionally, were seasoned or stuffed with several ingredients before baking. In Jordan and Egypt, instead, flat breads were only plain.

Table 1. Occurrence of flat breads in the different categories.

Country	Flat Bread Category							
	Plain				Garnished (Seasoned, Stuffed)		Fried	
	Single-Layered		Double-Layered					
	Number	%	Number	%	Number	%	Number	%
Croatia	5	3.5	2	1.4	7	4.9	-	-
Egypt	6	4.2	2	1.4	-	-	-	-
France	-	-	-	-	3	2.1	-	-
Greece	6	4.2	1	0.7	12	8.4	4	2.8
Italy	23	16.1	7	4.9	39	27.3	6	4.2
Jordan	5	3.5	1	0.7	-	-	-	-
Lebanon	3	2.1	1	0.7	3	2.1	-	-
Malta	-	-	1	0.7	1	0.7	-	-
Spain	3	2.1	-	-	1	0.7	1	0.7
Total	51	35.7	15	10.5	66	46.2	11	7.7

Among the plain types, the single-layered category, easier to be prepared, accounted for 35.7%. The double-layered (10.5%) are, instead, those characterized by the typical "pocket", such as the Jordan *Kmaj* [21] (Figure 2A), the Egyptian *Shamy* and *Baladi* [22], and the common Arabic bread or *Khobz* (*Khobz* means "bread" in Arabic) (Figure 2B), which are all known in western countries as "*Pita*" bread.

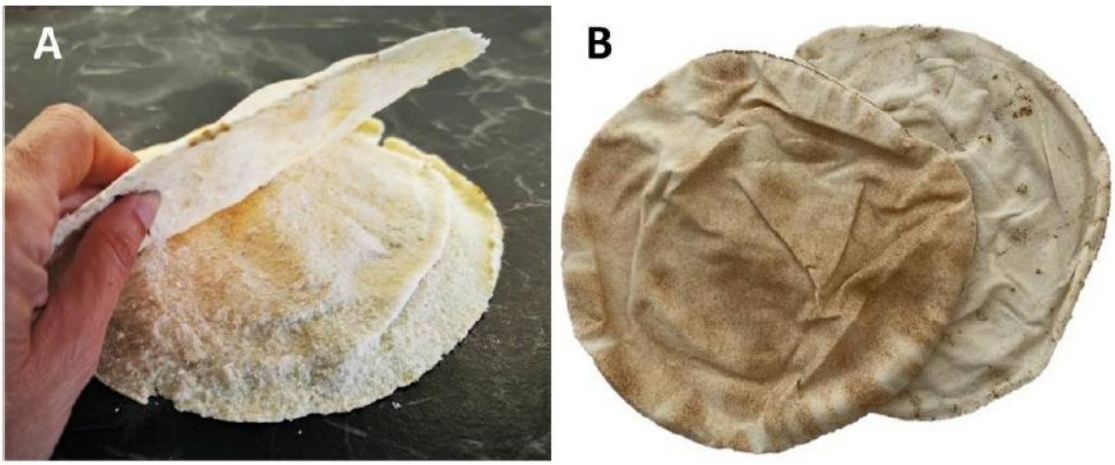

Figure 2. (**A**) Jordan *Kmaj* opened to show the internal "pocket". (**B**) Lebanese *Khobz*.

The pocket is the visible result of the thermal expansion of the fermentation gas into a thin dough layer, which takes place during baking (Figure 3).

Figure 3. Jordan *Kmaj* in an automatic baking line. The inflation of bread due to the thermal expansion of the gases is clearly visible.

Fried flat breads accounted for 7.7%. They were recorded in Italy (6 breads, namely *Gnocco fritto*, *Crescenta fritta*—also named *Crescentina fritta*—*Pinzini ferraresi*, *Cresciolina*, *Pizza fritta*, *Pitt'ajima*), Greece (4 breads: *Pisia*, *Tiganopsomo*, *Sfakianopita* and *Fyllota*), and Spain (one bread: *Arepas Canarias*).

Figure 4 shows, per each country, the number of flat bread types marketed outside the area of origin (town, subregion), compared to the total number flat breads produced in the country. In Italy, Greece, and Croatia, only a minority of flat breads were found to be marketed through the entire country, outside the area where they are originally produced and consumed, which was generally a very limited geographic area.

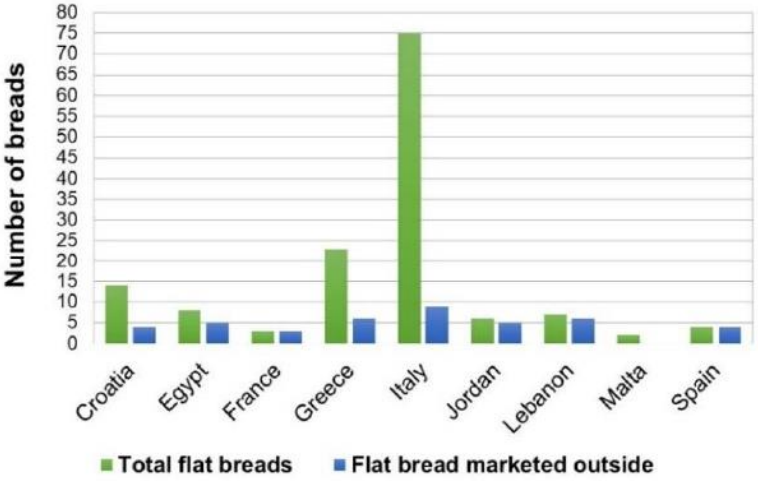

Figure 4. Number of flat bread types marketed outside the area of origin, compared to total flat breads per country (n = 143).

On the contrary, in countries such as Jordan, Lebanon, and Egypt, the distribution and consumption of flat breads was homogenous throughout the entire country and three flat breads from these countries were also exported abroad (namely the Egyptian *Baladi* and *Shamy*, and the Lebanese *Khobz*). These findings agreed with the different character, previously discussed, that flat breads assume in different areas: local specialties vs. national staple foods.

3.2. Flour Type and Quality

Refined soft wheat flour was widely used (67.4%) in the preparation of the surveyed flat breads (Table 2).

Table 2. Type of flour used in the preparation of flat breads. Multiple answers were admitted, because breads could be prepared with different flours or with flour blends.

Country	Type of Flour											
	Soft Wheat				Durum Wheat [a]		Corn [a]		Rye [a]		Other Species [a,b]	
	Refined		Whole Meal									
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	14	8.0	2	1.1	-	-	3	1.7	2	1.1	-	-
Egypt	5	2.9	1	0.6	-	-	2	0.6	-	-	2	1.1
France	3	1.7	-	-	-	-	-	-	-	-	-	-
Greece	20	11.4	2	1.1	4 [c]	-	3	1.7	-	-	-	-
Italy	59	33.7	2	1.1	14	8.0	5	2.9	2	1.1	3	1.7
Jordan	6	3.4	-	-	5	2.9	-	-	-	-	-	-
Lebanon	7	4.0	1	0.6	1	0.6	1	0.6	-	-	-	-
Malta	2	1.1	1	0.6	-	-	-	-	-	-	-	-
Spain	3	1.7	-	-	-	-	2	1.1	-	-	-	-
Total	118	67.4	9	5.1	24	13.7	16	8.6	4	2.3	5	2.9

[a] Refined flour, unless otherwise specified. [b] Sorghum, chickpea, chestnut. [c] Durum wheat whole meal is used in the preparation of *Koulouri* (Greece).

Whole meal flour of soft wheat was used only in 5.1% of cases. Earlier studies, dating the late nineties, reported that flat breads were commonly made of wheat flour at high extraction levels [4], so this flour has been progressively substituted by the refined one.

The use of durum wheat flour (more precisely, re-milled semolina) was reported in 13.7% of cases. Durum wheat cultivation is common in semiarid zones of the Mediterranean basin, and its use in bread making has been already reported [23]. In fact, durum wheat breads (not flat), such as Altamura bread [24] and Dittaino bread [25], have been awarded by the Protected Designation of Origin (PDO) European Union (EU) mark for their peculiar characteristics, such as a compact and yellowish crumb (due to carotenoid pigments). Durum wheat whole meal represented an exception and was found only in the preparation of the Greek *Koulouri*.

The use of corn refined flour accounted for 8.6%. In three cases it was subjected to thermal treatments: pre-cooked corn flour was recorded in the production of the Spanish *Arepa Canarias*, and up to 30% extruded-cooked corn flour could be optionally added to soft wheat refined flour to prepare the Croatian flat breads *Pogača* and *Kukuruzna miješana ciabatta* ("corn composite *ciabatta*"). The thermal treatment causes starch gelatinization, increasing dough viscosity [26]. In the presence of wheat flour, this effect is not strictly needed because good viscoelasticity is ensured by gluten; however, the thermal treatment could be made also because it is able to slow down bread aging [27,28].

Corn has long been cultivated in the Canary islands and Eastern Europe, including Croatia [29], so in the past the exclusive use of corn in the preparation of these breads could be hypothesized, explaining the need of pre-gelatinizing flour. The use of pre-cooked corn flour in the preparation of *Arepa Canarias*, indeed, resembles the procedure adopted for its Venezuelan counterpart, *Arepa*, which is made from corn only [30]. Probably a return cultural contamination took place in the Canary islands following migrations to America, including Venezuela.

Without a thermal pretreatment, instead, corn flour is used in the preparation of the Egyptian *Bataw* and *Meraharah*, as well as in the *Talo*, a traditional bread from the Mungia subregion of Basque Countries. Interestingly, the latter has been associated by archeobotanists to an ancient flat bread of the same area, made of acorn [31], which is being rediscovered recently for its high nutritional value [32]. Corn flour can be used as an

alternative to wheat flour in the Greek *Souvlakopita* and *Plakopita*, while *Bobota*, which was the most consumed bread in Greece during the German-Italian occupation during World War II, was and is still made exclusively from corn flour [16]. In Italy, corn flour is used to prepare the *Carchiola*, *Torta al Testo*, *Pizza di granone*, *Pizza con farina di mais*, and *Pizza di farinella bacolese*. Only a very small amount of corn flour, about 5%, is mixed with wheat flour for preparing the Lebanese *Markouk*.

Rye flour was found to be used in colder areas—to which is more adapted [33]—such as the alpine Italian regions and part of Croatia. A mixture of rye and soft wheat flour, indeed, is used to prepare the Italian *Puccia ladina* (typically consumed in the mountain huts of the Dolomites) and *Schüttelbrot*. Rye flour is optionally added to soft wheat flour to prepare the Croatian *Pogača* and *Kruh ispod peke* ("bread under the lid").

Like other commodities, cereals and particularly wheat, have long since become fully globalized. Egypt, for example, has become the world's largest importer of wheat, exposing the country to significant vulnerabilities, not to mention that more than half of the consumption of wheat in the Mediterranean countries comes from Russia and Ukraine [34]. The use of alternative flours should therefore be strengthened. Among them, sorghum flour was traditionally used to prepare the Egyptian *Khobz min el dorra al rafi'ah* and *Zallut*; however, these breads are now only prepared at home for family consumption and not for commercial purposes, with a serious risk of losing their knowledge.

Pulses, though nutritionally valuable, with an amino acid profile complementary to that of cereals, are underrepresented. Chickpea flour is used only in the Italian *Farinata*, a typical street food from Liguria region with variants in Tuscany and Piedmont [35]. However, the addition of pulse flours to bakery products, including flat bread, has been the object of a rising attention in the recent years [36–39].

Chestnut flour is used for preparing the Italian *Neccio*. Besides their high antioxidant activity, chestnuts are rich in minerals, polyunsaturated fatty acids, fiber, and vitamins [40]. In Italy, indeed, this crop has an important economic value [41] and specific chestnut cultivars (Carpinese, Pontecosi, Capannaccia, and Morona) grown in the Garfagnana subregion of Tuscany are used to prepare the flour named "*Farina di Neccio della Garfagnana*" which has been recognized as a PDO food product. According to tradition, before milling, the chestnuts are dried in small stone buildings named "*metati*", where a hearth on the ground floor heats and dries the chestnuts placed on the upper floor [42].

Regarding the quality of flours used for flat breads, a limited technical knowledge was observed in all the surveyed countries, especially (but not exclusively) for the most artisanal productions. Consequently, this information often remained undefined in the database (where "not specified" is indicated), reaching 71.6% of missing data, which was the highest percentage among all collected data. Where information on the quality features was available, protein and gluten content and gluten quality were the most frequently reported. For refined wheat flour the quality parameters generally were: protein content $\geq$ 9%, wet gluten content $\geq$ 25%, alveograph W $\geq$ 180 $\times$ 10^{-4} J, and P/L $\leq$ 1. For *Pizza Napoletana* Traditional Specialty Guaranteed (STG) more detailed quality information was available: dry gluten 9.5–11%, alveograph W 220–380 $\times$ 10^{-4} J, P/L 0.50–070, water absorption 55–62%, farinograph stability 4–12 min, farinograph drop off of consistency $\leq$60 Brabender Units [43]. For corn flour only protein content $\geq$7% was reported.

It should be mentioned that data collection at bakers faced obstacles related to the COVID-19 pandemic restrictions and to the successive flour shortage following the Ukraine crisis. The economic value of bread and its scarcity have always important social repercussions, as evidenced by past and recent history. Bread availability provides a sense of security, while the lack of bread can be the cause of violent social movements. For example, in Lebanon, where the COVID-19 pandemic economic loss overlapped with a pre-existing crisis [44], further wheat shortage exacerbated the situation, making wheat and bread availability an extremely hot topic. Especially small producers suffered from the financial crisis on full blow, which took its toll on their sales to the point that they cannot afford even proper maintenance for their equipment. A similar situation was observed in

Egypt and Jordan [45,46]. At various levels, lockdown-related economic losses and flour shortage were common to all countries, making bakers not really inclined to cooperate with the interviews. These issues were reduced, but not totally solved, with the help of local associations of bakers which introduced the researchers, or relying on direct knowledge with them.

3.3. Additional Ingredients

Though many plain flat breads (54.3%) did not contain any lipid, olive oil was used in 19 cases, 10 of which were characterized by the use of the extra virgin category (Table 3).

Table 3. Type of fat eventually used in plain flat breads. Multiple answers were admitted, because breads could be prepared with different oils and fats or with blends.

Country	Olive Oil		Sunflower Oil		Vegetable Oil (Not Specified)		Lard		None	
	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	1	1.4	2	2.9	1	1.4	-	-	3	4.3
Egypt	-	-	-	-	1	1.4	-	-	7	10.0
France	-	-	-	-	-	-	-	-	-	-
Greece	2	2.9	-	-	-	-	-	-	5	7.1
Italy	13 [a]	18.6	-	-	-	-	8	11.4	12	17.1
Jordan	-	-	-	-	-	-	-	-	6	8.6
Lebanon	1	1.4	-	-	1	1.4	-	-	3	4.3
Malta	1 [a]	1.4	-	-	-	-	-	-	-	-
Spain	1	1.4	-	-	-	-	-	-	2	2.9
Total	19	27.1	2	2.9	3	4.3	8	11.4	38	54.3

[a] Extra virgin olive oil is used in nine Italian flat breads and in the Maltese one.

Two flat breads included sunflower oil in their formulation, and three were prepared with lard, namely the Italian *Piadina Romagnola*, *Crescentina di Modena*, and *Torta al testo*, whose official technical sheets [8,47–49], however, report also the possibility to use olive oil. The use of lard is traditional in the area of origin of these three flat breads, which is approximately the same area of *Prosciutto di Parma* PDO ham and is characterized by the presence of numerous pig farms.

However, besides the obvious nutritional and health implications related to the reduction of saturated fatty acids, the substitution of lard with olive oil (possibly extra virgin olive oil), could eventually overcome religious restrictions for pork-derived ingredients.

All the garnished flat breads contained vegetable oils or lard. Vegetable oils, when specified, were represented by olive, sunflower, or rapeseed oil, and their blends (Table 4). Greek, Italian, and French garnished flat breads were prepared with olive oil, and in particular extra virgin olive oil was used in the Italian *Focaccia di Recco* and *Pizza Napoletana* STG, agreeing with their official technical sheet [43,50]. Sunflower oil was used in the Croatian *Rudarska greblica* and *Zlevanka*, as well as in the *Poljički soparnik*, where, however, it was used in 50:50 mixture with olive oil. The French *Flammekueche*, of Alsatian origin (with German influence), was prepared with rapeseed oil. Lard, instead, was used in two Croatian (*Rudarska greblica* and *Zlevanka*) and ten Italian garnished flat breads (*Gnocco ingrassato*, *Focaccia Novese*, *Focaccia di Voltri*, *Crescia d'la stacciola*, *Crescia brusca*, *Pizza a scannatur di Carbone*, *Scarcedda*, *Pizza con i cingoli di maiale*, *Pizza con le sfrigole*, *Crescenta bolognese*).

During kneading and baking several reactions take place, including lipid oxidation. Studies carried out in several types of Italian *focaccia* have shown that the level of oxidation may change by varying the type of toppings, with moist ingredients able to mitigate the rise of temperature during baking, thus exposing the lipid fraction to a less severe heat stress [8].

Table 4. Characteristic ingredients of garnished flat breads. Multiple answers were admitted, because breads could be prepared with different fats and ingredients.

Country	Fats				Additional Ingredients											
	Vegetable Oil [a]		Lard		Plant-Based Ingredients [b]		Dairy Products		Eggs		Cured Meat		Canned Fish		Meat	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	5	8.3	2	3.3	7	6.3	3	2.7	2	1.8	-	-	3	2.7	-	-
Egypt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
France	3	5.0	-	-	2	1.8	1	0.9	-	-	1	0.9	1	0.9	-	-
Greece	10	16.7	-	-	9	8.1	5	4.5	6	5.4	-	-	-	-	-	-
Italy	28	46.7	10	16.7	23	20.7	12	10.8	5	4.5	15	13.5	6	5.4	1	0.9
Jordan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lebanon	1	1.7	-	-	3	2.7	2	1.8	-	-	-	-	-	-	1	0.9
Malta	-	-	-	-	1	0.9	1	0.9	1	0.9	-	-	-	-	-	-
Spain	1	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	48	80.0	12	20.0	45	40.5	24	21.6	14	12.6	16	14.4	10	9.0	2	1.8

[a] When specified, olive oil, sunflower oil, rapeseed oil, or blends of these were used. [b] Spices, vegetables, cereals, seeds.

Excluding oils and fats, the garnished flat breads contain also other ingredients which impart them well-defined and recognizable sensory characteristics and, by varying in nature and quantity, differentiate them into a myriad of nuanced variations. These ingredients are used to stuff or season the dough before baking, and their combination generates a pleasant palatability which makes the final product become much more than a staple food. The additional ingredients can be of plant and/or animal origin. The plant-based ones, which include spices, various vegetables, cereals, or seeds, accounted for 40.5%, while the sum of the ingredients having animal origin (dairy products, eggs, cured meat, canned fish, and meat) totalized 59.5%. Cured meat includes several charcuterie products such as salami and ham, while fresh meat is typically pork meat, added for example to Italian *Pizza a scannatur di Carbone* prepared on the day of the pig slaughter.

3.4. Leavening

Leavening agents were: compressed bakers' yeast (*Saccharomyces cerevisiae*) (55.8%), sourdough (16.7%), and baking powder (9.0%), while 18.6% flat breads were unleavened (Table 5).

Table 5. Type of yeast, if any, used in the preparation of flat breads. Multiple answers were admitted, because breads could be prepared with different types of yeast or yeast mixtures.

Country	Type of Yeast							
	Baking Powder		Compressed Yeast		Sourdough		None	
	Number	%	Number	%	Number	%	Number	%
Croatia	2	1.3	9	5.8	2	1.3	3	1.9
Egypt	1	0.6	5	3.2	1	0.6	-	-
France	-	-	3	1.9	-	-	-	-
Greece	1	0.6	17	10.9	1	0.6	5	3.2
Italy	7	4.5	40	25.6	19	12.2	16	10.3
Jordan	3	1.9	4	2.6	-	-	1	0.6
Lebanon	-	-	6	3.8	-	-	1	0.6
Malta	-	-	1	0.6	2	1.3	-	-
Spain	-	-	2	1.3	1	0.6	3	1.9
Total	14	9.0	87	55.8	26	16.7	29	18.6

Sourdough, indeed, despite being the most traditional leavening method in the past, has overtime been partly replaced by compressed yeast, which is easier to handle and reduces leavening times. However, there is a renewed interest by consumers toward sourdough-leavened flat breads, also based on research results that prove nutritional and qualitative improvements, such as a reduction of phytic acid in whole meal flat breads [51],

an increase of selenium bioavailability [52], and improved shelf life [53], texture and sensory properties [54,55].

More "modern" leavening aids, such as baking soda, are used in the Italian flat breads *Piadina Romagnola*, *Crostolo*, *Torta al Testo*, *Pizza scima*, *Gnocco fritto*, *Crescenta fritta*, in the Croatian *Pogaca z oreji* and *Zlevanka*, and in the Greek *Plakopita* (*Pita* bread baked on a stone). In the preparation of *Crescentina di Modena* baking soda can be used as an alternative to the most used compressed yeast. Similarly, the Egyptian *Shamy* bread is leavened with baking powder as an alternative to compressed yeast. In three Jordan breads (*Mashrouh*, *Tannur*, and *Taboun*), instead, baking soda is used together with compressed yeast.

Among the unleavened flat breads there is an Italian one whose multiple names all remind the absence of fermentation: "*Pizza azzima*" (which is the original name, literally meaning "unleavened pizza" in Italian), and its naming variations "*Pizza ascima*", "*Pizza scive*" and "*Pizza scime*". Another unleavened Italian flat bread, similar to the former, is the "*Pitt'ajima*", whose name is also clearly related to the original "*Pizza azzima*".

The Egyptian *Shamsi* bread (whose name derives from the Arabic word "*shams*", meaning "sun"), instead, is a leavened one. Its leavening phase is interesting from the ethnographic point of view, because traditionally it takes place with the help of sun heat, i.e., open air, under the direct sunlight [16] (Figure 5).

Figure 5. Preparation of Egyptian *Shamsi* bread: dough exposed to the sun for fermentation.

This bread is decorated by making three crescent-shaped cuts, that form three angles as the dough rises. Coptic Christians, instead, make four cuts to obtain a roughly cross-shaped loaf [56] (Figure 6).

Figure 6. Egyptian *Shamsi* bread.

3.5. Baking

When specified, the declared baking temperatures were <250 °C (50.3%), between 250 and 300 °C (7%), and >300 °C (28.7%) (Table 6).

Table 6. Baking temperature adopted in the baking process of flat breads.

Country	Temperature (°C)							
	<250		250–300		>300		Not Specified	
	Number	%	Number	%	Number	%	Number	%
Croatia	7	4.9	1	0.7	2	1.4	4	2.8
Egypt	-	-	1	0.7	7	4.9	-	-
France	3	2.1	-	-	-	-	-	-
Greece	13	9.1	-	-	2	1.4	8	5.6
Italy	48	33.6	4	2.8	22	15.4	1	0.7
Jordan	-	-	1	0.7	4	2.8	1	0.7
Lebanon	1	0.7	3	2.1	3	2.1	-	-
Malta	-	-	-	-	1	0.7	1	0.7
Spain	-	-	-	-	-	-	5	3.5
Total	72	50.3	10	7.0	41	28.7	20	14.0

In the past, flat breads were baked only in wood-burning ovens, at very high temperatures, above 300 °C. Nowadays, the adopted baking temperature tends to be lower, below 250 °C, due to greater awareness on the risks related to the formation of thermal contaminants, such as polycyclic aromatic hydrocarbons (PAHs) [10,57] and acrylamide [58].

Besides the modern electric or gas ovens (belt conveyor tunnel ovens or batch ovens), several traditional baking systems are still used in the preparation of flat breads. This survey recorded 16 different traditional ways to bake flat bread (Table 7).

The method that most resembles the way flat breads were probably baked in antiquity was recorded for the Jordanian *Arbood*, the traditional bread of the Bedouins. This bread is prepared in the easiest possible way, i.e., unleavened and baked under hot ashes (a very rational way to cook, when an oven is not available). A fire is lit in a sandy area and, after the wood has burned, the dough disc is placed over hot ashes and covered with other ashes. During baking, bread is turned with the help of a stick, to cook evenly on both sides.

Another simple baking tool is a metal grill placed on the embers. The one used for baking the Italian *Carchiola*, named *r'ticula*, has a pivot in the center, so that it can be turned without removing it from the embers of the fireplace. The convex circular metal griddle (named *Saj* in Middle East and Egypt and *Satsi* in Greece) (Figure 7) is used in a similar way to the metal grills, being placed on the embers. It has a large diameter, approximately 50 cm, and it is suitable to bake very large flat breads such as the Jordan *Shrak* (also named *Saaj* bread from its baking system) and *Mashrouh* and the similar Lebanese *Markouk* (named also *Saj* bread).

Table 7. Traditional baking systems used in the preparation of flat breads.

Traditional Baking System	Country and Breads
On a hot sandy ground, under hot ashes and embers	Jordan: *Arbood*
Metal grill (*R'ticula*) on embers	Italy: *Carchiola, Crostolo*
In the fireplace (named *Komin* in Croatia, *Camino* in Italy), under hot ashes and embers	Croatia: *Poljički soparnik*; Italy: *Crescia sotto la cenere*
On the hearth, under a bell-shaped iron lid (*Peka*) covered with embers	Croatia: *Kruh ispod peke* ("Bread under the lid" or "The *Peka*")
In the fireplace, under a terracotta lid (*Coppo*) covered with embers	Italy: *Pizza scime (or Pizza scive, Pizza ascima, Pizza azzima); Pizza somma*
Baking stone	Greece: *Plakopita, Spargana tou Christou*
Terracotta plate (*Tégia*) [a]	Italy, *Piadina Romagnola*
Terracotta plate, smaller than *Tégia* (*Tigella*) [b]	Italy, *Crescentina di Modena*
Terracotta plate with a terracotta lid (*Testo*) [a]	Italy: *Testarolo Pontremolese*, *Panigaccio* [c], *Torta al Testo* [d], *Neccio* [d], *Crescia sfogliata* [d]
Iron griddle	Spain: *Talo*
Circular convex metal griddle (*Saj* or, only in Greece, *Satsi*)	Jordan: *Mashrouh, Saaj*; Lebanon: *Saj;* Egypt: *Farasheeh*; Greece: *Fylla Perek (Perek sheets)*
Metal pan	Greece: *Pisia, Tiganopsomo, Sfakianopita, Fyllota* Italy: *Farinata*
Tinned copper pan (*Sole*)	Italy: *Borlengo di Guiglia*
Igloo-shaped clay oven (*Tabun*)	Jordan: *Tabun*
Vertical, tubular-shaped clay oven (*Tannur*)	Jordan: *Tannur*; Lebanon: *Tannur*
Refractory stone oven, dome-shaped	All surveyed countries, with many breads each

[a] Modern versions made of metal are currently used; [b] Modern versions of this cooking system consist of two flat cast iron discs, between which the dough is cooked; [c] *Panigaccio* is cooked without the lid, between two *testo* plates. Multiple *testo* plates can be stacked; [d] *Torta al Testo*, *Neccio* and *Crescia sfogliata* are cooked without the lid, but have to be flipped during baking to cook homogeneously on both sides.

Figure 7. A domestic *saj*.

The most traditional types of oven are those which retained the greatest differences among countries, being tightly related to local history and habits. Basically, two main baking systems were observed: baking plates (originally made of raw clay or terracotta, but nowadays generally substituted by iron cast), either coupled with lids or not, and vertical ovens: *tannūr* (transcribed also as *tannur* or *tannour*; pl. *tananir*) and *tabūn* (or *tabun*, *taboun*; pl. *tawabeen)*.

Baking plates, i.e., large, open and shallow vessels, have been documented for cooking food in various contexts—temporal, spatial, and cultural, in parts of Europe and the eastern Mediterranean [59]. Late Neolithic baking pans of the Balkans (early 5th millennium B.C.) and the subsequent baking plates of central Europe (late 5th and early 4th millennia B.C.), are small- and medium-sized flat trays interpreted as being used for baking bread [60]. Furthermore, baking trays (25–40 cm diameter) with elaborate molded rims, appear in Syria during the Early Bronze Age (3200–2000 B.C.) and are found throughout the Levant during the Middle (2000–1600 B.C.) and Late Bronze Ages (1600–1100 B.C.). They are interpreted as vessels used on special occasions for baking bread or flat pies [61].

Baking lids have a documented ancient root in the Roman *testum.* Indeed, Cato reports in the *Liber de agri cultura* (chapters 74 and 75) that bread and other foods were baked "*sub testum*", i.e., on the hearth and under a terracotta lid named *testum* [62]. Instead, terracotta baking plates to be placed on the embers, named *testelli* (sing. *testello*), were reported in the Middle Age in central-northern Italy [62]. The influence of the original Latin word on the name "*Testo*" given to the terracotta plate with a terracotta lid traditionally used to bake the Italian *Testarolo Pontremolese* and *Torta al Testo* is evident, as is the influence on the name of the corresponding flat breads.

Another naming similarity is between *tigella* (pl. *tigelle*), the terracotta plate used to bake the *Crescentina di Modena* (which is very often named also *tigella*, after its baking system), and *taguella*, the typical flat bread prepared by the Touareg of Central Sahara [63]. The *tigella* terracotta plate derives its name from the Latin verb *tegere*, meaning "to cover", because the traditional way of cooking the *Crescentina di Modena* involved to place the dough on the red-hot *tigella*, to cover it with another *tigella*, and to stack several of them in the fireplace. Chestnut or walnut leaves were used to avoid the direct contact of the dough with terracotta, as well as to flavor it and keep it clean from the ash.

Tannur and *tabun*, instead, are vertical ovens [2]. The *tannur* consists of a truncated conical structure (Figure 8).

Figure 8. Dough discs pressed onto the inner walls of *tannur* for baking.

Archeological remains of these ovens are widespread in the Middle East, Central Asia, northern India, North Africa, and along the Mediterranean coasts [2]. The *tannur* is still used in the rural areas of the Middle East, particularly in Syria and Iraq, whereas it disappeared in Egypt, where it was used until the 19th century [64].

Embedded in the masonry, which acts as a workbench to prepare the dough and lay the bread loaves for cooling, the *tannur* is placed in a slightly inclined position to facilitate the introduction of food to be baked, including bread, through the circular opening at the top, or "mouth" [2]. The dough discs are rapidly pressed onto the inner walls of *tannur* with the aid of a "bread cushion" or directly by hand. The adhesion to the vertical walls can be helped by wetting the surface of the dough discs just before slapping them into the oven [2]. After about 1–2 min of cooking, the bread is taken out by metal tongs. The vertical ovens, such as all wood-burning ovens, reach very high temperatures, exceeding 300 °C.

The *tabun*, instead, has an upper opening as the *tannur*, but has an "igloo" shape, wider than tall [2] (Figure 9). This kind of oven, of Palestinian origin, is present in Jordan also due to large presence in this country of Palestinian refugees [46,65–67]. It is used in a different way than *tannur* because, instead of slapping onto the inner walls, the loaves are placed on the oven floor, next to the embers, usually on a layer of hot pebbles [2]. During baking, the top opening of the oven is closed with a metal lid.

Figure 9. *Tabun* oven.

The wood-fired refractory stone oven, dome-shaped, was present in all the surveyed countries, but with a different "perception". In countries such as Italy, for instance, the presence of a wood-fired refractory stone oven ("*forno a legna*") in a *pizzeria* is perceived very positively and attracts customers.

On the contrary, the traditional "*furn fallahi*" (farmer oven) used to bake the *Bataw* bread in the rural areas of Egypt is perceived as obsolete and has been almost abandoned (although it will likely make a comeback due to the sharp rise in the price of gas following the current Ukraine crisis).

3.6. Bread Characteristics

Regarding size and shape (Table 8), 50 breads had a diameter between 10 and 40 cm, while seven breads were larger than 40 cm: the Italian *Borlengo* and *Testarolo*, the Croatian *Poljički soparnik*, the Jordan *Saaj* (or *Shrak*) and *Mashrouh*, the Lebanese *Saj* (or *Markouk*) and *Khobz*. Only one bread was smaller than 10 cm, namely the Italian *Crescentina di Modena*. Not all flat breads are circular: 34.2% of them were oval or rectangular.

Table 8. Shape characteristics of flat breads.

Country	Circular (Diameter, cm)								Other Shapes [a]	
	<10		10–40		>40		Not Specified			
	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	-	-	4	2.8	1	0.7	-	-	9	6.3
Egypt	-	-	5	3.5	-	-	3	2.1	-	-
France	-	-	-	-	-	-	-	-	3	2.1
Greece	-	-	18	12.6	-	-	-	-	5	3.5
Italy	1	0.7	17	11.9	2	1.4	28	19.6	27	18.9
Jordan	-	-	3	2.1	2	1.4	1	0.7	-	-
Lebanon	-	-	5	3.5	2	1.4	-	-	-	-
Malta	-	-	2	1.4	-	-	-	-	-	-
Spain	-	-	3	2.1	-	-	-	-	2	1.4
Total	1	0.7	57	39.9	7	4.9	32	22.4	46	32.2

[a] Rectangular or oval.

An important quality characteristic of flat breads was golden color (45.4%). Moreover, texture was relevant, which should be crunchy (12.2%) for the hard flat bread types and soft (14.1%) for those pliable and rollable (Table 9).

Table 9. Principal quality characteristics of flat breads. Multiple answers were admitted, because breads could show more quality characteristics at the same time.

Country	Quality Characteristics							
	Golden Color		Crunchy Texture		Soft Texture and/or Pliability		Not Specified	
	Number	%	Number	%	Number	%	Number	%
Croatia	13	8.6	2	1.2	1	0.6	-	-
Egypt	5	2.9	-	-	-	-	3	1.8
France	2	1.3	-	-	-	-	1	0.6
Greece	13	8.6	7	4.1	11	6.5	-	-
Italy	21	15.4	8	5.2	8	4.7	41	24.1
Jordan	5	2.9	-	-	-	-	1	0.6
Lebanon	6	3.6	1	0.6	3	1.8	-	-
Malta	1	0.7	2	1.2	1	0.6	-	-
Spain	2	1.3	-	-	-	-	2	1.2
Total	68	45.4	30	12.2	24	14.1	48	28.24

In the marketing classification, bread is included in the group of products of frequently purchased products, characterized by short shelf life and subject to high risk of waste [15,68,69]. The shelf life of flat breads ranged from shorter than 3 days (58%), between 3 and 7 days (27.3%) and up to one year (9.1%) in case of hard, dry flat breads (Table 10). Hard breads were obtained by means of a two-step baking: the first to cook and the second to dry them. Traditionally, this procedure was typical of breads to be carried during the transhumance of sheep [2]. These flat breads were the Croatian *Mlinci* (meaning "Mills") and *Zagorski mlinci*, the Egyptian *Bataw* and *Merahrah*, the Greek *Fylla Perek*, the Italian *Pane Carasau*, *Schuttelbrot*, *Guttiau*, *Pistoccu*, *Zichi* and *Puccia ladina*, the Lebanese *Mullat al smeed* and the Spanish *Torta Cenceña* (or *Torta de Gazpacho Manchego*). The latter only shares its name with the cold vegetable soup named *gazpacho* consumed during the summer mainly in the Andalusia region. *Gazpacho Manchego*, indeed, is a game meat stew from the Castilla La Mancha region, traditionally eaten with unleavened bread cakes. Its most peculiar ingredient is the unleavened bread cake (*Torta Cenceña*) which, originally, was the plate for *Gazpacho Manchego*.

Table 10. Shelf life of flat breads.

Country	Shelf Life							
	<3 Days		3–7 Days		Up to 1 Year [a]		Not Specified	
	Number	%	Number	%	Number	%	Number	%
Croatia	3	2.1	1	0.7	2	1.4	8	5.6
Egypt	1	0.7	5	3.5	2	1.4	-	-
France	2	1.4	1	0.7	-	-	-	-
Greece	19	13.3	3	2.1	1	0.7	-	-
Italy	48	34.6	21	14.0	6	3.7	-	-
Jordan	5	3.5	1	-	-	-	-	-
Lebanon	3	2.1	3	2.1	1	0.7	-	-
Malta	2	1.4	-	-	-	-	-	-
Spain	-	-	4	2.8	1	0.7	-	-
Total	83	58.0	39	27.3	13	9.1	8	5.6

[a] Dry breads.

In 5.6% of cases the shelf life was not specified because the product was prepared at a very small scale level and marketed unpackaged.

Studies showed that modified atmosphere packaging (40% carbon dioxide and 60% nitrogen), coupled with an oxygen absorbent sachet, prolonged the shelf life of *pita* bread up to 28 days [70]. Alternatively, sodium propionate (0.3%) can be added [71]. Innovative solutions for extending the shelf life of bread are under study, such as ethanol and/or essential oil emitters, antimicrobial films, nanopackaging, biodegradable and renewable packaging, and edible coatings [72].

3.7. Artisanal vs. Industrial Breads

Typically, the surveyed flat breads showed an artisanal character (82%) (Figure 10). Those produced at industrial level accounted for 7%, and were: *Piadina* (Italy), *Kmaj* (Jordan), *Mlinci* and *Kukuruzna miješana ciabatta* (Croatia), *Souvlakopita* (*Pita* bread) (Greece), *Baladi* and *Shami* bread (Egypt), *Khobz* (Arabic bread) (Lebanon). Another 11% was produced either way.

The Egyptian *Bataw*, which was the most traditional farmer bread in Egypt, can be considered a case study. It was not standardized, being made in a different way in different places. Farmers could prepare *Bataw* bread with wheat, corn, or mixture of these flours (the most common option), with or without fenugreek. Furthermore, this kind of bread was produced either in soft form or, to prolong shelf life, in hard, dry form.

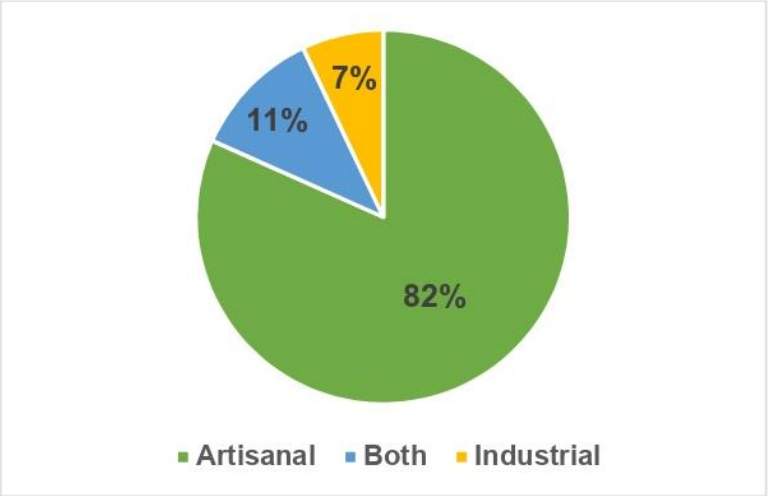

Figure 10. Percentage of flat breads produced in artisanal or industrial mode.

These findings demonstrate that the formulation of *Bataw* bread, a traditional product made mostly on a family basis, depends on the local availability of raw materials and personal preference of the family. However, this bread is currently not as widely consumed as in the past, mostly due to the subsidization of another bread, namely *Baladi* bread. It should be considered that in Egypt, bread consumption is one of the highest in the world, so bread subsidies have a strong influence on consumer choice [34]. Likely, changes in lifestyle and increasing urbanization may further enhance the abandonment of *Bataw* breadmaking. Therefore, there is a concrete risk of losing the memory and knowledge behind this kind of bread. This happens continuously to many food products, everywhere, but since bread is much more than simply a nutritious food, being linked to identity and local knowledge [3], abandoning a certain type of artisanal bread, in favor of a more industrialized one, is a phenomenon with important cultural repercussions.

Besides the *Bataw*, other breads which were found to be made on a family basis were the Egyptian *Zallut*, *Khobz min el dorra al rafi'ah, Farasheeh,* and *Merahrah*; the Jordan *Arbood*; the Lebanese *Mishtah el jreesh* and *Mullat al smeed*; the Italian *Carchiola*, *Puccia alla spasa* and *Pitt'ajima*; the Greek *Lambropsomo*, *Christopsomo*, *Spargana tou Christou* and *Vasilopita trifti* (whose cultural importance and ethnographical aspects are highlighted in the next paragraph).

3.8. Specific Consumption Patterns

The analysis of data also provided a cultural view of flat breads within each country. Preferences for food, including bread, are influenced by culture, family habits, traditions, religious believes, and income [68]. One of the characteristics of contemporary consumer behavior and habits is that people often purchase products (including food products) not because they are "used for something", but because they symbolize something, as described by Solomon et al. [73] and Jones [74]. The role a product plays in people's lives, indeed, extends beyond the practical functions it fulfils. Bread is a "soul food" and is the symbolic food *par excellence*, to be treated with the respect due to what it represents.

A strong link has been observed between the consumption of a specific flat bread type and certain periods of the year having a religious meaning, highlighting the iconic nature of cooking and eating [74]. The Greek *Lambropsomo* (Easter Bread) and *Lagana* are related to Easter [16]. The *Lambropsomo* is prepared by intertwining three long cylinders of dough, symbolizing the Holy Trinity, and on the surface of the dough four hard-boiled red-colored eggs are placed, which remind the blood of Jesus Christ. The *Lagana* is specifically consumed on Clean Monday (*Kathari Deutera*), i.e., on the first day of Lent of the Eastern Christianity, when sinful attitudes and non-fasting foods are left behind. That day is also named "Ash Monday", by analogy with Ash Wednesday (the day when the Western

Churches begin Lent). In Greece, the *Christopsomo* is the typical Christmas Bread, eaten on Christmas' eve. The surface of this bread is decorated with a cross of dough and walnuts in shell [16]. The latter symbolizes rebirth and are believed to bring prosperity to the family. Moreover, the Greek *Spargana tou Christou* is related to Christmas. The name of these very thin pancakes, prepared during Christmas lent, literally means "The swaddling clothes of Jesus" [16]. The *Vasilopita trifti* or "New year's bread", instead, is a traditional Greek bread served at midnight on New Year's Eve to celebrate the life of Saint Basil of Caesarea (*Agios Vasilis*), who is Santa Claus according to Greek Christmas traditions [16]. After baking, a coin is inserted through the base of the bread, and whoever finds it is said to be granted luck for the rest of the year.

In Croatia, *Zagorski mlinci* are traditionally eaten with turkey at Christmas and New Year. Moreover, *Poljički soparnik* is known as the "poor man's dish" because it was always prepared on fasting days (All Saints' day or Good Friday) and holidays such as Christmas.

The Lebanese *Mishtah el jreesh*, now become a rare bread prepared only at home, is particularly associated with the Muslim month of Ramadan, for breaking the fast, while the *Mullat al smeed*, another rare Lebanese bread, was traditionally brought during the *Hajj* (pilgrimage to Mecca) because of its long shelf life (being dried) but, as travel times became shorter, *Mullat al smeed* began to disappear [75]. The meat variant of *Manouche*, instead, called *Lahem b aajin* (or "meat in dough"), is a "go to" meal for funerals and condolences in certain regions of Lebanon.

In Italy, the Sardinian double-layered flat bread *Spianata* was traditionally prepared in a decorated version for weddings, by using a special stamp named *pintadera*. The Italian *Borlengo* was typically consumed at Carnival, so its name is related to the Italian word "*burla*", meaning "joke" [16]. The *Pizza a scannatur di Carbone*, instead, was prepared on the day of the pig slaughter which, in the past, represented an important day, to be celebrated as a collective rite ending with a common lunch for those who helped in the slaughter [16].

3.9. Promoting the Tradition

The application of quality schemes to flat breads deserves a specific discussion. As a way for promoting the most traditional and artisanal foods, several quality marks have been set up. This action is aimed at keeping their knowledge alive, reducing the erosion of food diversity, which is a modern problem induced by the globalization of food products.

The EU Regulation No 1151/2012 [20] provides the legal framework on quality schemes to protect the name of food and beverages having unique characteristics linked to their geographical origin as well as traditional know-how [20]. These quality schemes include the PDO, granted to products whose production, processing, and preparation are entirely made within a particular geographical area; the Protected Geographical Indication (PGI), awarded to products for which at least one of the stages of production, processing or preparation takes place within a particular geographical area; the TSG, for products whose quality is not linked to a specific geographical area but is based on traditional processing methods or recipes. These quality marks are all recognizable by the presence of specific logos in the label of food and bring benefits to producers, who keep the exclusive right to use the protected name and usually get a premium price. At the same time, the consumers have a proof of heritage and tradition of food specialties they buy.

Many of the surveyed flat breads have been awarded of quality marks (Table 11). Five were PGI (namely the Italian *Piadina Romagnola*, *Focaccia di Recco* and *Schuttelbrot dell'Alto Adige*, as well as the Croatian *Poljički soparnik*' and *Zagorski mlinci*) and one was TSG (the *Pizza Napoletana*). Furthermore, being the "art" of the Neapolitan pizza-makers (*pizzaiuoli napoletani*) globally renowned, it has been inscribed on the Representative List of the Intangible Cultural Heritage of Humanity by the United Nations Educational, Scientific and Cultural Organization (UNESCO). Similarly, "the culinary art and culture of flattened sourdough flat bread *Ftira"*, was added by the UNESCO to the same list, being a key part of the cultural heritage of the inhabitants of the Maltese archipelago.

Table 11. Flat breads awarded by quality marks. PGI = Protected Geographical Indication; TSG = Traditional Specialty Guaranteed; PAT = *Prodotti agroalimentari tradizionali* (Traditional Agri-food Products); DeCO = *Denominazione Comunale di Origine* (Municipal Designation of Origin).

Quality Mark		Releasing Organism	Geographic Validity	Number of Breads	Bread Names and Country
Name	**Acronym**				
Protected Geographical Indication	PGI	European Commission	EU	5	*Piadina Romagnola* (Italy); *Schuttelbrot* (Italy); *Focaccia di Recco* (Italy); *Poljički soparnik'* (Croatia); *Zagorski mlinci* (Croatia)
Guaranteed Traditional Specialty	TSG	European Commission	EU	1	*Pizza Napoletana* (Italy)
Intangible cultural heritage of humanity	-	UNESCO	Global	2	The culinary art and culture of flattened sourdough flat bread *Ftira* (Malta); The Art of Neapolitan Pizza-makers (Italy)
Slow Food presidium	-	Slow Food Foundation for Biodiversity	Global	2	*Testarolo Pontremolese* (Italy); *Mungia Talo* (Spain)
Intangible cultural goods	-	Ministry of Culture, of the Republic of Croatia	Croatia	1	*Pogača z oreji* (Croatia)
Croatian Quality	-	Croatian Economy Chamber	Croatia	1	*Pogača Pogacha* (Croatia)
Municipal Designation of Origin	DeCO	Italian Municipalities	Italy	2	*Crostolo di Urbania* (Italy); *Farinata di Imperia* (Italy)
Traditional Agri-food Products	PAT	Italian Ministry of Agriculture, Food and Forestry	Italy	63	See detailed list in Table 12

Table 12. Italian flat breads awarded by the Italian quality mark "*Prodotti Agroalimentari Tradizionali*" (PAT, meaning "Agri-food Traditional Products"), geographically subdivided based on their origin in northern, central, southern Italy or its islands.

Region	Number	Bread Names
Northern Italy		
Aosta Valley	-	-
Piedmont	2	*Farinata, Focaccia Novese*

Table 12. *Cont.*

Region	Number	Bread Names
Trentino-Alto Adige	-	-
Friuli-Venezia Giulia	-	-
Veneto	-	-
Lombardy	1	*Schiacciatina*
Emilia-Romagna	8	*Crostolo, Borlengo di Guiglia, Crescentina, Gnocco fritto, Crescenta fritta, Focaccia con ciccioli, Erbazzone, Crescenta*
Liguria	3	*Testarolo della Lunigiana, Farinata, Focaccia*
Total	14	-
Central Italy		
Tuscany	6	*Testaroli, Panigaccio, Neccio, Farinata, Schiaccia grossetana, Focaccia con i friccioli*
Umbria	2	*Torta al Testo, Schiacciata al formaggio*
Marches	5	*Crostolo, Cresciolina, Crescia sotto la cenere, Crescia d'la stacciola, Crescia brusca*
Abruzzo	2	*Pizza Scime (or Pizza scive, Pizza ascima, Pizza azzima), Pizza con le sfrigole*
Lazio	7	*Pizza bianca romana, Pizza con farina di mais, Pizza somma, Pizza rossa, Pizza fritta, Pizza sotto la brace, Pizza a fiamma*
Molise	3	*Pizza coi cicoli di maiale, Pizza di granone, Pizza scimia*
Total	25	-
Southern Italy		
Apulia	10	*Puccia salentina, Focaccia barese, Focaccia di S. Giuseppe, Calzone di Ischitella, Focaccia a libro di Sammichele di Bari, Paposcia, Pitilla, Pizza sfoglia, Scannatedda, Sceblasti*
Basilicata	4	*Carchiola, Pizza a scannatur di Carbone, Scarcedda, Pizza con i cingoli di maiale*
Calabria	1	*Pizza di maggio*
Campania	3	*Pizza, Pizza di farinella bacolese, Pizza di scarola*
Total	18	-
Islands		
Sardinia	6	*Carasau bread, Guttiau, Spianata, Pistoccu, Zichi, Focaccia Portoscusese*
Sicily	1	*Sfincione*
Total	7	-

According to the Slow Food Foundation for Biodiversity, Slow Food Presidia should be good tasting, sustainably produced, should have a local and social dimension, and represent a sense of place. This recognition requires that producers join a project to safeguard biodiversity and form a community. Two flat breads of this survey were Slow Food presidia, i.e., the Italian *Testarolo Pontremolese* and the Spanish *Talo*.

In Croatia, the quality schemes "Intangible cultural goods" (awarded by the Ministry of Culture, of the Republic of Croatia), and "Croatian quality" (by the Croatian Chamber of Commerce) applied to *Pogača z oreji* and *Pogača*, respectively.

The "*Denominazione Comunale di Origine*" (DeCO, meaning "Municipal Designation of Origin"), instead, is granted by the Italian Municipalities to recognize, promote, and protect high quality artisanal agri-food products indigenous to their municipal territory. Two Italian breads were DeCO, while the remarkable number of 63 were *Prodotti Agroalimentari Tradizionali* (PAT, meaning "Traditional Agri-food Products"), which is another (more prestigious) Italian recognition awarded by the Italian Ministry of Agriculture to foods prepared according to traditional processing systems, homogeneous in the geographic area and consolidated through a period of time not inferior to 25 years (Table 12).

A list of PAT is released yearly, with new entries and eventually deletions for products which have been upgraded to PDO, PGI, or TSG [76]. PAT flat breads were homogeneously distributed throughout the country.

Rare breads, instead, prepared only by the household cooks for family consumption and not for sale, were surveyed by the Slow Food Foundation for Biodiversity within the "Ark of Taste" project. These breads were the Egyptian *Zallut*, *Khobz min el dorra al rafi'ah, Farasheeh,* and *Merahrah* [77], the Lebanese *Mishtah el jreesh* and *Mullat al smeed* [75], the Jordan *Arbood* [78] and the Italian breads reported in Table 13. The Ark of Taste project is aimed at keeping alive small-scale quality productions tightly linked to local culture, history and tradition [79,80].

Table 13. Breads surveyed by Slow Food Foundation for Biodiversity as "Ark of Taste".

Country	Number	Bread Names
Egypt	5	*Khobz min el dorra al rafi'ah, Farasheeh, Zallut, Merahrah, Shamsi*
Italy	7	*Carchiola, Pizza scime (or Pizza scive, Pizza ascima, Pizza azzima), Pitt'ajima, Pizza a fiamma, Puccia alla spasa, Puccia ladina, Focaccia a libro di Sammichele*
Jordan	1	*Arbood*
Lebanon	2	*Mishtah, Mullat al smeed*
Total	15	-

It is indeed fundamental to draw attention to these local rare foods which are at risk of extinction, to prevent the reduction of food and cultural biodiversity. The Egyptian *Shamsi* bread, for example, which was another rare bread, has recently increased its popularity and has now gained certain market, as proved by the large number of webpages released by searching its name in Google. A possible means for keeping alive these food products is also to show their production to the tourists, who are attracted by local traditions, such as for the Jordan *Arbood* bread. This bread is typically baked for tourists enjoying the Wadi Rum tours. In this way, the promotion of traditional foods can generate an income to the local communities [81].

Another means to enhance the knowledge of rare flat breads is represented by the food blogs which attract the interest of many people prompt by the will of learning new recipes and rediscovering old ones. These tools, as well as YouTube videos, or even Wikipedia, easily carry the information also far from the area of origin. For example, the Italian breads inserted in the Ark of Taste (namely *Carchiola, Pizza scime—or Pizza scive, Pizza ascima, Pizza*

azzima—Pitt'ajima, Pizza a fiamma, Puccia alla spasa and *Puccia ladina*) are all the object of several blogs and are mentioned in Wikipedia pages, with *Carchiola* having its own entry.

In Lebanon, instead, the popular garnished flat bread *Manouche* is the object of the "World *Manoucheh* Day", on November 2nd, though this bread has not an official quality label yet.

4. Conclusions

This survey provides an overview of the different recipe, process, and product quality specifications of flat breads produced in the examined countries, as well as an insight on the related local culture. It appears that flat breads are really a large and diverse family of food products, with a rich history and ethnographical dimension.

Their main technical characteristics could be summarized as follows. Flours were from soft wheat (67.4%), durum wheat (13.7%), corn (8.6%), rye, sorghum, chickpea, and chestnut (together 5.2%). All garnished flat breads contained vegetable oils or lard, while 54% of plain flat breads did not contain fats. Leavening agents were: compressed yeast (55.8%), sourdough (16.7%), or baking powder (9.0%); 18.6% flat breads were unleavened. The baking temperatures were <250 °C (50.3%), between 250 and 300 °C (7%), and >300 °C (28.7%). Sixteen old-style baking systems were recorded, classified into baking plates and vertical ovens (*tannur* and *tabun*). Artisanal flat breads accounted for 82%, while the industrial for 7%. The diameter of breads was between 10 and 40 cm (39.9%), <10 cm (1%), >40 cm (4.9%). Not all flat breads were circular: 34.2% of them were oval or rectangular. The shelf life ranged from shorter than 3 days (58%), between 3 and 7 days (27.3%) and, in case of hard, dry flat breads, up to one year (9.1%). The main quality characteristics were golden color (45.4%), crunchiness (12.2%), and softness (14.1%) for hard and rollable flat breads, respectively. Quality schemes (national, European or global) applied to 91 flat breads.

In addition to the technical aspects, a clear social, ethnographical, and cultural dimension was identified. Twelve flat breads were strongly associated to religious celebrations, and 15 flat breads were rare, prepared only by the household cooks in a rural environment, for family consumption.

The collected information, gathered in a publicly available database, will be fundamental for allowing further valorization and dissemination activities, and will be useful for the selection, within each one of the examined country, of the most suitable flat breads for nutritional fortification and technical innovation within the FlatBreadMine or any other research project.

Criteria for selecting breads can be proposed, as follows:

(1) The type of bread chosen must be native to the country and widely consumed throughout the national territory. To maximize the nutritional impact on the general population, it would be of little usefulness to fortify a flat bread produced on a small-scale, consumed only in a restricted geographical area.

(2) If different categories of flat bread are available in the considered country, all equally diffused, more than one bread should be selected, to represent the single-layered, double-layered, and garnished or fried products.

5. Future Perspectives

Traditional and rare flat breads, prepared in a genuine and not globalized way using local raw materials, in addition to not risking the shortage of imported flours for geopolitical or climatic reasons, are well adapted to the territory and sustainable. However, these breads may progressively disappear due to changes in lifestyle and increasing urbanization, and their loss would lead to genetic erosion. Actions are therefore needed to prevent the reduction of cultural and biological diversity related to their disappearance. The strategy for such preventing actions, and directions for future research, should involve: (1) Periodically surveying the existing flat bread diversity, with the same approach used in this article. It is worth noting that the FlatBreadMine database can be easily updated, as well as extended to include many other countries; (2) applying quality schemes to the most genuine and

high quality products; (3) disseminating information and raising awareness by promoting the products both remotely (food blogs, Youtube video) and in person, also among tourists (because food has to be tasted, to see it online is not enough).

Traditional flat breads produced on a small-scale and those, more industrialized, on a large-scale, have totally different features and should follow distinct paths, with the first as a specialty dedicated to a niche consumer, carrying a strong cultural message, and the latter for mass consumption, able to guarantee a real impact on the population if nutritionally improved.

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Chapter 2

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Article

Sourdough Fermentation of Oat and Barley Flour with Bran and Its Application in Flatbread Made with No-Time and Dough Retardation Methods

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Abstract: Dough retardation is commonly used to extend dough shelf-life, but it poses a challenge for flatbreads due to their large surface. This study explored the sourdough fermentation of oats and barley, addressing challenges in the retardation of dough for flatbread. Sourdough, using flour only or flour blended with bran (3:1), was fermented with a LIVENDO LV1 starter at 30 °C for 24 h. The pH value, microbial viable cell count, total titratable acidity and organic acids concentration of the sourdough were measured. The properties of dough and flatbread, depending on the retardation time (24 h and 48 h), sourdough type (oat or barley) and sourdough level (30% or 50% dough weight), were investigated. Oat flour's limited acidification improved with the inclusion of bran, resulting in a desirable pH, TTA, and lactic to acetic acid ratio after 15 h of fermentation, which were comparable to results achieved with barley sourdough. The sourdough addition slowed down the enzymatic browning of dough during retardation. Dough retardation at 24 h reduced the phytates content (32–38%) and crumb hardness (9–16%), depending on the sourdough type and level. In dough retardation, β-glucans were degraded by up to 9% in the case of oats and by up to 28% in the samples with barley. Overall, adding oat or barley sourdough at a 30% dough weight can be recommended to enhance flatbread's nutritional value and prolong its shelf life.

Keywords: oat; barley; retarding; sourdough flatbread; phytates; β-glucans

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1. Introduction

Bread is the most consumed food in the world and is therefore an important product for nutritional improvement [1]. The oldest bread in the world is flatbread, a traditional staple food from the Mediterranean region [2]. The production of flatbread, having its roots in traditional methods, has now moved to mechanical industrial processes. Flatbreads are categorized as a single- and double-layered, whereby single-layered flatbreads can be leavened or unleavened [3]. In the Mediterranean region, the most common are single-layered flatbreads (77%) made of refined wheat flour leavened with baker's yeast [2] that can be additionally garnished. Flatbread production includes steps such as kneading, optional dough proofing, shaping, and baking [4]. Flatbread products are characterized by a low dietary fiber (DF) content, due to the fact that refined wheat flour is the main ingredient [5]. Oat, barley, millet, rye, sorghum, or legume flour have been used as enriching ingredients to increase the protein, mineral, and DF content of wheat-based flatbreads [6–11]. By using different enriching ingredients and breadmaking processes, flatbreads with increased nutritional value and tailored to specific diets can be produced, while preserving their overall quality [6].

Alternative cereals, such as oats and barley, possess several valuable properties that meet the requirements for functional diet components. Oat and barley flour, but also bran as a by-product of flour milling, are a good source of DF, especially soluble ones such

as mixed-linkage (1 → 3), (1 → 4)-β-D-glucans. The consumption of β-glucans has been associated with a reduction in diabetes symptoms, the ability to lower plasma cholesterol, the improvement of lipid metabolism and a reduction in colon cancer risk [12,13]. Although barley flour was traditionally used in breadmaking, its usage declined over time. Several studies addressed the challenge of using barley or oat flour in making flatbread. Wheat flour was supplemented with barley flour at levels of 15, 30, 45 and 60% (flour weight) in the development of Balzama flatbreads with a high β-glucan content and a relatively low glycemic index [14]. The replacement of 50% wheat flour with barley flour also significantly lowered the glycemic index of roti flatbread [15]. To improve the quality and nutritional value of barbari bread, barley flour was used together with wholewheat flour in a 1:1 ratio [16]. The use of oat flour at 25 and 50% to wheat flour was tested in chapatti flatbread, which increased the content of bioactive compounds [17]. The use of bran, as a by-product of the milling industry, could additionally play an important role in the environmental sustainability of flatbread production.

The fermentation process used influences the overall quality of the bread. In the modern bakery industry, sourdough is used not only as an alternative to baker's yeast, but also to reduce the need for additives, improve sensory properties, nutritional value, and prolong the shelf-life of bread [18,19]. Sourdough can be spontaneously fermented by naturally present microorganisms (type I) or with added lactic acid bacteria (LAB) and yeast (type II sourdough) [20]. The interaction between flour and inoculated LAB has the greatest influence on achieving the desired nutritional and technological properties of bread [21]. The sourdough fermentation of wholegrain flour or bran under suitable hydration conditions successfully increases the bioavailability of minerals, protein digestibility [18], and the solubilization of DF [22]. Wholegrain and bran contain a high amount of phytic acid (PA), an antinutrient which impairs mineral bioavailability by forming insoluble complexes with iron, magnesium, zinc, and calcium cations [18]. Prolonged fermentation enables the degradation of most of the PA, which is due to the microbial phytase activity, but also due to the endogenous cereal enzyme activated at lower pH values [16,23]. Pejcz et al. [13] fermented wholegrain barley flour with LV1 starter (0.5%), dough yield (DY) 200, at 30 °C for 18 h, aiming to enrich wheat bread with dietary fiber, mainly β-glucans. With the same aim, Rieder et al. [22] used sourdough obtained from barley flour and oat bran fermented with *L. plantarum* at 30 °C for 18 h. Whereas the acidification of barley flour was found to be mostly successful, it was inadequately characterized in the case of oat flour or bran. To our knowledge, the influence of bran addition on the acidification kinetics of oat and barley flour has not been investigated. Hence, this paper presents a comparative study of the sourdough fermentation of the two most important β-glucan sources among cereals, oats and barley.

A retarded dough method is generally used to improve the flavor of sourdough bread [24]. Retarded dough stored between 0 and 10 °C for up to 14–24 h results in better-tasting products with a pleasant, slightly sour flavor, a light chewy crumb, and a thick, brown crust [24]. Further on, the retardation method was introduced to extend the shelf-life of the dough and perform the baking on demand [7]. However, due to a large surface area, retarded flat doughs are more prone to enzymatic browning, which eventually affects the consumer's acceptance of the final product [25]. This browning is caused by the action of polyphenol oxidase (PPO) and peroxidase enzymes that are mainly concentrated in the bran fractions and catalyze the oxidation of free and reduced phenolic compounds to quinones, which interact and form brown pigments. Therefore, bran-containing dough is more susceptible to enzymatic browning [26].

Additives, such as ascorbic and citric acid, L-cysteine, and 4-hexylresorcinol, which is a competitive enzyme inhibitor, were tested as anti-browning agents, as well as heat and microwave treatment [25,27]. Discoloration of barley-based dough can be controlled by heat treatment, with oxygen exclusion, by lowering the phenolics content or PPO activity, and by using enzyme inhibitors [25,27]. A combination of chemicals and microwaves can slow down changes in the lightness of barley dough during 96 h of retardation for

multigrain Indian flatbread [25,27]. However, on the market, there is a strong trend of breads enriched with bioactive compounds and clean label breads without additives. The use of sourdough as a natural improver in a retarded dough and its potential to inhibit enzymatic browning in the making of healthier flatbreads has not yet been well addressed.

Thus, the aim of this study was to investigate the acidification kinetics of the sourdough fermentation of oat and barley flour with or without adding bran using a commercial starter. We hypothesized that the addition of bran will improve the acidification kinetics of oat and barley sourdough and that the addition of sourdough will slow down the browning of the dough during retardation and additionally reduce the phytates content. Hence, the influence of sourdough type II on the nutritive value and quality of single-layered flatbread made using the no-time method, and retarded dough method (24 and 48 h) was investigated at two addition levels (30 and 50% dough weight).

2. Materials and Methods

2.1. Ingredients

The semi-refined wheat flour (Čakovečki mlinovi Inc., Čakovec, Croatia) contained 11.0% protein, 11.9% moisture, and 2.3% fat. Its amylolytic activity was 1640 Brabender units (BU). The oat flour (Granolio Inc., Zagreb, Croatia) contained 12.4% protein, 11.5% moisture, and 7.9% fat. The barley flour (Ivan Varga family-run farm, Orehovica, Croatia) contained 11.2% protein, 12.4% moisture, and 1.8% fat. The oat bran (Eko-Jazo Ltd., Ivanovac, Croatia) and barley bran (Ivan Varga family-run farm, Orehovica, Croatia) contained 19.9 and 11.0% protein, 11.4 and 12.2% moisture, and 4.7 and 1.3% fat, respectively, as reported previously by Grgić et al. [28].

The particle geometric mean diameter was determined according to the ICC standard 207 by sieving at the mesh apertures of 670, 355, 282, 225, 180 and 125 µm [28], and was 231 µm for oat flour, 295 µm for barley flour, 531 µm for oat bran and 514 µm for barley bran.

A commercial freeze-dried starter LIVENDO LV1® (Lesaffre, France) was used to prepare the sourdough.

2.2. Chemical Analyses of Flour/Bran and Flatbread

The dry weight (d.w.) content of the flour and bran was determined in duplicate according to AOAC method 925.10 [29], whereas for the flatbread, AACC method 44-15.02 [30] was followed.

The total dietary fiber was determined in duplicate according to AOAC method 2011.25 using the Total Dietary Fibre Assay Kit (Megazyme, Bray, Ireland).

The concentrations of minerals in the oat, barley flour, and bran were determined using atomic absorption spectrometry (AAS). Ashing of a well-homogenized sample (5 g) was performed in a muffle furnace (KR-170, Heraeus, Hanau, Germany) at 550 °C according to AOAC method 923.03 [29]. After cooling to room temperature, the ash was weighed. Then, it was dissolved during heating with 5 mL of 5 M nitric acid, and quantitatively transferred to a 25 mL volumetric flask with deionized water. The sample solutions were further diluted to ensure that the concentration of each analyte was within the linear range of the method. Lanthanum (III)-chloride (1%, *v*/*v*) was added to the diluted solutions, standards, and to blank samples to avoid interference of the phosphates with magnesium. The atomic absorption spectrometer (Perkin Elmer 2380; Norwalk, CT, USA) was set to an acetylene flow of 2.2 L/min and an air flow of 14.5 L/min. The calibration curves were prepared for each analyte using standard solutions (Supelco, Darmstadt, Germany) at five concentration levels and the measurement was performed in at least five consecutive replicates at the following wavelengths: 324.7 nm for Cu, 248.3 nm for Fe, 213.9 nm for Zn, and 285.2 nm for Mg.

The activity of α-amylase was determined spectrophotometrically following the α-amylase SD method (K-AMYLSD 04/19, Megazyme, Bray, Ireland) and the manufacturer's instructions.

Polyphenol oxidase (PPO) activity was assessed in accordance with the AACC 22-85.01 method [30] with a slight modification [31]. Sample (50 mg) was vortexed with a 10 mM solution of L-DOPA in a 50 mM MOPS buffer (1.5 mL) at 1.000 rpm for 15 min. After

centrifugation at 14.800 rpm for 5 min, the resulting supernatant was used for spectrophotometric measurement at 475 nm. The PPO activity was calculated as the difference in the absorbance of sample and blank and expressed as Δ475/g sample.

The β-glucan content in flatbread was determined according to AOAC Method 995.16 and AACC Method 32-23 [30], using the Mixed-linkage β-glucan Assay Kit (Megazyme, Bray, Ireland). Phytic acid content was determined spectrophotometrically at 655 nm using the Phytic Acid Assay Kit (K-PHYT 05/19) following the manufacturer's instructions (Megazyme, Bray, Ireland).

All spectrophotometric analyses were performed in duplicates using the spectrophotometer PerkinElmer Lambda 35 UV/Vis (Waltham, MA, USA).

2.3. Sourdough Fermentation and Characterization

Sourdough was prepared using flour only or a blend of flour and bran. The amount of the bran ratio added to flour for sourdough fermentation was determined in the preliminary experiment. In the bran-including fermentations, oat or barley flour was mixed with the bran in a ratio of 3:1. To obtain DY 300, 240 g of sterile tap water was added to 120 g of floury material. The starter LIVENDO LV1® was added at 0.5 g/100 g of the floury material, and the dough was mixed for 5 min. The sourdough was fermented in sealed jars in a thermostat (INB 500, Memmert, Schwabach, Germany) at 30 °C for 15–24 h.

Measurement of pH, Total Titratable Acidity and Viable Cell Counts in Sourdough

The pH value during sourdough fermentation was recorded every 5 min during 24 h of fermentation using a PH-230SD pH meter equipped with a data logger (Lutron Electronic Enterprise Co., Ltd., Taipei City, Taiwan).

The total titratable acidity (TTA) of the sourdough (10 g) was determined after suspending it in distilled water (90 mL) by titration with 0.1 M NaOH to a final pH of 8.5 [32]. The TTA is expressed as the average volume (mL) of NaOH consumed for two replicate titrations.

The organic acids were determined according to the method of Lefebvre et al. [33] with slight modifications. Ten grams of sourdough were suspended in distilled water on a magnetic stirrer for 30 min and diluted to 50 mL in a flask. After centrifugation (Rottina, Hettich, Kirchlengern, Germany) at 13.081 rpm for 5 min, the supernatant (5 mL) was transferred to a test tube. Carrez-I solution (1 mL) and Carrez-II solution (1 mL) were added, after which the sample was centrifuged at 4000 rpm for 5 min. A filtered supernatant was used for determination of the organic acids content in duplicate using the D-lactic acid and L-lactic acid Assay Kit (K-DLATE 08/18) and Acetic acid Assay Kit (K-ACETRM 04/20) (Megazyme, Bray, Ireland). A molar ratio between the lactic acid and acetic acid represents the fermentation quotient (FQ).

The number of viable cells in the sourdough at the end of fermentation was determined according to ISO 7954:2002 [34] for yeasts (*Saccharomyces* spp.) and ISO 15214:98 [35] for LAB (*Lactobacillus* spp.). Three decimal dilutions were analyzed in two replicates. The results are expressed as colony-forming units (CFU) per g of sourdough.

2.4. Modelling of Sourdough Fermentation Kinetics

Acidification data (difference in pH value) were modelled according to the Gompertz equation as modified by Zwietering et al. [36]:

$$y = k + A\exp\{-\exp(\mu_{max}e/A)(\lambda - t) + 1\} \quad (1)$$

where y is log (dpH dt^{-1}, units of pH min^{-1}); k is the initial level of the dependent variable; A (ΔpH) is the difference in pH (units) between the initial value and the value reached in the stationary phase of the sourdough fermentation; μ_{max} is the maximum acidification rate, λ is the length of the latency phase expressed in minutes, and t is the time.

2.5. Experimental Design

After establishing the more desirable type of milling product (flour and bran) for sourdough fermentation, the application of sourdough in flatbread was investigated. Based on the results of selected properties of flatbreads, dough retardation lasting 24 h was further compared with a no-time process. A two-level full factorial design of the experiment involved three independent variables, i.e., substrate type, sourdough level, and retardation time (Table 1). The measured responses were specific volume, baking loss, spread ratio, crumb hardness, cohesiveness, resilience, crust and crumb color, PA, and β-glucans content.

Table 1. Experimental plan and samples code used in the breadmaking.

Sample Code	Flour and Bran Type	Sourdough Level (g/100 g Dough)	Retardation Time (h)
O-30-0 h	oat	30	0
O-50-0 h	oat	50	0
O-30-24 h	oat	30	24
O-50-24 h	oat	50	24
B-30-0 h	barley	30	0
B-50-0 h	barley	50	0
B-30-24 h	barley	30	24
B-50-24 h	barley	50	24

2.6. Breadmaking

A single-layered Croatian type of flatbread ('Pogača') was made using a no-time and retarded dough method. Four different types of control breads without sourdough and matching four types of breads with sourdough were prepared. Three pieces of each type of bread were baked either after a no-time (0 h) process or dough retardation (24 or 48 h) process. The sourdough, fermented for 15 h from a blend of oat or barley flour and bran, was used in the breadmaking within 1 h of storage in a refrigerator.

In the controls, 18 or 30% (*w*/*w*) of oat or barley flour/bran blend replaced wheat flour, while in the sourbreads, 30 or 50% of oat or barley sourdough was added at dough weight, respectively, which corresponded to the substituted flour weight in the controls. The other ingredients were semi-refined wheat flour (69.6 and 81.8%, respectively), water in an amount adjusted to 200 BU (82–88.5%, total flour), instant baker's yeast (0.8%), oil (4%) and salt (2%). Water in the sourdough was taken into account so that the total amount of water in the controls and sourbreads was the same. The dough was mixed in a spiral mixer (Diosna SP12, Osnabrück, Germany). First flour was mixed with water for 2 min at 90 rpm, then yeast and salt were added, and fast mixing was continued for 5 min at 120 rpm. In the third minute, oil was added. After the bulk fermentation (28 °C, relative humidity 75%, 20 min), dough was divided into round balls (450 g) and placed in metal pans (20 cm diameter). Dough was subjected to retardation at a temperature 2 ± 2 °C, up to 48 h or to direct proofing at 28 °C, and relative humidity 75% for 1 h (Wiesheu, Affalterbach, Germany). Breads were baked in triplicates at 240 °C for 30 min with 0.21 mL cm^{-2} of steam in a deck oven (Wiesheu, Affalterbach, Germany). After cooling for 1.5 h at room temperature (20–22 °C) and 50–60% relative humidity, the breads were used for the subsequent measurements.

2.7. Evaluation of Dough and Bread Physical Properties

The pH of the bread dough was measured (in duplicates) at room temperature using the pH meter Testo 206 (Testo, Berlin, Germany).

Dough weight loss (in triplicates) was calculated by measuring the weight of the dough before and after retardation according to the Equation below (2):

$$\text{Dough weight loss} = \frac{m_1 - m_2}{m_1} \times 100 \quad (2)$$

where m_1 is the weight of dough before retardation and m_2 is the weight after retardation (tempered at room temperature).

The color of the dough during 0, 1, 2, 4, 6, 24 and 48 h of retardation was measured in triplicates, whereas the color of crumb and upper crust of bread baked after 0 and 24 h of retardation was measured at six points. All measurements were performed with a colorimeter (CM-700d, Konica Minolta, Osaka, Japan). In addition to the L^* value, which expresses the brightness or whiteness of a sample with 0 as black and 100 as pure white, redness (a^*) and yellowness (b^*) were measured. The total color difference (TCD) before and after dough retardation was calculated using Equation (3):

$$\text{TCD} = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (3)$$

Baking loss was calculated from the weight (in triplicates) of the dough before proofing and the bread after baking and 1 h of cooling at room temperature, according to Equation (4):

$$\text{Baking loss} = \frac{m_1 - m_2}{m_1} \times 100 \quad (4)$$

The volume of the bread was determined in triplicates according to AACC 10-05.01 method (AACC International, 2010) [30]. The specific volume was calculated as a ratio of volume to weight.

The width (at 2 points) and height (at 4 points) of the triplicate breads were measured with a caliper and the spread was calculated as their ratio.

The texture profile of the crumb was determined using a TA1 texture analyzer (Ametek Llyod Instruments Ltd., West Sussex, UK) with an aluminum probe of a diameter of 55 mm [37]. Immediately before the analysis, the crumb, with a thickness of 12.5 mm, was cut into 36 mm diameter pieces, and two pieces were stacked together. The double-compression test was performed in six replicates under the following conditions: probe speed before, during and after the test 2 mm/s, trigger force 5 N, strain 50%, and pause duration 30 s. The results processed using the program Nexygen PLUS 3 Software (Ametek Lloyd Instruments Ltd., West Sussex, UK) are expressed as hardness, cohesiveness, and the resilience of the crumb.

2.8. Statistical Analyses

To determine the influence of flour type, sourdough level, and retardation time on flatbread physical and nutritive properties, recorded data were subjected to factorial analysis of variance (ANOVA). The Tukey test for honest significant differences was used to assess differences between means. ANOVA, Tukey's post hoc test, Pearson's correlation test, and principal component analysis (PCA) were considered statistically significant when $p < 0.05$. Analyses were carried out with Statistica 14.1.0 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Enzymatic Activity and Bioactive Components of Flour and Bran

Wholegrain cereals provide protein and energy, DF, minerals, vitamins, and antioxidants that are important for human health [38], but also possess a high enzymatic activity. Barley flour was richer in DF and minerals (magnesium in particular) compared to oat and wheat flour (Table 2). The DF content of oat bran was 121% higher than that in the oat flour, whereas it was 44% higher in barley bran compared to its flour. Unlike the barley samples, the mineral content (especially magnesium and iron) of oat bran was significantly higher than in oat flour. This suggested that bran samples were suitable for the enrichment of bread with DF and minerals.

Among flours, semi-refined wheat flour showed the lowest concentration of phenolic compounds but also the lowest PPO activity (Table 2). The total phenolics of barley and oat flour were 66% or 200% lower than those previously reported for their bran, respectively, which was 0.84 mg FAE/g d.w. and 0.75 mg FAE/g d.w., respectively [28]. A similar difference (50% for barley and 150% for oat) between flour and bran was found in the PPO

activity. Habuš et al. [26] obtained comparable results for the PPO activity of oat flour (2.17 Δ475/g), while the PPO activity of their barley flour was 2.5-fold lower (1.167 Δ475/g). Our results indicated a high risk of enzymatic browning of dough containing barley or oat flour, which was even higher when using bran.

Table 2. Total dietary fiber, mineral content, TPC and enzymatic activity of oat and barley flour and bran compared with semi-refined wheat flour (mean ± standard deviation).

Parameter	Wheat Flour	Oat Flour	Oat Bran	Barley Flour	Barley Bran
Total dietary fiber (g/100 g d.w.)	4.90 ± 0.01 ^{d}	9.29 ± 0.01 ^{c}	20.51 ± 0.03 ^{a}	13.77 ± 0.31 ^{b}	19.77 ± 0.25 ^{a}
Total minerals as ash (g/100 g d.w.)	0.70 ± 0.01 ^{e}	1.83 ± 0.00 ^{d}	3.02 ± 0.00 ^{a}	2.33 ± 0.03 ^{c}	2.57 ± 0.03 ^{b}
Mg (mg/100 g d.w.)	45.9 ± 2.89 ^{c}	71.30 ± 1.70 ^{b}	120.57 ± 5.46 ^{a}	112.21 ± 2.57 ^{a}	73.58 ± 0.07 ^{b}
Fe (mg/100 g d.w.)	1.53 ± 0.08 ^{b}	1.99 ± 0.01 ^{b}	6.99 ± 1.26 ^{a}	2.86 ± 0.25 ^{b}	3.28 ± 0.02 ^{b}
Zn (mg/100 g d.w.)	1.25 ± 0.01 ^{b}	2.35 ± 0.05 ^{ab}	3.76 ± 0.81 ^{a}	2.50 ± 0.01 ^{ab}	2.83 ± 0.07 ^{ab}
Cu (mg/100 g d.w.)	0.27 ± 0.02 ^{b}	0.44 ± 0.01 ^{ab}	0.56 ± 0.08 ^{a}	0.51 ± 0.01 ^{a}	0.48 ± 0.02 ^{ab}
TPC (mg FAE/g d.w.)	0.11 ± 0.00 ^{c}	0.28 ± 0.01 ^{b}	*	0.45 ± 0.01 ^{a}	**
Polyphenol oxidase (Δ475/g)	2.30 ± 0.00 ^{d}	2.52 ± 0.01 ^{d}	6.30 ± 0.01 ^{a}	3.00 ± 0.00 ^{c}	4.50 ± 0.02 ^{b}

TPC—total phenolic content; d.w.—dry weight; * 0.84; ** 0.75 mg FAE/g d.w. according to [28]. a–e Values within the same row marked with different letters differ significantly according to Tukey's test ($p < 0.05$).

3.2. Fermentation Kinetics of Oat and Barley Sourdough

The pH changes during 24 h of sourdough fermentation were well fitted to the Gompertz model to show the difference in acidification kinetics of oat and barley flour depending on the bran addition (Figure 1, Table 3). Overall, barley flour had a higher acidification power than oat flour (Table 3). The addition of bran resulted in a 22–26% reduction in lag time and 13–14% reduction in the time required to reach the maximum acidification rate of barley and oat flour, respectively (Figure 1, Table 3). The bran addition positively affected an increase (46%) in the acidification rate of oats, while it remained unchanged for barley. Related, the addition of bran resulted in a 7% lower pH and 55% higher TTA in oats, while the pH and TTA of barley were only slightly changed with bran addition (5 or 10% respectively). This is probably because of the initially higher mineral content of barley flour [39]. Hence, the pH value and TTA at the end of 24 h fermentation were similar between the blend of oat flour and bran with barley sourdoughs. A similar pH value (3.81) but higher TTA (19.53 mL 0.1 M NaOH) at the end of 24 h fermentation of barley flour (DY = 148, 30 °C, multi-strain starter culture) was obtained by Mariotti et al. [39]. Huttner et al. [40] previously reported slightly higher pH (4.36–4.39) and TTA values (9.9–10.8 mL 0.1 M NaOH) after 24 h fermentation of wholegrain oat flour at 28 °C, depending on the starter cultures.

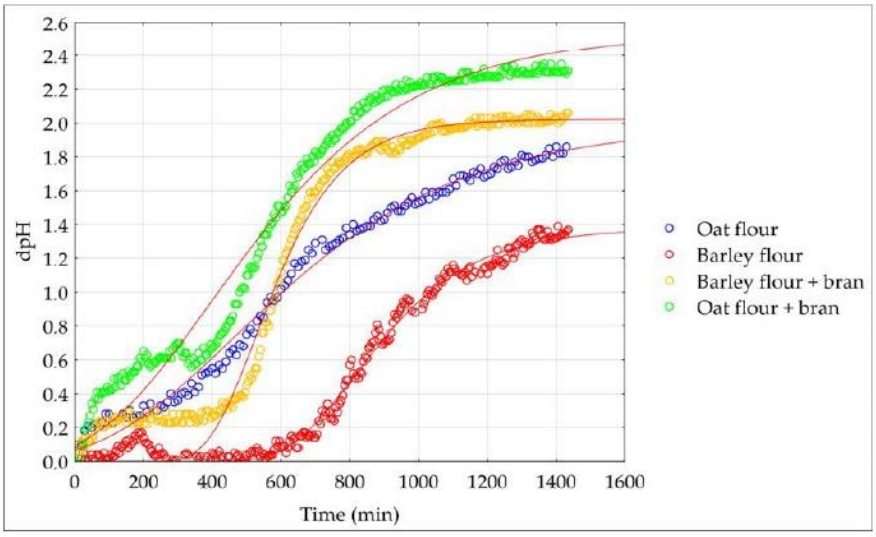

Figure 1. Acidification kinetics fitted to the Gompertz model for oat and barley flour and flour-bran blend. Fermented with starter LV1 at 30 °C for 24 h and DY = 300.

The optimum pH value of sourdough for breadmaking should be approx. 4, which requires a fermentation period of 6 to 24 h, depending on the temperature (25–35 °C) [41]. In our samples, after 15 h (900 min) of fermentation, a pH value in the range of 3.69 and 3.95 was reached (Figure 1, Table 3). This corresponded to a pH value of 1.43 and 1.53 for oat and barley flour, respectively, whereas the greater drop in pH (1.87 of oats and 2.17 in barley sample) was measured after adding bran (Figure 1).

Table 3. Total acidity (TTA, mL 0.1 M NaOH), pH value at the end of the 24 h fermentation, and the acidification kinetics according to the Gompertz model.

Substrate	pH	TTA (mL 0.1 M NaOH)	μ_{max} (h^{-1})	A (dpH)	λ (h)	T_i (h)	*p*-Value	R^2
Oat flour	3.95 ± 0.31 ^{a}	6.50 ± 0.42 ^{d}	0.116	1.99	1.42	7.7	<0.001	0.990
Barley flour	3.83 ± 0.22 ^{b}	9.33 ± 0.35 ^{b}	0.344	1.69	8.31	10.1	<0.001	0.978
Oat flour and bran	3.69 ± 0.18 ^{c}	9.95 ± 0.51 ^{a}	0.169	2.54	1.05	6.6	<0.001	0.970
Barley flour and bran	3.69 ± 0.23 ^{c}	8.35 ± 0.62 ^{c}	0.322	2.02	6.49	8.8	<0.001	0.973

μ_{max}—maximum acidification rate; A—difference in pH (units); λ—lag phase; T_i—time to reach μ_{max}. $^{a–d}$ Values within the same column marked with different letters differ significantly according to Tukey's test ($p < 0.05$).

The number of viable cells at the end of the 15 h fermentation was typical for the mature sourdough and similar between samples (Table 4). Huttner et al. [40] found a comparable LAB CFU after 24 h fermentation of wholegrain oat flour (7.7–8.9 × 10^8), while a small difference could be attributed to different amount of inoculum. In our study, the pH was lower and the TTA was higher for both oat and barley sourdough after adding bran (Table 4), regardless of the similar CFU.

Table 4. Colony-forming units (CFU), total acidity (TTA), pH value, organic acids content and fermentation quotient (FQ) after 15 h of sourdough fermentation of oat or barley flour with or without added bran.

Sourdough	LAB (CFU/g)	Yeast (CFU/g)	pH	TTA (mL 0.1 M NaOH)	Lactic Acid (mg/kg)	Acetic Acid (mg/kg)	FQ
Oat flour	1.85 × 10^9	2.00 × 10^7	4.45 ± 0.05 ^{a}	6.35 ± 0.41 ^{c}	474.88 ± 2.57 ^{b}	24.25 ± 0.65 ^{a}	13.05 ^{c}
Oat flour and bran	3.24 × 10^9	2.88 × 10^7	3.89 ± 0.18 ^{c}	7.53 ± 0.22 ^{a}	479.86 ± 2.47 ^{b}	25.19 ± 0.65 ^{a}	12.70 ^{d}
Barley flour	2.82 × 10^9	3.06 × 10^7	4.03 ± 0.06 ^{b}	5.86 ± 0.31 ^{d}	582.77 ± 0.41 ^{a}	12.54 ± 1.14 ^{b}	30.98 ^{a}
Barley flour and bran	2.26 × 10^9	3.81 × 10^7	3.85 ± 0.10 ^{d}	7.00 ± 0.11 ^{b}	481.50 ± 6.40 ^{b}	12.20 ± 2.28 ^{b}	26.31 ^{b}

$^{a–d}$ Values within the same column marked with different letters differ significantly according to Tukey's test ($p < 0.05$). LAB = lactic acid bacteria.

Both oat sourdough samples (flour only and flour and bran blend) had a 93–107% higher acetic acid concentration than barley sourdoughs (Table 4). On the other hand, sourdough from barley flour had the highest lactic acid concentration. The addition of bran reduced the amount of lactic acid in barley sourdough and the fermentation quotient of both types of sourdough. Therefore, the fermentation quotient of barley sourdough was 107–138% (2-fold) higher than that of oat sourdough. According to Arora et al. [18], the recommended fermentation quotient is below 5.0, but with large variations from 0.25 to 20.

Since the flour and bran blend had a higher acidification rate, a higher TTA and a lower pH value, it was selected for the breadmaking phase.

3.3. Changes in Color, pH, and Weight of the Dough during the Retardation Process

The color change of dough might affect the acceptability of the final baked product. The TCD can be classified as very distinct (TCD > 3), distinct (1.5 < TCD < 3) and as a small difference (TCD < 1.5) [42]. Figure 2a,b shows the lightness (L^*) parameter and TCD of the dough during retardation. As expected, doughs with a higher amount of barley or oat were darker. Moreover, barley doughs were darker than oat doughs which can be related to higher content of phenolics and higher PPO activity in barley flour

than in oat flour. Compared to the control without sourdough, the L^* values of doughs with sourdough were higher during the whole time of retardation. After 24 and 48 h, the L^* value was 17–21% and 15–23% higher in oat samples, while it was 11–20% and 15–27% higher in barley samples depending on the sourdough amount compared to the control dough (Figure 2a). All control doughs showed a very distinct (TCD > 3) color change already after 6 h of retardation (Figure 2b). In contrast, the TCD of samples with sourdough after 6 h was small ($\leq$1.5). Only after 24 h of retardation, the TCD of sourdough-containing doughs was distinct (between 1.5 and 3), while after 48 h it was very distinct, except for O-30-SD dough. Hence, during retardation for 24 or 48 h, TCD was reduced by 36 or 75%, respectively, in oat dough, and even more by 48 or 79%, respectively, in barley dough, with the use of sourdough. Similar dough-darkening was previously reported for multigrain Indian flatbread (*chapatti*) and barley-based dough due to the activity of PPO [25,27]. In the presence of oxygen, and phenolic substrates, PPO catalyzes melanin formation. PPO is a type of copper-containing protein that hydroxylates *p*-hydroxy monophenol to o-dihydroxy phenol (EC 1.14.18.1), and then dehydrogenates *o*-dihydroxy phenol to o-quinone (EC 1.10.3.1) [43]. The quinone products of PPO react with various components, such as amines, thiols, and phenols, and forms melanin, a colored metabolic end-product [44]. In general, the optimum pH of PPO ranges between 4 and 8, while wheat PPO has two pH optimums, 5.3 and 6.9 [45]. The addition of sourdough lowered the pH of the dough (Table 5) which may have slowed down the activity of PPO and dough-darkening. Banerji et al. [25] found that the most effective approach to prevent dough browning is a synergistic effect of chemical and microwave treatment, while the L^* values of the doughs with treated flours were stable and 16.5% higher after storage at 4 °C for 96 h [25]. Sourdough can therefore be used as a natural means of slowing down the darkening of the dough during short-term retardation to a comparable extent, as the chemical inhibitors were combined with the microwave treatment.

The pH of the control bread dough without sourdough was between 5.80 and 5.95, while it was 16–26% lower after adding sourdough (Table 5). When wheat, barley or wheat-barley sourdough was added at a concentration of 25 g/100 g, dough pH values of 4.23 to 4.63 were reported [39]. The slightly higher pH value of the bread dough found in this study, despite the higher sourdough content, could be due to the slightly higher pH value of the sourdough in this study than in the study by Mariotti et al. [30], which was in the range of 3.63–3.81. In our study, the pH value of the retarded oat dough was 5–7% lower than that of the no-time processed (non retarded) sample, while the difference in the barley dough was minimal, i.e., amounting to 1–2% (Table 5).

The dough weight loss during the retardation was lower than 1% (Table 5). In the first stages of retardation, the dough loses moisture from its surface as it tries to establish an equilibrium with the surrounding air [46]. It can be assumed that the elevated fiber content of our dough helped to maintain moisture and weight stability during refrigerated storage.

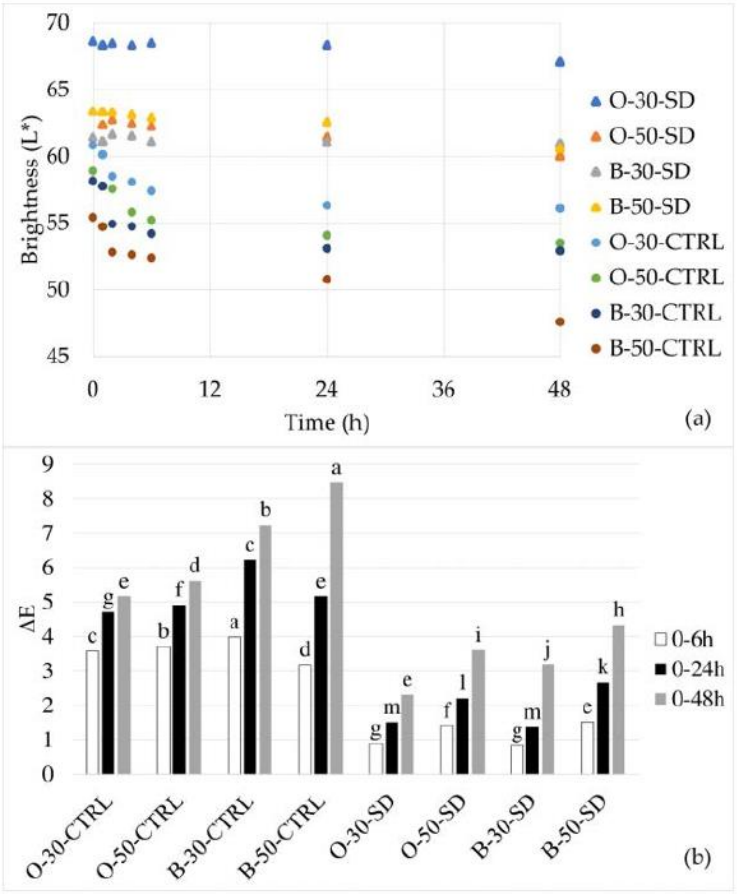

Figure 2. Color changes of dough with sourdough compared to the control without sourdough during 48 h of retardation: (**a**) brightness (*L**) of dough; and (**b**) total color difference (ΔE). CTRL—control sample without sourdough; SD—sample with sourdough. a–m Values marked with different letters differ significantly according to Tukey's test ($p < 0.05$).

Table 5. No-time (0 h) and retarded (24 h) dough properties depending on the oat (O) and barley (B) sourdough level (30 or 50% dough weight).

Parameter/Dough	O-30-0 h	O-30-24 h	O-50-0 h	O-50-24 h	B-30-0 h	B-30-24 h	B-50-0 h	B-50-24 h
pH of dough	4.72 ± 0.06 ^{ab}	4.40 ± 0.06 ^{b}	4.37 ± 0.06 ^{b}	4.14 ± 0.05 ^{b}	5.01 ± 0.16 ^{a}	4.97 ± 0.24 ^{a}	4.49 ± 0.03 ^{b}	4.42 ± 0.05 ^{b}
weight loss (%)	NA *	0.32 ± 0.06 ^{a}	NA	0.36 ± 0.02 ^{a}	NA	0.28 ± 0.04 ^{a}	NA	0.34 ± 0.18 ^{a}

^{a,b} Values within the same row marked with different letters differ significantly according to Tukey's test ($p < 0.05$). * NA—not applicable.

3.4. The Physical Properties of Flatbread

The properties of flatbreads made with the addition of sourdough fermented from oat/barley flour-bran blend using the no-time or retarded dough processes are shown in Table 6 and Figure 3. The physical properties of flatbreads were affected by the flour type, sourdough level, retardation time or their interactions (Table 7).

The increasing level of oat or barley blends in flatbreads resulted in a lower specific volume (Figure 3). An increasing amount of fiber, including β-glucans, which have a high-water binding capacity, limit the water for the formation of the gluten network and negatively affects bread volume [47]. In addition, the use of sourdough at the higher level reduced the bread volume, due to the acidic weakening of gluten [16]. Moreover, flatbreads made after dough retardation had a significantly lower specific volume with a bigger spread ratio than those from the no-time process (Figure 3, Table 6). After 24 h of dough retardation, the specific volume decreased by 9–12% with the 30% sourdough addition and by 12–18% with the 50% sourdough addition. After 48 h of dough retardation, the specific

volume of the flatbread with 30% of sourdough was 18–20% lower, while it decreased by 20–30% when using 50% sourdough. With a prolonged retardation time, although the volume of the dough piece increases, there is usually a progressive loss in the volume of the baked product [46]. As the gas fraction increases during fermentation, the thickness of the gluten–starch matrix surrounding the gas cells decreases, i.e., the gluten network becomes weaker to trap the CO_2, which leads to a significant decrease in the bread volume [48,49]. Wang et al. [24] reported the highest specific volume of sourdough steamed bread after 24 h of retardation compared to 15, 18, 21, 27 and 30 h. In their study, the type of sourdough as well as the type of bread was different. Although a high volume is not a prerequisite for high-quality flatbread, and the shape can be flattened due to the volume and color degradation, we found the dough shelf-life of 48 h in retardation unacceptable. Since the dough browning and volume reduction after the 24 h retardation was still acceptable for some samples, it was selected for further analyses.

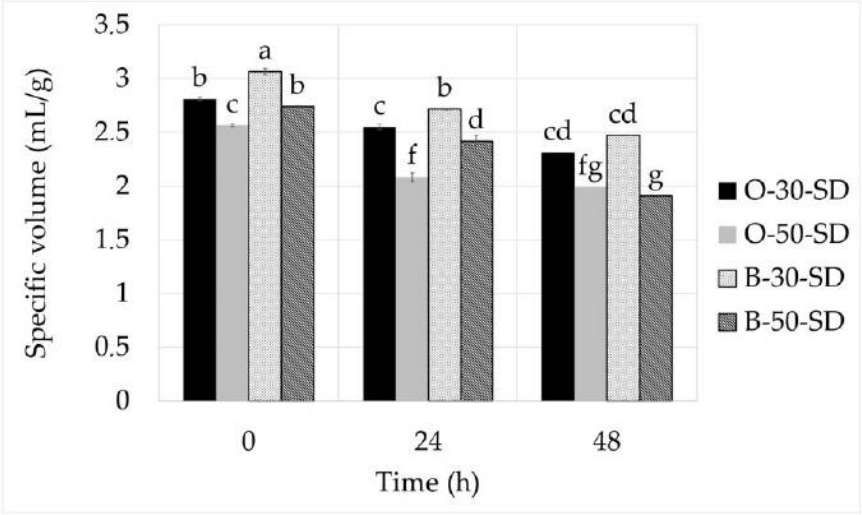

Figure 3. Specific volume of flatbreads from the no-time method and after 24 or 48 h of dough retardation, depending on the flour type and sourdough level. a–d, f, g Values marked with different letters differ significantly according to Tukey's test ($p < 0.05$).

Table 6. Baking loss, physical and nutritive properties of flatbread from the no-time (0 h) and retarded dough (24 h) processes, depending on the oat (O) and barley (B) sourdough level (30 or 50% dough weight).

	Parameter/ Bread Type	O-30-0 h	O-30-24 h	O-50-0 h	O-50-24 h	B-30-0 h	B-30-24 h	B-50-0 h	B-50-24 h
	Baking loss (%)	17.18 ± 0.31 ^{b}	18.76 ± 0.10 ^{ab}	17.92 ± 0.30 ^{b}	17.80 ± 0.84 ^{b}	18.05 ± 0.01 ^{b}	18.70 ± 0.22 ^{ab}	18.85 ± 0.49 ^{a}	20.23 ± 0.44 ^{a}
	Spread ratio	4.66 ± 0.08 ^{bc}	5.87 ± 0.07 ^{ab}	4.52 ± 0.03 ^{bc}	6.19 ± 0.44 ^{a}	4.76 ± 0.01 ^{c}	5.71 ± 0.16 ^{a}	5.25 ± 0.43 ^{bc}	6.11 ± 0.04 ^{a}
	Hardness (N)	31.30 ± 2.19 ^{bc}	28.58 ± 1.76 ^{c}	34.15 ± 3.10 ^{b}	28.72 ± 0.73 ^{c}	37.35 ± 3.06 ^{ab}	38.33 ± 2.27 ^{a}	38.73 ± 2.09 ^{ab}	33.27 ± 2.79 ^{bc}
	Cohesiveness	0.78 ± 0.03 ^{a}	0.70 ± 0.03 ^{b}	0.75 ± 0.02 ^{ab}	0.69 ± 0.02 ^{b}	0.79 ± 0.02 ^{a}	0.74 ± 0.05 ^{ab}	0.77 ± 0.03 ^{a}	0.70 ± 0.02 ^{b}
	Resilience	0.75 ± 0.04 ^{ab}	0.66 ± 0.04 ^{c}	0.71 ± 0.02 ^{b}	0.64 ± 0.04 ^{c}	0.78 ± 0.04 ^{a}	0.73 ± 0.04 ^{ab}	0.74 ± 0.02 ^{ab}	0.64 ± 0.01 ^{c}
crust	L^*	46.36 ± 0.00 ^{a}	39.12 ± 2.59 ^{b}	43.90 ± 2.30 ^{ab}	39.07 ± 2.60 ^{b}	39.34 ± 0.00 ^{b}	37.96 ± 0.58 ^{b}	37.87 ± 1.70 ^{b}	39.38 ± 1.08 ^{b}
crust	a^*	11.46 ± 0.00 ^{a}	5.72 ± 0.44 ^{d}	10.29 ± 0.07 ^{ab}	5.63 ± 0.21 ^{d}	10.94 ± 0.00 ^{ab}	6.38 ± 0.20 ^{c}	9.76 ± 0.79 ^{b}	5.42 ± 0.18 ^{d}
crust	b^*	28.62 ± 0.00 ^{a}	21.45 ± 0.89 ^{b}	27.31 ± 1.19 ^{a}	21.81 ± 0.56 ^{b}	22.58 ± 0.00 ^{b}	22.02 ± 0.04 ^{bc}	23.43 ± 0.16 ^{b}	17.72 ± 1.03 ^{c}
crust	TCD	NA	11.69	NA	8.68	NA	4.04	NA	7.35
crumb	L^*	52.52 ± 2.02 ^{a}	49.38 ± 1.23 ^{b}	50.43 ± 3.38 ^{a}	47.64 ± 1.79 ^{b}	41.31 ± 0.23 ^{bc}	41.78 ± 0.04 ^{bc}	40.00 ± 0.64 ^{bc}	36.30 ± 0.57 ^{c}
crumb	a^*	0.36 ± 0.12 ^{d}	0.64 ± 0.07 ^{cd}	0.63 ± 0.14 ^{d}	0.62 ± 0.11 ^{c}	2.00 ± 0.01 ^{b}	2.00 ± 0.08 ^{b}	3.13 ± 0.02 ^{a}	2.85 ± 0.11 ^{a}
crumb	b^*	14.16 ± 1.20 ^{a}	13.04 ± 0.71 ^{ab}	14.75 ± 1.34 ^{ab}	17.54 ± 4.43 ^{ab}	12.29 ± 0.07 ^{b}	13.87 ± 0.98 ^{ab}	12.62 ± 0.79 ^{b}	11.39 ± 0.56 ^{b}
crumb	TCD	NA	3.34	NA	3.95	NA	2.93	NA	3.91
	Dry matter (g/100 g)	53.67 ± 5.21 ^{a}	53.75 ± 3.19 ^{a}	53.69 ± 4.74 ^{a}	51.04 ± 5.02 ^{a}	55.82 ± 3.56 ^{a}	55.68 ± 4.60 ^{a}	55.56 ± 4.02 ^{a}	57.35 ± 2.72 ^{a}
	Phytic acid (g/100 g d.w.)	0.68 ± 0.02 ^{b}	0.42 ± 0.00 ^{c}	0.97 ± 0.02 ^{a}	0.60 ± 0.03 ^{bc}	0.59 ± 0.01 ^{bc}	0.40 ± 0.02 ^{c}	0.67 ± 0.00 ^{b}	0.49 ± 0.00 ^{c}
	β-glucans (g/100 g d.w.)	1.07 ± 0.01 ^{b}	0.97 ± 0.00 ^{c}	2.04 ± 0.05 ^{a}	1.96 ± 0.01 ^{a}	0.42 ± 0.01 ^{e}	0.30 ± 0.00 ^{f}	0.96 ± 0.00 ^{c}	0.88 ± 0.01 ^{d}

$^{a–f}$ Values within the same column marked with different letters differ significantly according to Tukey's test ($p < 0.05$). NA—not applicable; TCD—total color difference; d.w.—dry weight.

The crumb of the flatbread with 30 or 50% oat sourdough was harder (12–16%) compared to its barley counterpart. Compared to both flatbread types with 30% sourdough, the crumb was harder with the addition of 50% sourdough (4% in barley and 10% in oat bread). In addition, the crumb of flatbreads with 50% oat or barley sourdough was slightly less cohesive (4–11%), and resilient (5–18%) than in breads with 30% sourdough. This is in agreement with Flander et al. [50], who found that increasing the level of wheat sourdough increased the hardness and reduced the resilience of the mixed oat–wheat bread. Although sourdough is known for improving crumb texture, here, with the increasing sourdough levels, the amount of barley flour/bran was also increased, whereas the proportion of wheat flour decreased, which negatively affected the crumb texture. As the bran concentration increases, the hardness of the sourdough bread increases, since it is influenced by the ingredients added to the dough [51]. Our oat and barley flour, and particularly the bran, was high in DF (Table 2). The high fiber content, especially the high-molecular-weight β-glucan in oat bran and the high arabinoxylan content in barley bran, can interfere with the formation of the reaction between starch and protein in wheat flour, resulting in a harder crumb [52].

The crumb texture of the flatbreads made from the retarded dough was less hard (9–16%), less cohesive (6–10%), and less resilient (6–14%) compared to breads made from the no-time process, which agrees with Wang et al. [24]. One possible explanation is that the lower pH value of the dough (Table 5) and the retardation reduced the strength of the wheat gluten. The acidification caused by the growth of LAB changes the gluten network. At a pH of around 4.0, the solubility of the proteins is increased, and the formation of new bonds is prevented, i.e., the reduction of inter- and intramolecular disulfide bonds solubilizes the gluten proteins, resulting in gluten weakness [53]. In addition, an acidic pH (4.1–5.0) during dough retardation is favorable for proteolytic enzymes, which allows for greater proteolysis of the gluten [53]. On the other hand, the solubility and swelling properties of dietary fiber (mainly β-glucans, arabinoxylans) increases at the low pH value characteristic of the sourdough and retardation process, which also aids in crumb softening [53].

Table 7. ANOVA *p*-values for the influence of flour type, sourdough level and retardation time on flatbread physical and nutritive properties.

Dependent Variable	Flour Type	Sourdough Level	Retardation Time	Flour Type × Sourdough Level	Flour × Retardation Time	Sourdough Level × Retardation Time	Flour × Sourdough Level × Retardation Time
Specific volume	0.000 *	0.000 *	0.000 *	0.202 *	0.291	0.011 *	0.003 *
Baking loss	0.001 *	0.034 *	0.003 *	0.016 *	0.513	0.275	0.019 *
Spread ratio	0.227	0.049 *	0.000 *	0.164	0.049 *	0.447	0.262
Hardness	0.000 *	0.752	0.001 *	0.026	0.066	0.001 *	0.063
Cohesiveness	0.034 *	0.006 *	<0.001 *	0.336	0.578	0.950	0.236
Resilience	<0.001 *	<0.001 *	<0.001 *	0.031 *	0.608	0.370	0.067
L^* of crust	0.011 *	0.161	0.027 *	0.945	0.002 *	0.468	0.530
a^* of crust	0.751	0.000 *	0.000 *	0.039 *	0.010 *	0.521	0.040 *
b^* of crust	0.000 *	0.045 *	0.000 *	0.232	0.008 *	0.101	0.006 *
L^* of crumb	0.000 *	0.005 *	0.000 *	0.043 *	0.000 *	0.024 *	0.149
a^* of crumb	0.000 *	0.000 *	0.002 *	0.000 *	0.000 *	0.332	0.002 *
b^* of crumb	0.005 *	0.054	0.036 *	0.637	0.031 *	0.476	0.018 *
Phytic acid	0.000 *	0.000 *	0.000 *	0.005 *	0.008 *	0.121	0.246
β-glucans	0.000 *	0.000 *	0.000 *	0.000 *	0.050	0.008 *	0.107

* Significant at $p < 0.05$.

After the dough retardation, color differences in the crust and crumb of the flatbreads were observed (Table 6). The TCD of the crumb was very distinct, with values ≥ 3 for all flatbreads. Only parameter a^* of the dough was positively correlated with the a^* of the crumb ($r = 0.871$, $p = 0.005$). This means that the TCD of bread could not be attributed only to a higher phenolic content and higher PPO activity, but also to other enzymes of

cereal and microbial origin (such as amylases, proteases, and peptidases) as well as to non-enzymatic browning (e.g., Maillard reactions, dextrinization, and caramelization).

The phenomenon of darkening was even more pronounced in the crust than in the crumb, as shown by the TCD values (Table 6). The intensification of the crust color was more pronounced in oat bread than in barley bread which could be attributed to a three-fold higher activity of α-amylase in oat (322 U/kg d.w.) than in barley (101.6 U/kg d.w.) flour. The α-amylase activity during fermentation and baking (high temperatures and low moisture conditions) is responsible for enhancing Maillard reactions, as the resulting reducing sugars react with amino acids (proteins), which leads to a darkening of the bread surface during baking [54]. The darkening of the breadcrust is not only attributed to α-amylase activity but can also be caused by other enzymes activated in the dough retardation process. Glycoside hydrolases, in general, and proteases, increase the content of reducing sugars or free amino groups, i.e., precursors for Maillard reactions [55]. In addition, Olaerts et al. [56] found that the endoxylanase activity of flour has a significant correlation with breadcrust color. The amount of the Maillard reaction precursors that are initially present in the flour or added in the form of sugar, and enzyme activities, determine the intensity of the color change of the breadcrust the most. As with the crumb color, the exposure to high temperatures leads to the breakdown of starch and the formation of brown dextrins, which are responsible for the dark color of the breadcrust, but also for the caramelization of sugar and the formation of the brown color.

3.5. Nutritive Value of Bread

The type of flour, the sourdough addition level, the retardation time, or their combination had a significant influence on the PA and β-glucans content of the flatbreads (Table 6). The content of PA and β-glucans was higher with higher amounts of oat or barley.

Barley-containing flatbreads had a 13–31% lower PA content than oat flatbreads, probably because barley bran had a 33.6% lower PA content than oat bran [28]. The 24 h dough retardation significantly reduced the PA content by 38% in oat breads and by 27–32% in barley breads compared to the no-time process (Table 6). Fermentation is the crucial step in degrading PA in bread and can reduce its content by 31 to 85% [16]. Previous studies showed that sourdough fermentation successfully reduces the PA content of bread. It was demonstrated that the application of 30% *Lactobacillus brevis* and *Lactobacillus plantarum* sourdough (dough weight) resulted in PA degradation in the range of 31% to 67% for the whole-wheat bread [56,57], and that *Lactobacillus plantarum* sourdough with DY 300 and a 30% addition resulted in a 45% reduction in PA content of Iranian sangak flatbread [56,57]. Our study shows that the retardation of dough that contains sourdough further contributes to the degradation of PA. The retardation process resulted in a decrease in dough pH of 1.05 to 1.39 units (Table 6), which may correspond to the optimum pH (4.5–5.0) of cereal phytase [57]. The reduction in PA content in flatbreads from the retarded process could contribute to improving the bioavailability of minerals in flatbreads.

The β-glucan content was higher in oat than in barley flatbreads, since the oat flour–bran blend had a 21% higher β-glucan content compared to the barley flour–bran blend (4.35 vs. 3.60 g/100 g d.w.). Following this, an increasing trend was observed when increasing amounts of oat or barley sourdough were incorporated in the flatbread. Nevertheless, after dough retardation, the β-glucan content decreased by 4–9% in oat-containing flatbreads and even more, by 8–28%, in the barley flatbreads (Table 6). This was only partly caused by the activity of endogenous β-glucanase, whose activity in our semi-refined wheat flour was 1.68 U/MBG4 kg d.w. This is in agreement with a previous finding showing that wheat flour has low β-glucanase activity [58]. In our case, β-glucans were mostly degraded due to the activity of oat or barley β-glucanase, which was especially high in bran (13.3 and 9.1 MBG4 U/kg d.w., respectively), as reported by Grgić et al. [28]. β-glucans preservation was more successful with the higher (50%) addition of sourdough, most likely due to the lower pH of the dough, which may have slowed down the activity of β-glucanase. Flander et al. [50] found 12% less β-glucans in wheat–oat mixed bread containing wheat

sourdough compared to straight dough bread. They concluded that the degradation of β-glucans occurred during the fermentation, which was not influenced by variations in the acidity of the breads between pH 4.9 and 5.8, but by endogenous β-glucanase activity and the fermentation time. Gamel et al. [59] also demonstrated a reduction (18–23%) in β-glucan content in whole-wheat/oat bread containing sourdough in different proportions (40, 60 and 80%, dough weight). In our study, the activity of β-glucanase, whose optimum is at 30 °C (approximate dough-proofing temperature), was reduced at the low retardation temperature (0–4 °C), which explains the small changes in the glucan concentrations of the retarded flatbread.

3.6. Principal Component Analysis

The PCA extracted 15 factors, and the first two components with eigenvalues of 6.864 and 5.425, accounting for 82% of the total variance, were considered (Figure 4). The first component contrasts variables crumb *a** and baking loss with crumb *L** and *b** crust color parameters (Figure 4a). The second component contrasts the β-glucans with specific volume, dough pH, hardness, cohesiveness, and resilience. The first component separates samples from the no-time-processed and from the retarded-dough flatbread, although the B-50-0h flatbread was confused with the retarded samples (Figure 4b). Overall, oat flatbreads were better differentiated along the process than barley flatbreads. Flatbreads from the no-time process were contrasted to the retarded samples due to a darker crust and crumb color. Also, the separation of oats from barley flatbreads was evident. Oat flatbreads were characterized by higher β-glucans content whereas higher crumb hardness, spread ratio, and crumb redness were attributed to barley flatbreads.

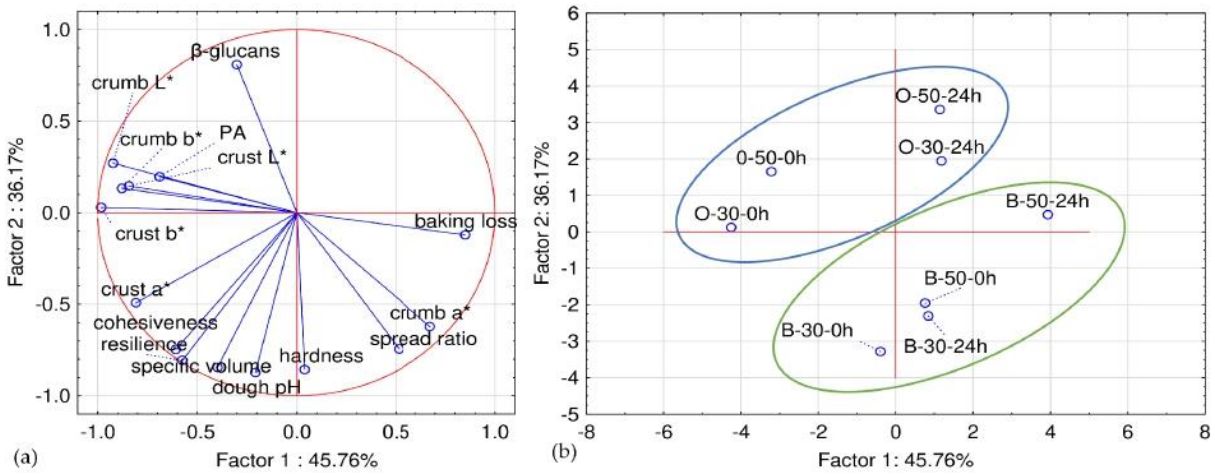

Figure 4. (**a**) the projection of responses on the factor plane according to principal component analysis; and (**b**) the projection of samples.

4. Conclusions

In this study, the sourdough fermentation of oat and barley flour with bran and commercially available starter cultures was comparatively investigated, and the quality of a mixed flatbread was examined as a function of the sourdough level and the dough retardation time. Oat flour showed a low acidification capacity when fermented with the commercially available starter. The partial replacement of flour with bran for sourdough fermentation proved to be advantageous due to its higher dietary fiber and minerals content, which enabled the desired pH value, total acidity, and lactic acid/acetic acid ratio to be achieved in a shorter timeframe. While the addition of oat or barley sourdough at the higher level (50% of dough weight) contributed to the higher β-glucan content of the mixed bread, sourdough addition at a lower level (30% of dough weight) resulted in a more desirable bread volume, crumb texture and a lower amount of phytic acid. Dough retardation in breadmaking was also shown to improve texture and reduce phytic acid

content. Enzymatic browning of the dough during retardation, which is primarily caused by polyphenol oxidase activity in the bran, can be effectively delayed by adding sourdough, providing a natural approach to breadmaking. Future research should investigate the use of non-thermally treated cereal ingredients to reduce glucans and volume degradation, and to maximize the nutritional value of the bread.

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Chapter 2

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Bojana Voučko: Experimental procedure, Project administration, Revision of the manuscript

Nikolina Čukelj Mustač: Writing original draft, Review and editing, Revision of the manuscript

Duška Ćurić: Resources, Supervision, Revision of the manuscrip

Alain Le-Bail: Conceptualization, Funding acquisition, Revision of the manuscript

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RESEARCH

Ultrasound-assisted Modification of Enzymatic and Antioxidant Activities, Functional and Rheological Properties of Oat and Barley Bran

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Abstract

Cereal bran possesses enzymes and antinutrients which should be reduced for their usability as a food ingredient high in dietary fiber, minerals, and antioxidants. This study investigated the influence of high-intensity ultrasound on enzymatic and antioxidant activities, phytic acid content (PA), and functional and rheological properties of oat and barley bran. Ultrasonication was performed at three specific energies (87 kJ/kg, 217.5 kJ/kg, and 348 kJ/kg), without or with pulsation (5 s emission, 10 s pause). Bran was assessed for β-glucanase, phytase and antioxidant activities, PA and total phenolic content, hydration, and rheological properties. β-glucanase from oat bran was inactivated up to 82% and from barley bran up to 55%, depending on the ultrasound-specific energy and pulsation. PA and antioxidant activities were higher in native oat bran (PA 17.35 mg/g d.w., FRAP 6.96 µmol TE/g d.w., 3.30 µmol FAE/g d.w.) compared to barley bran (PA 11.53 mg/g d.w., FRAP 2.27 µmol TE/g d.w., 5.30 µmol FAE/g d.w.). In both bran types, phytase activity increased (40–44%) after treatments at 87 kJ/kg, and on average, PA was reduced by 17% in oat bran and by 39% in barley bran. Depending on the energy and pulsation, bran ultrasonication reduced total phenolic content (27–55%), antioxidant activity (by 28–48%), complex viscosity (62–71%), and maximum stress tolerance (46–68%) while increased water swelling (42–48%) and retention capacity (44–59%). Hence, high-intensity ultrasound is a useful technique in reducing antinutrients while altering the enzymatic activity and functional properties of the bran. These results could help the wider application of bran in food production.

Keywords Cereal β-glucanase activity · Complex viscosity · Phytase activity · Phenolic compounds · Phytic acid

Introduction

Nowadays, there is a growing interest in the recovery of cereal by-products for developing new food products with high nutritional value (Guido & Moreira, 2017). One of the main cereal by-products is bran, which is obtained after grain milling. According to the Food and Agriculture Organization (FAOSTAT, 2022), the total production of barley bran reached 1.3 million tons in 2019, while the production of oat bran reached 1 million tons. In this context, 98.7% of barley bran and 99.8% of oat bran are used as animal feed (FAOSTAT, 2022). Due to the high content of soluble dietary fiber,

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high-quality proteins, free lipids, minerals, vitamins, polyphenols, and phytosterols in bran, their application in foods such as wheat bread, cookies, extrudates, noodle, and pasta has been studied (Baumgartner et al., 2018; Ebrahimi et al., 2022; Levent et al., 2020; Sibakov et al., 2015). Oat and barley bran contain substantial amounts of soluble dietary fiber, of which β–glucans predominate (Soukoulis & Aprea, 2012; Valoppi et al., 2021). The European Food Safety Agency (EFSA, 2011) has approved the health claim that the consumption of foods that contain barley or oat fiber in the minimum amount of 6 g per 100 g kcal of product leads to a reduction in plasma cholesterol levels and a reduction in the postprandial glycemic response. However, the physiological effects of oat and barley bran are affected by food processing, which may influence the solubility, extractability, and viscosity of dietary fiber as well as other bioactive compounds (such as phenolic acids, avenanthramides, phytosterols, and phytic acid).

Bran fractions are characterized by swelling, high absorption, binding, and retention of water, as well as gel formation which in turn affects their rheological behavior (Elleuch et al., 2011; Soukoulis & Aprea, 2012). These technological properties are linked to the physiological functionality of dietary fiber, e.g., water swelling capacity is associated with lowering blood cholesterol levels, water retention capacity is associated with lowering blood glucose levels, and viscosity is associated with reducing the risk of chronic diseases such as coronary heart disease, diabetes, obesity, and some forms of cancer (Elleuch et al., 2011). Sensory properties such as texture and appearance, as well as the physiological effects of β-glucan containing foods, are highly dependent on their water solubility and ability to form viscous solutions (Johansson et al., 2018). The viscosity of β-glucans is directly related to its concentration, molecular weight, and molecular structure (Pérez-Quirce et al., 2017). Activated endogenous β-glucanase degrades the molecular weight of β-glucans and lowers their viscosity (Pérez-Quirce et al., 2016, 2017; Tiwari et al., 2012). The content of water and β-glucanase levels, bran particle size, and the incubation time are critical factors for β-glucan degradation in food production (Johansson et al., 2018; Vatandoust et al., 2012). Thus, the precise knowledge of β-glucanase activity may be useful for technological reasons as it can improve dough and bread quality (Li et al., 2020), but it is doubtful from a physiological perspective. Some approaches that have previously been used to inactivate β-glucanase in flour include ethanol refluxing, scalding, autoclaving, oven heating, microwave processing, or the addition of organic acid salts (calcium propionate, potassium sorbate, and sodium benzoate) (Lazaridou et al., 2014; Pérez-Quirce et al., 2016; Rieder et al., 2015a; Tosh et al., 2012). Nevertheless, thermal and chemical methods may have negative effects on the technological properties of the flour, sensory aspects, and consumer acceptance of the final products (Rieder et al., 2015a). Hence, the processing of oat and barley bran using novel techniques needs to be further investigated.

The main antinutrient of oat and barley is phytic acid (PA), which binds phosphorus, iron, calcium, magnesium, and other minerals, thereby hindering their bioavailability (Baumgartner et al., 2018; Valoppi et al., 2021). Oat and barley bran have a low amount of phytase, the enzyme that can hydrolyze PA (Baumgartner et al., 2018; Steiner et al., 2007). Like glucanase, endogenous phytase is activated during prolonged soaking of bran or fermentation (Baumgartner et al., 2018). In that manner, Baumgartner et al. (2018) used hydrothermal treatment and fermentation to dephytinize oat bran. Guo et al. (2015) obtained high phytate degradation (around 87%) using steam flash explosion pretreatment of wheat bran. An effective method to reduce the PA content of finger millet grain was demonstrated by Yadav et al. (2021), who used probe-type ultrasound-assisted hydration (700 W, 20 kHz) with three different amplitudes (30%, 50%, 70%), treatment times (10 min, 20 min, 30 min), and different grain-to-water ratios. Mohammadi et al. (2021) found that the proper combination of pH (2, 6, and 9) and ultrasonic treatment (28 kHz) was a successful method for the dephytinization of rice bran. However, to our knowledge, no studies explored the effects of ultrasound on phytase activity and PA in oat and barley bran.

Ultrasound is a highly adaptable, relatively simple, environmentally friendly, and non-toxic food processing technology that is used due to low energy consumption and short processing time (Ali et al., 2023; Estivi et al., 2022). On a cereal matrix, the effects of ultrasound treatments on starches, proteins, hydration and germination, different enzymatic activity, and also food technological and sensorial quality have been explored (Estivi et al., 2022). Depending on the cereal matrix and process (equipment, frequency, intensity, amplitude, reactor geometry, and waves distribution), ultrasound is used to activate or deactivate enzymes, hydrate, or modify the main biopolymer through acoustic cavitation and free radical formation (Čukelj Mustač et al., 2019; Estivi et al., 2022; Habuš et al., 2021a; Vela et al., 2021). Previously, the effects of ultrasound on the activity of wheat or millet polyphenol oxidase, lipase, lipoxygenase, and peroxidase have been investigated (Čukelj Mustač et al., 2019; Habuš et al., 2021a, c). In addition, ultrasound has been shown to affect the functional, thermal, and rheological properties of proso millet bran (Čukelj Mustač et al., 2019), wheat bran (Habuš et al., 2021a, b), rice bran (Mohammadi et al., 2021), rice flour (Vela et al., 2021), and quinoa flour (Zhu & Li, 2019). Ultrasound has also been used for the extraction of bioactive compounds, β-glucans from oat and barley grain and by-products (Chen et al., 2018; Guido & Moreira, 2017; Sourki et al., 2016). To our knowledge, the effect of ultrasonication

on the enzymatic and antioxidant activity of oat and barley bran or their functional properties has not been explored to this date. Hence, this study aimed to investigate the effect of high-intensity ultrasonic treatment depending on the applied specific energy and pulse mode on the β-glucanase, phytase and antioxidant activities, PA and phenolics content, hydration, and rheology properties of oat and barley bran.

Materials and Methods

Materials

Oat bran (O) (Eko-Jazo Ltd., Ivanovac, Croatia) and barley bran (B) (Ivan Varga family-run farm, Orehovica, Croatia), which have not been previously thermally treated, were bought directly from producers. The bran was stored at − 18 °C until use to ensure their stability.

Methods

The proximate composition of bran samples determined according to the AOAC Official Methods (2012) is shown in Table 1. Dry weight (d.w.) content was determined according to the AOAC Method 925.10, and ash content was measured by Method 942.05. Protein content was determined by the AOAC Method 920.87 and calculated using a factor of 6.25, while fat content was determined according to Method 922.06. Soluble (SDF) and insoluble dietary fiber (IDF) were measured according to the AOAC Method 2011.25 using the Integrated total dietary fiber Assay Kit (Megazyme, Bray, Ireland). Carbohydrates content (%) was calculated according to Eq. (1):

$$\text{carbohydrates}(\%) = \%\text{dry matter} - (\%\text{protein} + \%\text{fat} + \%\text{fiber} + \%\text{ash}) \quad (1)$$

Particle size was determined by sieving according to the ICC standard 207 using the following mesh aperture: 670 µm, 355 µm, 282 µm, 225 µm, 180 µm, and 125 µm. The geometric mean diameter (d_{gw}) and the geometric standard deviation (S_{gw}) of the particle diameter were calculated according to Eqs. (2) and (3) (Patwa et al., 2014):

$$d_{gw} = log^{-1}\left[\frac{\sum_{i=1}^{n}\left(W_i log d_i\right)}{\sum_{i=1}^{n} W_i}\right] \quad (2)$$

$$S_{gw} = \frac{1}{2} d_{gw}\left[log^{-1} S_{log} - \left(log^{-1} S_{log}\right)^{-1}\right] \quad (3)$$

Experimental Design

After the independent variables range was defined in preliminary experiments, a full factorial design of the experiment involved three independent variables, i.e., specific energy input, pulse mode, and bran type (Table 2). The measured responses were β-glucanase and phytase activity, phytic acid (PA), total phenolic content (TPC), ferric reducing antioxidant power (FRAP), radical-scavenging capacity (DPPH), water swelling capacity (WS), water retention capacity (WRC), and rheological properties (complex viscosity, maximum stress tolerated by the sample (τ_{max}), behavior index (n', n''), consistency coefficients (K', K'')).

Ultrasound Treatment

Bran suspension (15%) was prepared by mixing 30 g of bran with 200 mL of distilled water in a 250 mL beaker. Samples were treated by a high-intensity ultrasound system consisting of a Hielscher UP400St sonicator (Hielscher Ultrasonics, Germany) and an H22D titanium probe, operating at a constant frequency of 24 kHz, 400 W, and 100% amplitude. The probe was immersed 2 cm in the bran suspension. Each treatment was performed in duplicate. The temperature rise during ultrasonication was recorded with a PT100 temperature probe immersed in the sample and is shown as an average of both bran types (Supplementary Fig. 1S). The control sample of oat and barley bran (O-C and B-C) was prepared as a water-bran suspension stirred for 30 min at room temperature on a magnetic stirrer (C-MAG HS-7; IKA Works, Inc., Wilmington, NC, USA) at 500 rpm. After treatment, bran samples were freeze-dried (Alpha 1–4 LSCplus; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 48 h and further analyzed.

Table 1 Proximate composition and mean particle size (d_{gw}) of native barley and oat bran

Bran type	Moisture (g/100 g)	Protein(g/100 g)	Fat (g/100 g)	Carbohydrates (g/100 g)	Soluble fiber(g/100 g)	Insoluble fiber (g/100 g)	Ash (g/100 g)	d_{gw} (µm)
Barley	12.24 ± 0.02	11.02 ± 0.15	1.28 ± 0.01	49.32 ± 0.22	9.16 ± 0.11	14.41 ± 0.38	2.57 ± 0.03	514 ± 1.40
Oat	11.44 ± 0.03	19.90 ± 0.18	4.73 ± 0.08	36.17 ± 0.03	12.31 ± 0.02	12.43 ± 0.04	3.02 ± 0.00	531 ± 1.43

Table 2 Experimental plan and samples code (actual values in brackets), treatment duration, and measured temperature at the end of treatment

Sample code	Bran type	Specific energy input (kJ/kg)	Pulse mode	Treatment time (min)	End temperature (°C)
O-87	1 (Oat)	1 (87)	1 (Off)	2.82	41
O-87-P	1 (Oat)	1 (87)	2 (On)*	7.75	41
O-217.5	1 (Oat)	2 (217.5)	1 (Off)	7.00	67
O-217.5-P	1 (Oat)	2 (217.5)	2 (On)	20.57	60
O-348	1 (Oat)	3 (348)	1 (Off)	12.83	81
O-348-P	1 (Oat)	3 (348)	2 (On)	34.50	75
B-87	2 (Barley)	1 (87)	1 (Off)	2.77	40
B-87-P	2 (Barley)	1 (87)	2 (On)	8.50	40
B-217.5	2 (Barley)	2 (217.5)	1 (Off)	7.87	65
B-217.5-P	2 (Barley)	2 (217.5)	2 (On)	22.00	60
B-348	2 (Barley)	3 (348)	1 (Off)	13.37	85
B-348-P	2 (Barley)	3 (348)	2 (On)	36.02	72

*5 s of emission with 10 s pause in between

Enzymatic Activity

The β-glucanase activity was determined spectrophotometrically with the Malt β-glucanase/lichenase assay kit (K-MBG4 08/18 method) (Megazyme, Bray, Ireland) following the manufacturer's instructions. After β-glucanase extraction from samples with malt β-glucanase/lichenase extraction buffer for 15 min with occasional shaking (MS3 shaker, IKA, Germany), the suspension was centrifuged at 6200 rpm for 5 min (Rotina, Hettich, Germany). The obtained β-glucanase extract was incubated with MBG4 substrate at 30 °C for 20 min. The absorbance was read at 400 nm. The phytase activity was determined with the Phytase Assay Kit (K-PHYTASE 09/21) (Megazyme, Bray, Ireland) according to the manufacturer's instructions. Briefly, samples were prepared according to procedure B, and free phosphate was removed using ion exchange resins (procedure D). In step 1, the extracted enzyme was incubated with phytic acid at 40 °C for 40–90 min (depending on the sample). The sample from step 1 was used in step 2, after which absorbance was measured spectrophotometrically at 360 nm. The spectrophotometer PerkinElmer Lambda 35 UV/Vis (Waltham, MA, USA) was used for all spectrophotometric measurements. All samples were analyzed in duplicate.

Phytic Acid Content, Total Phenolic Content, and Antioxidant Activity

PA content was determined spectrophotometrically in duplicate using the Phytic Acid Assay Kit (K-PHYT 05/19) (Megazyme, Bray, Ireland) according to the manufacturer's instructions. Briefly, the samples were extracted overnight with 0.66 M hydrochloric acid. The extracts obtained passed all steps of the A and B manufacturer's procedure, after which the absorbance was measured at 655 nm.

Free phenolics were extracted in duplicate according to Čukelj Mustač et al. (2019). The sample (250 mg) and 1 ml of 80% ethanol were shaken for 10 min in a horizontal position (shaker MS 3, IKA, Germany) and for another 10 min in an ultrasonic bath (Bandelin Electronic RK 100 H, Sonorex, Berlin, Germany), and then centrifuged at 8000 rpm for 15 min (MicroCL 21, Thermo Fisher Scientific, USA). The combined supernatants of three subsequent extractions were evaporated under a stream of nitrogen and dissolved in 500 µl of methanol.

The Folin-Ciocalteu (FC) assay for the determination of TPC was performed according to Čukelj et al. (2019). The test consisted of pipetting distilled water (0.4 mL), the obtained extract (0.02 mL), and FC reagent (0.1 mL) into a cuvette, adding 30% Na_2CO_3 (0.3 mL) and distilled water (1.18 mL) after 3 min of reaction time. After mixing the solution, it was incubated for two hours in the dark. The calibration curve was constructed with the standard of ferulic acid (> 99% Sigma-Aldrich, Merck), and results are expressed as mg of ferulic acid equivalent (FAE) per gram of sample d.w.

The antioxidant activity of free phenolics was measured with the FRAP test (Čukelj Mustač et al., 2019). The FRAP reagent (1 mL) was added to the methanolic extract (0.01 mL). After 4 min of incubation, the absorbance was measured at 593 nm in duplicate. The standard solution of Trolox (> 97%; Sigma-Aldrich, Merck) was used to construct a calibration curve, and results are expressed in µmol Trolox equivalent (TE) per gram of sample d.w. Antioxidant activity was also determined by DPPH radical-scavenging capacity using an electron paramagnetic resonance (EPR) spectrometer (MS 5000-X Magnettech; Freiberg Instruments GmbH, Freiberg, Germany) working at X-band and set as follows: the magnetic field from 331.10 to 343.10 mT, 30 s sweep time at 100 kHz modulation frequency, and 0.2 mT modulation amplitude. Phenolic extracts (0.01 mL) were

added to 0.95 mL of methanolic DPPH solution (0.06 mM), vortexed, and left standing for 20 min at ambient temperature in the dark. The spectra were recorded for the last 10 min of the reaction. DPPH radical-scavenging capacity (%) was calculated from peak-to-peak amplitude and expressed as micromoles of ferulic acid equivalents (FAE) per gram of bran d.w. using Eq. (4) (Chandrasekara & Shahidi, 2011):

$$\text{DPPH radical scavenging capacity (\%)} = \frac{\text{EPR signal intensity for the control} - \text{EPR signal intensity for the sample}}{\text{EPR signal intensity for the control}} \times 100 \quad (4)$$

Hydration Properties of Bran

The WRC of samples was determined in duplicate using a slightly modified method described by Guzmán et al. (2015). To a pre-weighed 2 mL centrifuge tube, 0.3 g of the bran sample and 1.5 mL of distilled water were added. The tubes were vortexed at 1500 rpm for 20 min and then centrifuged at 9122 rpm for 5 min. The supernatant was decanted, and the tube was allowed to drain on a paper towel for 10 min by placing the tubes at a 180° angle. After re-weighing the test tube, the WRC was calculated on d.w.

The WS of the bran sample was determined in duplicate in the graduated cylinder on exactly 200 mg of the sample to which 10 mL of distilled water was added (Habuš et al., 2021b). After 18 h of hydration at ambient temperature, the bed volume was determined and expressed on the d.w. of bran.

Rheological Properties

To assess the viscoelastic properties, samples were first heated under stirring at a constant paddle rotating speed (160 rpm) using a Micro Visco-Amylo-Graph (MVA, Brabender GmbH & Co. KG, Duisburg, Germany) following a modified method by Liu et al. (2022). Suspensions (15 g of bran mixed with 105 mL of distilled water corrected at 14% solids, w/w) were equilibrated at 50 °C for 1 min, heated to 95 °C at a rate of 6.0 °C/min, maintained at 95 °C for 5 min, cooled to 50 °C at a rate of 6.0 °C/min, and held at 50 °C for 2 min. The measurements were carried out at a measuring range of 150 cmg. Samples from the MVA were cooled to 25 °C overnight and loaded on MCR 92 rheometer (Anton Paar, Graz, Austria). All oscillatory measurements were performed in duplicate using a parallel plate geometry of 50 mm diameter with a 1 mm gap. Stress sweeps were performed from 0.1 to 100 Pa at a constant frequency of 6.28 rad/s and a shear strain of 0.05%. Frequency sweeps were performed from 6.28 to 68.2 rad/s, at 0.1% of shear strain, within the linear viscoelastic region. The tests were carried out at a constant temperature (25 °C) controlled with a Peltier temperature device P-PTD200/AIR (Anton Paar, Graz, Austria). The loss factor (tan δ) was calculated as a ratio of lost energy to stored energy measured in the test (G''/G'). The storage (G') and loss (G'') modulus were fitted to the power law model using Eqs. (5) and (6) (Liu et al., 2021):

$$G' = K' \times \omega^{n'} \quad (5)$$

$$G'' = K' \times \omega^{n''} \quad (6)$$

where K' and K'' are consistency coefficients (Pa.s$^{n'}$, Pa.s$^{n''}$), n' and n'' represent behavior index, and ω is the angular frequency.

Statistical Analyses

To determine the influence of specific energy input, pulse mode, and bran type on outcome variables, data were subjected to factorial analysis of variance (ANOVA). The Tukey post-hoc test was used to assess honest significant differences between samples. ANOVA, Tukey test, Spearman correlation test, Pareto diagrams, and principal component analysis (PCA) were performed using Statistica 14 (TIBCO Software Inc., CA, USA) at a significance level of $p < 0.05$.

Results and Discussion

Enzymatic Activity

Control oat bran (13.3 MBG4 U/kg d.w.) had 1.5 times higher β-glucanase activity than barley bran (9.1 MBG4 U/kg d.w.) (Fig. 1). Vatandoust et al. (2012) found similar β-glucanase activity in wheat bran (8–19 U/kg) depending on the cultivar. Depending on the interaction between bran type and ultrasound energy or pulse mode (Table 3), β-glucanase activity changed (Fig. 1). However, the individual influences of bran type and specific energy were most pronounced (Fig. 2a). In oat bran, β-glucanase activity decreased proportionally with increasing specific energy. The highest decrease (81.6%) was observed after O-348-P treatment (Fig. 1). Barley bran β-glucanase activity showed a nonlinear behavior; it initially increased (10.2–25.1%) but decreased after treatments with higher specific energy B-217.5, B-348, and B-348-P. Barley bran β-glucanase was more inactivated after continuous sonication, whereas the pulsed mode favored the inactivation of oat bran glucanase. Thus, the highest inactivation (55.3%) of barley bran β-glucanase was found in B-348, but its

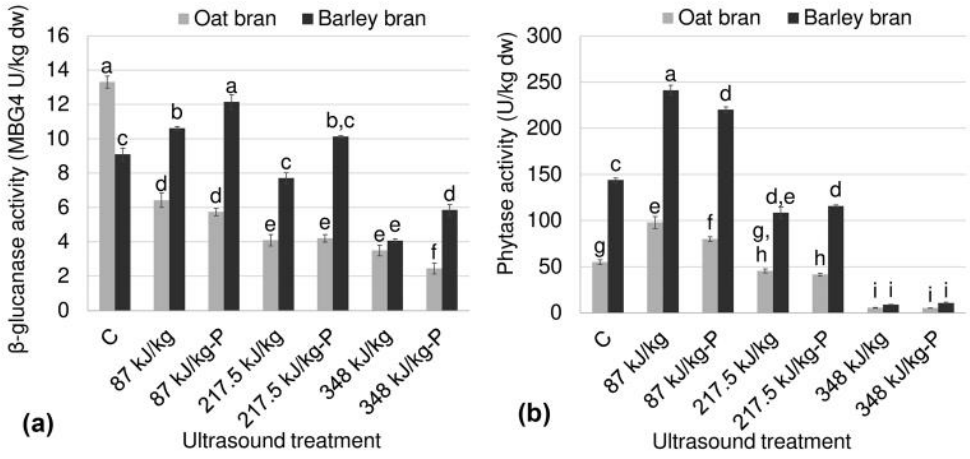

Fig. 1 Changes in (**a**) β-glucanase and (**b**) phytase activity for oat and barley bran depending on ultrasonic treatment compared with the control (C)

activity was still 1.7 times higher than in oat bran with the lowest β-glucanase activity. Previously, microwave heating was found to inactivate 87–100% of β-glucanase activity depending on the moisture content of rice flour (Pérez-Quirce et al., 2016), while oven heating decreased 70-fold the activity of β-glucanase of wholegrain barley flour (Rieder et al., 2015b). In this study, the β-glucanase activity of both bran types was inversely correlated with specific energy ($r = -0.760$, $p = 0.002$) and temperature at the end of treatment ($r = -0.847$, $p < 0.0001$). Specific energy input and pulse mode had a significant effect ($p < 0.005$) on the temperature recorded at the end of treatment. A strong positive correlation was found between temperature and specific energy ($r = 0.969$, $p < 0.001$), as well as with treatment time ($r = 0.839$, $p < 0.001$). In the case of oat bran, the greater reduction of β-glucanase activity after treatment with high specific energy and pulse mode was probably due to the longer exposure (for about 12 min) to high temperatures above 70 °C. On the other hand, barley β-glucanase is probably located farther from the cell membrane, making it less susceptible to ultrasound waves which disrupt cells and release enzymes (Mawson et al., 2011). This could be the reason why barley bran showed increased β-glucanase activity after milder treatments (B-87, B-87-P, and B-217.5-P) while reduced activity after more intensive treatment with higher end temperature (Fig. 2a).

The control barley bran (143.9 U/kg d.w.) had 2.6 times higher phytase activity than the oat bran (55.1 U/kg d.w.)

Table 3 ANOVA results for the influence of specific energy input, pulse mode, and bran type on the output variables

Dependent variable	Bran type	Specific energy input	Bran type × specific energy	Bran type × pulse mode	Specific energy × pulse mode	Bran type × specific energy × pulse mode
β-glucanase activity	< 0.001	< 0.001*	< 0.001*	< 0.001*	0.014*	0.512
Phytase activity	< 0.001*	< 0.001*	< 0.001*	0.249	< 0.001*	0.130
PA	< 0.001*	0.016*	0.033*	0.03*	< 0.001*	0.001*
TPC	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.313
FRAP	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
DPPH	< 0.001*	< 0.001*	< 0.001*	0.055	< 0.001*	< 0.001*
WS	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
WRC	0.011*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Complex viscosity	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
τ_{max}	< 0.001*	< 0.001*	< 0.001*	0.002*	< 0.001*	< 0.001*
n'	< 0.001*	0.042*	0.125	0.479	0.326	0.655
n''	< 0.001*	0.003*	0.146	0.757	0.17	0.1
K'	< 0.001*	< 0.001*	< 0.001*	0.083	< 0.001*	< 0.001*
K''	< 0.001*	< 0.001*	0.002*	0.135	0.005*	0.009*

PA phytic acid, *TPC* total phenolic content, *FRAP* ferric reducing antioxidant power, *DPPH* radical-scavenging capacity, *WS* water swelling, *WRC* water retention capacity, τ_{max} maximum stress tolerated by the sample; n' and n'', behavior index; K' and K'', consistency coefficient

*Significant at $p < 0.05$

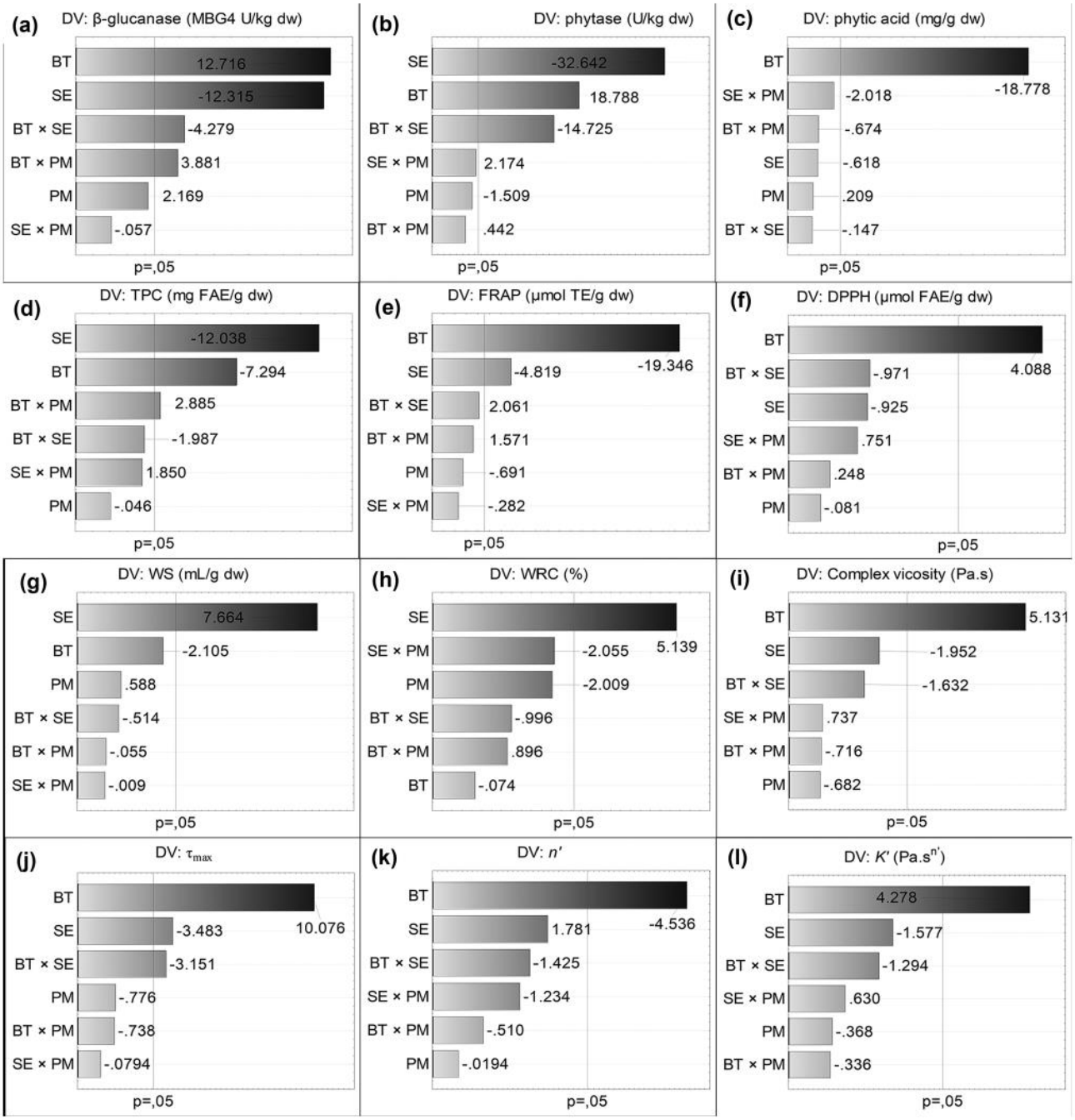

Fig. 2 Pareto charts for the estimation of independent variables (bran type (BP), ultrasound specific energy (SE), and pulse mode (PM)) on dependent variables (DV): (**a**) β-glucanase activity, (**b**) phytase activity, (**c**) phytic acid (PA), (**d**) total phenolic content (TPC), (**e**) ferric reducing antioxidant power (FRAP), (**f**) radical-scavenging capacity (DPPH), (**g**) water swelling (WS), (**h**) water retention capacity (WRC), (**i**) complex viscosity, (**j**) maximum stress tolerated by the sample (τ_{max}), (**k**) behavior index (n'), and (**l**) consistency coefficient (K'). Values situated on the right of the vertical (red) lines represent significant effects ($p<0.05$), and larger values of standardized effect estimate represent a more pronounced effect on the response, while the values on the left represent effects with no statistical significance ($p>0.05$)

(Fig. 1b). In both bran types, phytase showed a similar trend of activity changes depending on the applied ultrasound specific energy and pulse mode (Table 3, Fig. 2b). After the treatments with low specific energy (87 kJ/kg), phytase activity increased by 31–44% in oat bran and similarly by 35–40% in barley bran, depending on the pulse mode. Nevertheless, phytase

activity was reduced after ultrasonication at 217.5 kJ/kg, significantly in both treatment modes of barley bran (19.5–24.7%, respectively), while only the pulsation mode significantly reduced (17.5–24.9%) the oat bran enzyme. The final temperature after these treatments ranged from 60 to 66 °C (Table 2, Supplementary Fig. 1S). Phytase of both brans was almost completely inactivated (> 90%) after ultrasonication at the highest specific energy (348 kJ/kg), reaching final temperatures between 72 and 86 °C. Phytase activity positively correlated with β-glucanase activity ($r = 0.833$, $p < 0.001$) and negatively correlated with the specific energy ($r = -0.747$, $p = 0.002$), the temperature at the end of treatment ($r = -0.794$, $p < 0.001$), and the time of treatment ($r = -0.550$, $p = 0.042$). Thus, we can conclude that temperature was the main trigger for the reduced activity, as phytases (from wheat) are stable between 50 and 60 °C (Senwo et al., 2005), but are inactivated at higher temperatures and prolonged heating (Vashishth et al., 2017). The reduction in enzyme activity with high-intensity ultrasound is mainly attributed to the chemical and mechanical effects of cavitation and heat generation due to the extreme local temperature and pressure rise (Kumari et al., 2018; Mawson et al., 2011). Cavitating bubbles and shock waves cause strong shear and microflow in the fluid. As a result of these extreme conditions, there is a loss of biological activity of the enzyme due to the breakdown of hydrogen bonds and van der Waals interactions in the polypeptide chains, as well as changes in the secondary and tertiary structure of the protein (Bhargava et al., 2021; Mawson et al., 2011). This study shows that ultrasound changes phytase activity in a similar manner in both oat and barley bran, while the change in β-glucanase activity highly depends on its origin.

Phytic Acid Content, Total Phenolic Content, and Antioxidant Capacity

Phenolics and PA act as antinutrients that impair the digestibility of minerals or macronutrients in cereal foods, but also have antioxidant activity (Zajdel et al., 2013). The PA content of the control oat bran was 33.6% higher than that of the control barley bran (Table 4). These results are consistent with a previous study by Reddy (2002), although Baumgartner et al. (2018) and Levent et al. (2020) found a slightly higher PA content (18 mg/g and 20 mg/g, respectively) of oat bran. In our study, ultrasonication reduced the PA content of all samples depending on the interaction between bran type, ultrasound-specific energy, and pulse mode (Table 3). The PA content was reduced by up to 38.8% in barley bran, whereas by only up to 17.3% in oat bran. Possible explanations could be a higher phytase activity in each barley bran compared to the oat counterpart. The small difference between samples could be due to the fact that higher applied energies are followed by longer treatments, i.e., a longer soaking time during which phytase is activated, but they also end with a higher temperature at which phytase is inactivated (Fig. 1). In comparison, native oat or barley bran did not differ in their PA content (17.63 mg/g d.w. and 11.73 mg/g d.w., respectively) from their counterparts that were stirred with water for 30 min at room temperature (17.35 mg/g d.w. and 11.53 mg/g d.w.). Yadav et al. (2021) reported that the PA content of finger millet samples decreased by 67% with increasing ultrasound amplitude and soaking time (from 10 to 30 min). Mohammadi et al. (2021) also reported a decrease in PA (by 7%, 11%, and 23%) of rice bran due to the combined effect of pH (2, 6, and 9) and ultrasound (150 W, 28 kHz,

Table 4 Phytic acid (PA), total phenolics (TPC), and antioxidant activity (FRAP, DPPH) of ultrasonically treated oat (O) and barley (B) bran compared to the control (C)

Sample	PA (mg/g d.w.)	TPC (mg FAE/g d.w.)	FRAP (μmol TE/g d.w.)	DPPH (μmol FAE/g d.w.)
O-C	17.35 ± 0.31^{a}	0.84 ± 0.02^{ab}	6.96 ± 0.17^{a}	3.30 ± 0.04^{c}
O-87	15.01 ± 0.69^{bc}	0.89 ± 0.02^{a}	7.03 ± 0.04^{a}	2.63 ± 0.06^{d}
O-87-P	15.73 ± 0.46^{b}	0.83 ± 0.02^{b}	6.38 ± 0.04^{b}	2.10 ± 0.01^{e}
O-217.5	14.32 ± 0.00^{c}	0.76 ± 0.00^{bc}	5.60 ± 0.02^{d}	2.08 ± 0.08^{e}
O-217.5-P	16.27 ± 0.00^{ab}	0.65 ± 0.01^{d}	5.96 ± 0.04^{c}	2.70 ± 0.04^{d}
O-348	15.93 ± 0.02^{b}	0.61 ± 0.01^{d}	5.51 ± 0.02^{d}	2.59 ± 0.03^{d}
O-348-P	14.35 ± 0.18^{c}	0.63 ± 0.00^{d}	4.50 ± 0.02^{e}	2.17 ± 0.01^{e}
B-C	11.53 ± 0.28^{d}	0.75 ± 0.01^{bc}	2.27 ± 0.01^{g}	5.30 ± 0.20^{a}
B-87	7.66 ± 0.08^{e}	0.73 ± 0.01^{c}	2.31 ± 0.02^{fg}	4.12 ± 0.23^{b}
B-87-P	8.31 ± 0.32^{e}	0.75 ± 0.01^{c}	2.53 ± 0.02^{f}	3.54 ± 0.07^{c}
B-217.5	7.19 ± 0.01^{e}	0.59 ± 0.01^{d}	1.85 ± 0.03^{h}	4.26 ± 0.06^{b}
B-217.5-P	7.06 ± 0.25^{e}	0.63 ± 0.02^{d}	1.85 ± 0.03^{h}	4.46 ± 0.02^{b}
B-348	8.15 ± 0.01^{e}	0.34 ± 0.00^{f}	1.61 ± 0.02^{i}	2.76 ± 0.18^{d}
B-348-P	7.06 ± 0.11^{e}	0.46 ± 0.03^{e}	1.90 ± 0.01^{h}	3.32 ± 0.05^{c}

[a–i]Values within the same column marked with different letters differ significantly according to Tukey's test ($p < 0.05$)

1 h). They attributed the PA degradation to the generated heat and the resulting increased temperature, which initiates the chemical degradation of PA to lower inositol phosphate. Our study confirms the advantage of using ultrasound for the degradation of bran PA and extends the knowledge that the observed PA degradation is not only chemical but also enzymatic through the action of endogenous phytase. Thus, the effect of bran type on PA was the strongest compared to the other dependent variables considered in this study (Fig. 2c).

The TPC of the control oat bran was 10% higher than that of the control barley bran (Table 4). The TPC of ultrasonicated samples was dependent on the interaction of bran type and ultrasound-specific energy or pulse mode (Table 3). Yet, the specific energy input had the greatest influence on reducing TPC (Fig. 2d). Although TPC remained unchanged after the lowest specific energy treatments (Table 4), phenolics degradation occurred at 217.5 kJ/kg in both treatments of barley bran (approx. 20%), while only in the pulsing treatment of oat bran (23% decrease). The degradation of TPC of oat bran remained at a similar level (by 27% on average) at the higher specific energy input (Table 4), whereas barley bran TPC was even more degraded (55%) after treatments with the highest energy (348 kJ/kg) without a pulse, compared to the control. A negative correlation of TPC with specific energy ($r=-0.820$, $p<0.001$), treatment time ($r=-0.726$, $p=0.003$), and temperature ($r=-0.739$, $p=0.003$) was found. Our results are consistent with those of Habuš et al. (2021b), Yadav et al. (2021), and Zhu and Li (2019), who found that ultrasonic degradation of cereal phenolics is time, temperature, and amplitude dependent. In addition, we found that the phenolics of oat bran are more stable than those of barley bran.

Although the difference in TPC between brans was small, the FRAP antioxidant capacity of native oat bran was twofold higher than that of barley bran (Table 4). The reason could be the higher PA content in oat, but even more their unique phytochemicals, i.e., avenanthramides, acting as antioxidants (Hernandez-Hernandez et al., 2021; Schär et al., 2018). Both FRAP and DPPH antioxidant capacities were influenced by the interaction of bran type, ultrasound-specific energy, and pulse mode (Table 3), but the influence of bran type was the greatest (Fig. 3e, f). The FRAP antioxidant activity followed a similar trend as TPC. It decreased already at 87 kJ/kg in pulsing treatments and even more at higher ultrasound energies. Without pulsation during ultrasonication, the FRAP reduction (20%) of oat bran was the same at 217 kJ/kg as at 348 kJ/kg. The reduction of antioxidant activity (35% in FRAP, 34% in DPPH) of oat bran was the highest after ultrasonication at 348 kJ/kg with pulsation, while for barley bran, the antioxidants reduction (29% in FRAP, 48% in DPPH) was the highest at the same energy input but without pulsation. In agreement, Zhu and Li (2019) and Habuš et al. (2021c) also found that the FRAP antioxidant activity of quinoa flour or wheat bran was lower after ultrasonication. The synergistic effect of temperature and pressure rise, cavitating bubbles, and shock waves caused by the ultrasound leads to the formation of free radicals and the degradation of antioxidants (Kumari et al., 2018; Mawson et al., 2011). In this study, antioxidant activity measured by the FRAP assay showed a positive correlation with TPC results ($r=0.780$, $p=0.001$) and PA content ($r=0.859$, $p<0.0001$). Consistent with this, Ahn et al. (2004) reported the antioxidant activity of PA using the FRAP assay of irradiated PA. The DPPH values were inverse to those of the FRAP assay (Table 4) ($r=-0.609$, $p=0.021$) and PA content ($r=-0.591$, $p=0.026$). This was likely the result of the hydrophilicity of PA (Hong et al., 2018). Results of different tests for the same material may differ significantly due to the different types of antioxidants in the samples, which react differently with the radicals used (Shah & Modi, 2015). Unlike the FRAP assay, which is commonly used to measure the antioxidant capacity of hydrophilic compounds, the DPPH method is applicable to hydrophobic compounds (Pérez-Jiménez et al., 2008). Another reason could be the different principle of the EPR method used for determining DPPH scavenging capacity compared to the FRAP assay. Nevertheless, the application of the EPR method to oat and barley bran proved to be successful.

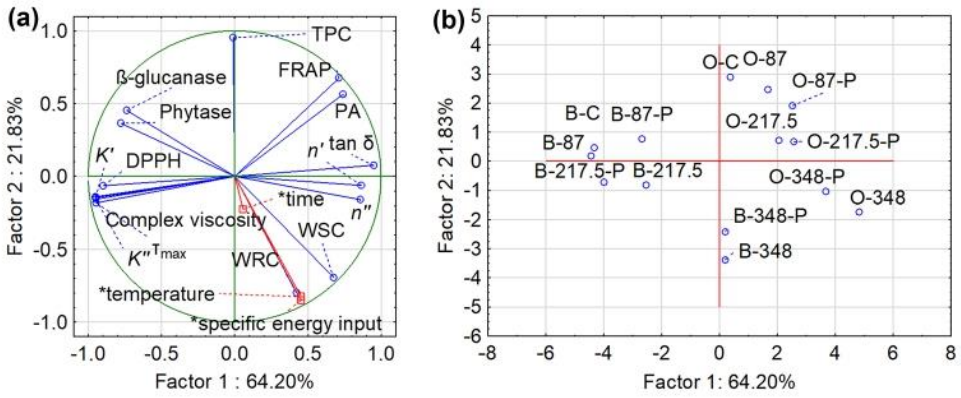

Fig. 3 (**a**) The projection of responses on the factor plane according to principal component analysis and (**b**) the projection of samples. DPPH, radical-scavenging capacity; FRAP, ferric reducing antioxidant power; n' and n'', behavior index; K' and K'', consistency coefficients; PA, phytic acid; TPC, total phenolic content; WS, water swelling; WRC, water retention capacity; τ_{max}, maximum stress tolerated by the sample

Hydration Properties

Hydration properties depend on cereal type, particle size, and concentration of protein, starch, and fiber (Elleuch et al., 2011; Godswill et al., 2019). The WS of the control oat bran was 21% higher than that of the control barley bran, while the WRC of barley bran was only slightly higher (11.6%) than the WRC of oat bran (Table 5). Barley bran contained more IDF (Table 1) than oat bran which could explain its slightly higher WRC. The higher WS of oat bran could be due to its higher protein content but also due to the different composition of its fiber compared to barley bran (Table 1). Although the WS and WRC of both bran types increased depending on the interaction of specific energy input and pulse mode (Tables 3 and 5), both were the most affected by the specific energy (Fig. 3g, h). Hence, at the highest energy input without pulsation, the increase in WS and WRC was the highest (respectively), 110% and 125% in oat bran while 124% and 70% in barley bran compared to their controls. Thus, WS and WRC were positively correlated with ultrasound-specific energy ($r=0.870$, for both), the temperature at the end of treatment ($r=0.875$ and 0.847, respectively), and the treatment time ($r=0.719$ and 0.579, respectively). This agrees with Elleuch et al. (2011) that hydration capacities increase with temperature. In agreement, Habuš et al. (2021b) showed a WS increase after ultrasonication of wheat bran, while Čukelj Mustač et al. (2019) found an increase in WRC of ultrasonicated millet bran. Ultrasound affects all cereal biopolymers such as starch, non-starch polysaccharides, and proteins including enzymes (Vela et al., 2021). Nevertheless, the WRC of our barley bran increased significantly only at the highest energy input, whereas the WRC of oat bran increased already at lower energies, although the treatment temperature did not differ between bran types. Furthermore, pulsation mode (i.e., treatment time and temperature) did not affect the WS of barley bran but did affect the WS of oat bran. Compared to oat bran, barley bran contained more starch than gelatinized and IDF that dissolved at higher temperatures (Elleuch et al., 2011). Nevertheless, the starch of the control barley bran was completely hydrolyzed by amylases, as evidenced by the absence of a gelatinization peak at MVA (Supplementary Fig. 2S a). On the other hand, the control oat bran showed a clear gelatinization peak at 61 °C (Supplementary Fig. 2S b), and the content of SDF was already high before the treatment. Ultrasound reduces particles size (Yadav et al., 2021) and hence increases the surface area of starch granules, and fiber and protein dissociation increases their water-binding sites, resulting in greater water absorption, retention, and solubility (Amini et al., 2015; Kaur & Gill, 2019). In addition, ultrasound inactivates amylases (Habuš et al., 2021a), which was confirmed here by improved gelatinization properties of treated oat and barley bran (Supplementary Fig. 2S). In addition, the higher values of hydration properties may indicate a shift in the solubility of β-glucans and other dietary fiber. Overall, the increased WRC and WS of ultrasound-treated barley and oat bran may indicate their improved physiological effect in terms of lower glycemic response and lower blood cholesterol levels (Elleuch et al., 2011).

Table 5 Hydration and rheological properties of ultrasonically treated oat and barley bran compared to the control (C)

Sample	WS (mL/g d.w.)	WRC (%)	Complex viscosity (Pa.s)	τ_{max} (Pa)	n'	n''	K' (Pa.s$^{n'}$)	K'' (Pa.s$^{n''}$)
O-C	5.65 ± 0.00^{Eg}	211.39 ± 7.31^{Df}	60.31 ± 1.55^{fg}	0.345 ± 0.01^{f}	0.136^{Ab}	0.171^{Bb}	288.79^{Ad}	77.80^{Ac}
O-87	5.55 ± 0.00^{Fg}	222.01 ± 0.96^{Df}	45.76 ± 4.43^{g}	0.291 ± 0.02^{f}	0.181^{Aab}	0.24^{ABb}	197.93^{Bd}	61.82^{ABc}
O-87-P	6.12 ± 0.00^{Df}	234.13 ± 8.08^{CDf}	37.52 ± 2.75^{g}	0.229 ± 0.03^{f}	0.231^{Aab}	0.276^{ABab}	142.66^{BCd}	52.15^{Bc}
O-217.5	7.49 ± 0.01^{Ce}	301.14 ± 2.42^{Bd}	45.42 ± 2.19^{g}	0.272 ± 0.00^{f}	0.161^{Aab}	0.207^{Bb}	204.67^{Bd}	64.88^{ABc}
O-217.5-P	8.10 ± 0.00^{Bd}	253.49 ± 3.59^{Cef}	47.91 ± 2.58^{g}	0.267 ± 0.00^{f}	0.201^{Aab}	0.253^{ABab}	196.72^{Bd}	74.05^{Ac}
O-348	11.84 ± 0.01^{Aa}	475.98 ± 3.59^{Aa}	22.94 ± 0.20^{g}	0.186 ± 0.00^{f}	0.336^{Aa}	0.389^{Aa}	69.05^{Cd}	25.57^{Cc}
O-348-P	11.82 ± 0.01^{Aa}	316.48 ± 8.89^{Bcd}	32.32 ± 2.10^{g}	0.24 ± 0.02^{f}	0.270^{Aab}	0.298^{ABab}	112.49^{Cd}	45.71^{Bc}
B-C	4.48 ± 0.2^{Ei}	239.05 ± 10.48^{Cef}	319.43 ± 18.10^{ab}	2.11 ± 0.08^{a}	0.095^{Ab}	0.133^{Ab}	1629.44^{Aa}	325.91^{Aab}
B-87	5.07 ± 0.00^{DEh}	264.31 ± 4.42^{Ce}	361.29 ± 22.04^{a}	2.03 ± 0.12^{a}	0.118^{Ab}	0.173^{Ab}	1782.92^{Aa}	364.66^{Aa}
B-87-P	5.25 ± 0.00^{Dgh}	253.96 ± 5.88^{Cef}	194.55 ± 18.21^{d}	1.69 ± 0.06^{b}	0.124^{Ab}	0.191^{Ab}	942.19^{Bbc}	212.73^{ABbc}
B-217.5	6.09 ± 0.00^{Cf}	265.03 ± 7.02^{Ce}	232.93 ± 15.55^{cd}	1.37 ± 0.01^{c}	0.124^{Ab}	0.171^{Ab}	1134.24^{Bb}	275.27^{ABab}
B-217.5-P	6.14 ± 0.01^{Cf}	264.64 ± 6.38^{Ce}	305.80 ± 12.54^{b}	1.67 ± 0.08^{b}	0.109^{ABb}	0.141^{Ab}	1735.33^{Aa}	381.82^{Aa}
B-348	10.04 ± 0.36^{Bc}	404.7 ± 8.99^{Ab}	148.19 ± 4.24^{e}	1.16 ± 0.04^{d}	0.144^{Ab}	0.206^{Ab}	686.37^{BCc}	179.95^{ABbc}
B-348-P	10.77 ± 0.00^{Ab}	340.68 ± 6.54^{Bc}	92.54 ± 0.30^{f}	0.658 ± 0.07^{e}	0.119^{Ab}	0.200^{Ab}	446.75^{Ccd}	95.54^{Bc}

[a–i]All values within the same column

[A–F]Values within the same bran type marked with different letters differ significantly according to Tukey's test ($p<0.05$)

WS water swelling, *WRC* water retention capacity, τ_{max} maximum stress tolerated by the sample; n' and n'—behavior index, K' and K' consistency coefficients

Rheological Properties of Gels

Bran fractions from oats and barley are known to have the highest viscosity due to higher β-glucan content compared with other types of cereal bran (Soukoulis & Aprea, 2012). The dynamic viscoelastic properties of control and processed oat and barley bran are shown in Supplementary Fig. 3S and Table 5. Compared to O-C, B-C exhibited higher complex viscosity (81.1%), τ_{max} (83.7%), G' (81.5%), and G'' (75.1%), K', K'' and slightly lower n' and n''. The viscoelastic properties of the gels depended on the interaction between bran type, ultrasound-specific energy, and pulsing (Table 3). Pareto diagrams showed that the rheological properties of gels were mainly influenced by the type of bran, except for τ_{max} which was the most affected by the specific energy (Fig. 3i–l).

Compared with the control, the G' value of all treated oat bran samples was lower while it was higher for some processed barley bran, i.e., B-87 and B-217.5-P (Supplementary Fig. 3S a). This indicates that barley bran B-87, B-217.5-P, and B-C as O-C had stronger viscoelastic solid properties than samples after treatments at the same energy with pulsing and those at higher specific energies. This is likely due to the breakdown of molecular chains of fiber or protein by ultrasound cavitation (Nwankpa, 2019). The lower G' value of treated samples may be attributed to the strong swelling ability of the processed bran, as indicated by the inverse correlation between G' and WS values ($r = -0.534$, $p = 0.05$). In agreement, Nwankpa (2019) correlated low G' of rice dispersion with its maximum swelling power. At higher frequencies, G' and G'' moduli of the control and processed bran showed frequency dependence (Fig. 4). Within the linear viscoelastic range, oat and barley bran (control and processed) revealed G' > G'', indicating gel-like characteristics, but not a true gel since tan δ (loss factor) values were > 0.1 and < 1 showing their property is between dense biopolymer and a real gel with a predominant viscoelastic solid behavior (Mandala et al., 2004). Tan δ was slightly increased at the higher frequencies for all treated and control samples, indicating increased elasticity. According to Nwankpa (2019), the increase in temperature during ultrasonication leads to an increase in the translational energy absorbed by the treated material which reduces the retained moisture resulting in lower flour viscosity.

In this study, sonication significantly decreased τ_{max} of several barley bran samples, but not oat bran samples (Table 4). Compared to the control, the first significant decrease in τ_{max} of barley bran occurred after 87 kJ/kg pulsing ultrasonication, whereas no significant difference was observed after the continuous ultrasonication at the same energy. However, the largest decrease of barley bran τ_{max} was recorded after treatment at 348 kJ/kg with pulse mode. Nwankpa (2019) reported that the behavior index (n' and n'') closer to 1 indicates a softer gel, while the exponent closer to 0 suggests a stiffer gel. Hence, the behavior index indicated that the gel created here from oat bran was softer than barley bran gel. This agrees with the higher τ_{max} for barley bran gel, indicating its stiffness. In addition, the behavior index increased, but τ_{max} decreased with increasing specific energy, i.e., the gel became softer and consequently could resist less force without deformation of the structure. The correlation between τ_{max} and relaxation exponents (for n', $r = -0.982$; for n'', $r = -0.882$, $p < 0.0001$) was proven.

Similar to τ_{max}, the complex viscosity of both bran types decreased after ultrasonication, except for B-87 (Table 5). A similar decreasing trend was found for consistency indices (K' and K'') of the treated samples. Complex viscosity positively correlated with consistency index (for K', $r = 0.987$; for K'', $r = 0.982$, $p < 0.001$) and τ_{max} ($r = 0.899$, $p < 0.001$). The lower viscosity of the gels could be explained by starch damage due to shear forces causing the straightening out of amylose molecules as well as the disruption of the chemical interactions and organization of the main constituents (starch and protein) (Nwankpa, 2019; Vela et al., 2021). The influence of ultrasonication was more substantial on the gel viscosity of barley than of oat bran. This denotes that sonicated barley bran led to a weaker gel that could not resist higher stress before its structure disruption. In our case, oat bran contained more protein and fiber, including β-glucans of higher molecular weight, than barley bran (Table 1) (Lazaridou & Biliaderis, 2007). The formation of a stable gel-like substance through the interaction between protein and β-glucan may explain the viscosity behavior of the treated oat bran (Liu et al., 2022). Thus, the choice of bran but also ultrasound processing conditions are crucial for the viscoelastic properties of their gels which have many potential food applications (fat replacers, beverages, bakery products, etc.).

Principal Component Analysis

The PCA extracted 15 factors, and the first two components with eigenvalues of 9.630 and 3.274, accounting for 86% of the total variance, were considered (Fig. 3). The first component contrasts variables phytase, β-glucanase, DPPH, complex viscosity, τ_{max}, K' and K'' with tanδ, and n' and n'' (Fig. 3a). The second component contrasts the TPC with WS and WRC. Furthermore, it contrasts specific energy, temperature, and treatment time with TPC but not with hydration properties. Yet, the treatment time made a small contribution. The first component essentially contrasts oat bran from barley bran samples, although the control oat and barley bran samples treated at 348 kJ/kg were confused with each other (Fig. 3b). The second component contrasts control samples from those treated at 348 kJ/kg (with or without pulse mode). Although oat bran samples were better

differentiated than barley bran samples (Fig. 3b). Component 1 contributes highly to samples B-C, B-87, and O-348, while component 2 contributes most to the samples O-C, O-87, O-87-P, B-348, and B-348-P. In particular, the oat bran samples O-C, O-87, and O-87-P which are characterized by the highest TPC, FRAP, and PA values are well separated from barley bran samples treated at 217.5 kJ/kg showing high DPPH values, complex viscosity, τ_{max}, and K' and K''. Furthermore, samples B-C and bran treated at 87 kJ/kg, characterized by the highest β-glucanase and phytase activities, are contrasted to both bran types treated at 348 kJ/kg showing the highest WS and WRC.

Conclusions

The effects of ultrasound-specific energy and pulse mode on enzyme and antioxidant activities, phytic acid concentration, total phenolic content, and rheological and hydration properties of oat and barley bran were studied. The ultrasonication of a water-bran suspension with higher specific energy input takes longer and results in a higher final temperature, while the pulsation mode increases treatment time and lowers the final temperature with the same energy input. Thus, the interactions between the bran type, specific energy, and pulse mode strongly influence enzymatic activity and functional properties. Nevertheless, bran type and specific energy (and final temperature) are the critical variables for enzyme activity, whereas pulsation mode has a minor influence. Ultrasound treatment with higher specific energy reduces β-glucanase and phytase activity, phenolic content, antioxidant activity, and complex viscosity while increasing water retention and swelling of both bran types. Similarly, as antioxidant activity and rheological properties, the content of phytic acid is highly origin dependent and can be reduced by 17% in oat bran and by 38% in barley bran in only 21 min of ultrasonication. Considering the specific energy input and the material origin, ultrasonication could be a simple and short pretreatment of bran, as a nutrient-dense ingredient, for various potential applications. The modified bran could be applied in liquid foods such as cereal beverages (smoothies, dairy products, etc.) as well as for the enrichment of baked goods. Future studies should investigate the effect of ultrasound on the molecular weight of β-glucans and the application of the treated bran in food products.

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Data Availability Additional data is available upon request.

Code availability Not applicable.

Declarations

Competing Interests The authors declare no competing interests.

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RESEARCH

Pulsed Electric Field Treatment of Oat and Barley Flour: Influence on Enzymes, Non-starch Polysaccharides, Dough Rheological Properties, and Application in Flat Bread

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Abstract

This study examined the effects of pulsed electric field (PEF) treatment on enzymes, non-starch polysaccharides, and bread-making potential of oat and barley flour. Enzyme activity, microstructure, β-glucan extractability, molecular weight (Mw) and structure of non-starch polysaccharides, dough rheology, and flat bread properties were determined. An exponential decay model explained better the residual activity of oat β-glucanase across electric field intensity than barley β-glucanase. PEF treatment of flour at 12 kV/cm for 162 ms significantly reduced β-glucanase activity (40.2–76.5%) while increasing the concentration of total β-glucans (33.5%) and water-extractable arabinoxylans (36–41%). Mw of linear β-d-glucans decreased (9%) while Mw of branched arabinoxylans increased (6–33%). Scanning electron microscopy showed changes in microstructure of barley proteins. Blending wheat flour (70%) with oat or barley flour (30% weight) after PEF treatment enhanced gluten aggregation energy (29–19%) and breakdown viscosity (18–43%) of dough, as well as increased β-glucan content (21–32%) but reduced specific volume (11–24%). The findings of this study provide a comprehensive insight into the PEF's potential for retarding enzymatic reactions and preserving integrity of cereal non-starch polysaccharides.

Keywords Cereal β-glucanase · Water-extractable non-starch polysaccharides · FTIR · NMR · Protein aggregation

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Introduction

Bread, a global staple, continues to evolve to meet the demands of modern life. Flat bread, one of the oldest types of bread, possesses consumers' interest due to its versatility and convenience. Increasing consumers' demand necessitates the development of nutritionally enriched flat breads (Garzon et al., 2022). Oat and barley flour, known for their richness in fiber, minerals, vitamins, and antioxidants, are underutilized ingredients in flat bread. Globally, in 2021, 1.46 billion tons of oat and 22.6 million tons of barley were produced, as reported by the Food and Agriculture Organization (FAOSTAT, 2023). Hence, oat and barley flour are interesting ingredients for fiber-enrichment of bread (Mansoor et al., 2019; Rieder et al., 2012), but certain challenges need to be overcome.

Barley and oat are characterized by their non-starch polysaccharides, β-glucans, and arabinoxylans (AX) in particular (Lazaridou et al., 2007; Zambrano et al., 2023). The β-glucan content in barley and oats ranges from 2.5 to 11.3% and 2.2 to 7.8%, respectively (Lazaridou et al., 2007). The proportion

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of AX in the endosperm is similar between barley and oats (1.2–1.3%), while their proportion in the bran is higher in barley (10.3%) than in oats (5.2%) (Zannini et al., 2022). Physiological and technological properties of β-glucans depend not only on their amount but also on their molecular weight (Mw), extractability, viscosity, and solubility (Lazaridou et al., 2007). The fine structure of cereal (1 → 3) and (1 → 4) β-D-glucans consists mainly of β-D-glucopyranosyl (β-d-Glc*p*) units that are 30% linked via 1,3-glycosidic bonds and 70% via 1,4-glycosidic bonds (Lazaridou et al., 2007; Zhang et al., 2019). β-Glucans of high Mw show high viscosity in the aqueous solution and pronounced gelation properties, which ultimately increases the physiological benefits (Goudar et al., 2020; Zhang et al., 2019). The AX is a heteroglycan whose structure consists of 1,4-linked β-d-xylopyranosyl residues (β-d-Xyl*p*) with α-l-arabinofuranosyl units (α-l-Ara*f*) (Zambrano et al., 2023). The AX is primarily consisting of insoluble dietary fiber, where the higher degree of *Araf* substitution to the *Xylp* backbone plays a crucial role in its solubility (Zambrano et al., 2023). β-Glucans and AX can lead to a weakening of the gluten network during bread production depending on their solubility and Mw (Cao et al., 2023; Courtin & Delcour, 2002).

The endogenous cereal β-glucanase depolymerizes β-D-glucans, i.e., it degrades the Mw of the β-glucans increasing its solubility but lowering the viscosity and reducing their physiological effect (Pérez-Quirce et al., 2017). Approaches used so far for inactivating β-glucanase in flour include high-intensity ultrasound, autoclaving, ethanol refluxing, scalding, and microwave processing (Grgić et al., 2023; Lazaridou et al., 2014; Pérez-Quirce et al., 2017; Pérez-Quirce et al., 2016). A harsh thermal or chemical processing can cause severe structural changes, e.g., disorder of the protein network, deformation of starch granules, and reorganization of amylose and amylopectin chains (Duque et al., 2020). Such modifications constraint the application of treated flour as a food ingredient. Therefore, innovative processing of flour to inactivate endogenous enzymes, i.e., β-glucanase, while preserving functional structure of its biopolymers, is needed.

The pulsed electric field (PEF) is emerging as a non-thermal technology for food processing. A food material located between two electrodes is subjected to a high-voltage electric field in short pulses (μs-ms) (Duque et al., 2019). The added energy is absorbed by the carbon backbone of biomacromolecules and is changing their configuration (Martín-Belloso & Elez-Martínez, 2005). Thus, PEF treatment can enhance the extraction of important compounds (Kumari et al., 2018, 2019) and alter the physicochemical properties of biomacromolecules (Duque et al., 2019, 2020; Jaeger, et al., 2010; Maniglia et al., 2021). Due to no chemical residues, low energy, and time consumption, PEF has already been successfully used to modulate the activity and stability of several enzymes (Jaeger et al., 2010; Li et al., 2022; Ohshima et al., 2007; Zhang et al., 2017). Moreover, the effects of PEF technology on polysaccharides such as corn, wheat and cassava starch, sugar beet pectin, and chitosan have been investigated (Han et al., 2012; Ma et al., 2012; Maniglia et al., 2021; Rivero-Ramos et al., 2023). To our knowledge, no study has yet investigated the influence of PEF processing on the activity of β-glucanase or non-starch polysaccharide structure of oat or barley flour.

The aim of this study was to investigate the influence of PEF treatment on the β-glucanase activity, the extractability of β-glucans, and the Mw of non-starch polysaccharides of oat and barley flour, but also to validate its functionality in further processing, i.e., making of composite bread. Therefore, we first investigated the kinetics of β-glucanase inactivation depending on the electric field intensities (EFI), the treatment time at selected EFI, and the specific energy input of the PEF treatment. Then, the changes in molecular structure and properties of its non-starch polysaccharides (β-glucans and arabinoxylans) after selected PEF treatment were determined. Finally, the application of PEF-treated flour on dough rheology and flat bread properties was investigated.

Materials and Methods

Materials

Oat flour was provided by Granolio Plc. (Zagreb, Croatia), barley flour by the family-run farm Ivan Varga (Orehovica, Croatia), and semi-refined wheat flour by Čakovečki mlinovi Plc. (Čakovec, Croatia) and stored at − 20 °C beforehand usage. Samples were not thermally treated beforehand. The proximate composition of flour samples was determined according to the AOAC Official Methods (2012). Oat, barley, and semi-refined wheat flour contained 11.0, 9.8, and 11.0% of protein (AOAC Method 920.87); 1.8, 2.3, and 0.8% of ash (AOAC Method 942.05); and 7.0, 1.6, and 2.3% of fat (AOAC Method 922.06), respectively. The particle geometric mean diameter, determined according to the ICC standard 207 (1998) by sieving at the mesh apertures of 670, 355, 282, 225, 180, and 125 μm and calculated as reported previously by Grgić et al. (2023), was 231 μm for oat flour and 295 μm for barley flour.

Methods

Pulsed Electric Field Treatment

The oat and barley flour suspended in water (in a ratio of 2:1, w/w) was treated in a high-intensity PEF system (HVG60/1, Impel Ltd., Zagreb, Croatia). The batch treatment chamber was equipped with two parallel stainless-steel electrodes

covering an area of 201 cm^2 and a distance of 25 mm. The system administered bipolar square wave pulses with a uniform pulse width of 2 µs and a pulse frequency of 150 Hz.

First, the suspensions of both flour types were PEF-treated for 18 ms at four levels of EFI: 4.8, 8.4, 12, and 16 kV/cm. After defining the EFI which inactivated the most of β-glucanase, the influence of treatment time was investigated with the same aim at five levels: 18, 54, 108, and 162 ms.

Specific energy input (W_S, kJ/kg) was calculated according to Lohani and Muthukumarappan (2016) following the equation (Eq. 1):

$$W_s = \frac{1}{2} \times \frac{CV^2 n}{m} \quad (1)$$

where C is the capacitance (F), V is the voltage (V), n is the number of pulses, and m is the sample weight (kg). The capacitance was measured using LCR meter (DE-5000, Deree Electrical Instrument Co., Ltd., New Taipei City, Taiwan). The W_S of the oat and barley flour samples varied from 0.1 to 11.48 kJ/kg and 0.1 to 13.11 kJ/kg, respectively throughout the PEF treatments. Samples were treated at the initial temperature of 20 ± 0.1 °C which minimally increased during the treatment (Table 1). The temperature and conductivity of flour-water suspension were measured before and after PEF treatment using a handheld conductivity meter (S230, Mettler-Toledo, Greifensee, Switzerland).

The PEF-treated samples were freeze-dried (Alpha 1–4 LSCplus; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and stored at −20 °C for subsequent analyses or used directly for bread making.

Enzymatic Activity

The activity of β-glucanase was measured using the malt β-glucanase/lichenase assay kit (K-MBG4 08/18 method) (Megazyme, Bray, Ireland) according to the previously described protocol (Grgić et al., 2023). Extraction of β-glucanase from the samples was performed using the malt β-glucanase/lichenase extraction buffer with an MS3 shaker (IKA, Germany) for 15 min. After centrifugation at 6.200 rpm for 5 min (Rotina, Hettich, Germany), the extracted β-glucanase was incubated with the MBG4 substrate at 30 °C for 20 min, and the absorbance was measured at 400 nm.

According to Giner et al. (2000), the inhibition of enzyme activity by PEF was described with an exponential, first-order kinetics model (Eqs. 2 and 3):

$$RA = RA_0 \times \exp(-k \times Ws) \quad (2)$$

$$RA = RA_0 \times \exp(-k \times EFI) \quad (3)$$

where RA is the residual β-glucanase activity (%); RA_0 is the intercept of the curve, k (ms^{-1}) is the first-order kinetic constant, and Ws is specific energy input (kJ kg^{-1}) or EFI (kV cm^{-1}).

The α-amylase activity was determined according to AOAC Method 2002.01 and ICC Standard No. 303 using α-amylase SD assay kit (K-AMYLSD 04/19 method) (Megazyme, Bray, Ireland) according to the manufacturer's instructions. The sample (0.5 g, particles < 0.5 mm) was extracted with 8 mL of extraction buffer (pH = 5.4) at 40 °C for 10 min with occasional mixing. The extract was then centrifuged at 10.696 rpm for 3 min (Rotina, Hettich, Kirchlengern, Germany), and 0.4 mL of the extract was used in a reaction with 0.1mL of amylase SD reagent solution at 40 °C for 10 min. After completion of the reaction, the absorbance was measured at 400 nm.

Polyphenol oxidase (PPO) activity was assessed in accordance with the AACC 22-85.01 method (AACC, 2000). To 50 mg of flour, 1.5 mL of a 10 mM solution of L-DOPA in a 50 mM MOPS buffer at pH 6.5 was added. After vigorous vortexing (MS 3 shaker, IKA, Germany) at 1.000 rpm for 15 min, the samples were subsequently centrifuged at 14.800 rpm for 5 min. The resulting supernatant was utilized for spectrophotometric analysis at 475 nm. PPO activity is reported as the average absorbance value obtained from three replicates.

The spectrophotometer PerkinElmer Lambda 35 UV/Vis (Waltham, MA, USA) was used for all spectrophotometric measurements done in duplicates.

Table 1 PEF-specific energy input (W_s), temperature, electrical conductivity, kinetics constant (K) of the residual β-glucanase activity as a function of W_s or EFI (with coefficient of determination and p-value according to first-order kinetic model) of water suspension of oat and barley flour at the selected electric field intensity (EFI 12 kV/cm), 81,000 pulse number, and 2 µs pulse width during 162 ms

Flour	W_s (kJ/kg)	Temperature (°C)		Electrical conductivity (mS/cm)		W_s			EFI		
		Initial	End	Initial	Post-process	K	R^2	P	K	R^2	P
Oat	4.48	20.0 ± 0.00	21.05 ± 0.07	0.793 ± 0.002	0.997 ± 0.004	1.937	0.867	0.006	0.078	0.961	< 0.001
Barley	5.53	20.1 ± 0.00	21.60 ± 0.14	1.802 ± 0.005	2.022 ± 0.012	0.607	0.683	0.044	0.030	0.540	0.075

Scanning Electron Microscopy

A scanning electron microscope (SEM, Vega 3 LMH, Tescan, Brno, Czech Republic) was used to observe microstructure of untreated and PEF-treated barley and oat flour samples. The samples were coated with a thin layer of a gold-palladium alloy prior to imaging with SEM operating at an electron acceleration voltage of 2.5 kV.

β-D-Glucan Content and Characterization of Polysaccharidic Fractions

Total β-D-glucan contents of control and PEF-treated flour as well as bread samples were determined in duplicate according to AOAC Method 995.16 using the enzymatic assay kit (K-BGLU 07/23, Megazyme, Bray, Ireland).

Prior to the extraction and characterization of polysaccharides, the homogenized material underwent a series of washing steps to remove lipids, pigments, and other ballast molecules. To remove water residues, samples were successively washed with hexane, acidified ethanol (0.2 mol L^{-1} HCl in 80% aqueous ethanol), aqueous ethanol (80%, v/v) until reaching a neutral pH, and finally with ethanol (96%, v/v) and acetone. After washing, the re-homogenized solids were first subjected to cold water extraction at 20 °C, followed by hot water reflux extraction at 100 °C.

Fourier Transform Infrared (FTIR) Measurement

The FTIR spectra of spectra and all obtained fractions were recorded using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, the sample was mixed with 10 times (v/v) potassium bromide (KBr for IR spectroscopy, Supelco Sigma-Aldrich, MA, USA) and ground thoroughly into fine powder in a mortar. The mixture was then pelleted with a hand press (Pike Technologies, Madison, WI, USA). The FTIR spectra were collected in the transmission mode at wavenumber region from 400 to 4000 cm^{-1} with a spectral resolution of 2 cm^{-1} and in 64 scans on average. FTIR spectra were recorded and subsequently processed (smoothed, baseline corrected, and averaged) using Omnic 8.0 software (Thermo Fisher Scientific, Waltham, USA) and then exported in ASCII format to the Origin 8.0 software (OriginLab, Northampton, MA, USA) for the preparation of output graphs. The spectra are represented as an average value of two repeated measurements for each sample.

Determination of β-Glucan Molecular Weight

A Mw of samples was analyzed using GPC/SEC chromatography system Omnisec Reveal coupled with a multi-angle light scattering (low-angle light scattering (LALS) and right-angle light scattering (RALS)), viscosity (DP), and refractive index (RI) detectors (Malvern Panalytical, Westborough, USA). Two GPC/SEC columns YMC-Pack Diol-200/S-5 µm/8 × 30 mm and YMC-Pack Diol-120/S-5 µm/8 × 30 mm (YMC, Japan) and a guard column (DL12S05-0308WTG, YMC, Japan) were eluted by 0.1 M sodium nitrate at a flow rate of 0.7 mL/min. The detectors and the columns were tempered at 35 °C. Calibration was performed with a Polycal pullulan/dextran standards (Malvern Pananalytical, Westborough, USA). The samples and standards were injected twice at volume 100 µL. The chromatographic system, as well as the acquisition and data analysis, was controlled by OMNISEC software v11.35 (Malvern Pananalytical, Westborough, USA).

Monosaccharide Composition and Linkages (Methylation)

The monosaccharide composition was determined after hydrolysis of sample (1–2 mg) with 72% H_2SO_4, followed by reduction and acetylation (Passos and Coimbra, 2013). The resulting compounds were analyzed as alditol acetates by GC-FID (Shimadzu GC2010, Kyoto, Japan) on a capillary column DB-225 (30 m length, 0.25 mm internal diameter, and 0.15 µm film thickness).

The AX concentration was estimated according to Zambrano et al. (2023) following Eq. 4:

$$\mathrm{AX}\ (\%) = (\mathrm{Ara}\ (\%) + \mathrm{Xyl}\ (\%)) \times 0.88 + \mathrm{Gal}\ (\%) \times 0.9 \quad (4)$$

The yield calculation for AX also considered galactose, as small quantities of galactose have been suggested to be present in AX by Paz-Samaniego et al. (2019).

Polysaccharides were methylation in dry DMSO (1 mL) with CH_3I, concentrated to dryness, hydrolyzed with 2 M TFA (120 °C for 1 h), reduced with $NaBD_4$, and subsequently acetylated. Partially methylated alditol acetates were analyzed with GC–MS (Shimadzu 2010 SE, Kyoto, Japan) on a capillary column HP-5 (30 m length, 0.25 mm internal diameter, 0.15 µm film thickness) according to Passos and Coimbra (2013). Duplicate samples were prepared for both analyses.

NMR Spectroscopy

The purified water-soluble fractions (F1 and F2) were selected and analyzed on a Bruker Avance III™ 500 MHz NMR spectrometer (Bruker, Billerica, MA, USA). Proton NMR and ^{13}C APT NMR spectra were recorded for D_2O solutions at 20 °C and 80 °C and processed using MestReNova 10.0 software (Mestrelab Research, Santiago de Compostela, Spain). Correlation 2D NMR experiments ^{1}H, ^{1}H COSY, ^{1}H, ^{13}C HMQC and ^{1}H, ^{13}C HBMC were used for the proton and carbon signal assignment. The ^{1}H NMR spectra of the chosen purified fractions were exported

as the ASCII data files to the Origin 6.0 (Microcal Origin, Northampton, MA, USA) software and normalized using least-squares curve-fitting (PeakFit module of Origin 6.0) and multiple Voigt (Gaussian–Lorentzian mix) curves in the region of 4.40–4.85 ppm. The Voigt components centered at 4.53–4.56 and 4.74–4.76 ppm were used to calculate the ratio between the 1,4- and 1,3-linked β-D-glucopyranosyl units in the mixed-linkage β-D-glucan.

Determination of Gluten Aggregation and Pasting Properties of Wheat Flour and Oat/Barley Flour Blend

The pasting characteristics of the flour blend were evaluated following the AACC (2000) method 76–21.02 using a Micro-ViscoAmylograph (Brabender GmbH & Co. KG, Duisburg, Germany) in a measuring range of 235 cmg. Suspensions of flour blend (10 g of flour 14% moisture basis suspended in 105 mL of distilled water) were equilibrated at 30 °C, heated to 93 °C at a rate of 7.5 °C/min, upheld at 93 °C for 5 min, cooled to 50 °C at a rate of 7.5 °C/min, and held at 50 °C for 1 min. The parameters derived from the recorded curve included maximum viscosity (MV), cold paste viscosity at the end of the test (CPV), breakdown viscosity (BV), and set-back viscosity (SV), all measured in Brabender Units (BU).

The wheat flour (70%, w/w) was blended with oat or barley flour (30%, w/w) both corrected to the 14% moisture. Gluten aggregation was evaluated using the GlutoPeak instrument (Brabender GmbH & Co. KG, Duisburg, Germany), following the method by Wang et al. (2018). The flour blend (8 g) was introduced into a 0.5 mol/L $CaCl_2$ solution (10 g). The test was conducted at a stirring speed of 3.000 rpm, at a temperature of 20 °C, lasting for 3 min. The obtained parameters were encompassed Peak Maximum Time (PMT, in minutes) and Maximum Torque (BEM, in Brabender Units, BU), representing the time prior to maximum torque decline and the peak resistance during mixing, torque before maximum (AM), and gluten aggregation energy (AGGEN), respectively.

Experimental Bread Making

A control wheat single layer flat bread (typical for Croatia), as well as four types of composite breads in which 30% of wheat flour was replaced with oat or barley flour, untreated or PEF-treated was prepared. The addition of water was adjusted until a farinograph consistency of 200 BU was achieved in each formulation (75% for wheat control, 82% for barley, and 86% for oat composite bread) considering the amount of water already contained in PEF-treated samples. Instant yeast (Lesaffre Adriatic Ltd., Croatia, 0.4%), table salt (2%), and oil (4%) were added on flour weight basis.

Dough was kneaded in a spiral mixer (Diosna SP12, Osnabrück, Germany) in several stages. Flour was mixed with water for 2 min at 90 rpm; then, yeast and salt were added, and fast mixing was continued at 120 rpm for 5 min. After this time, oil (4% at flour weight) was added, and mixing continued for 2 more minutes. The resulting mixture was subjected to bulk fermentation (28 °C, relative humidity 75%, 1.5 h), divided into round balls (350 g), placed in metal pans (20 cm diameter), and subjected to proofing at 28 °C, RH 75%, 1 h (proofing cabinet, Wiesheu, Affalterbach, Germany). The flat bread was baked in a deck oven (Wiesheu, Affalterbach, Germany) at a temperature of 240 °C for 30 min with 0.21 mL cm^{-2} of steam. Breads were baked in triplicate and cooled for 1.5 h at ambient conditions for subsequent measurements.

Oscillatory Rheology Measurement

To assess the viscoelastic properties of dough prepared as previously described, oscillatory measurements were performed in duplicate with parallel plate geometry of 25 mm diameter and 2 mm gap (MCR 92 rheometer, Anton Paar, Graz, Austria). A constant frequency of 6.28 rad/s, a shear strain of 0.05%, a pressure of 0.1 to 100 Pa, and 0.1% of the shear strain, within the linear viscoelastic region, were set for the stress sweep test. The Peltier temperature control device P-PTD200/AIR (Anton Paar, Graz, Austria) was used to maintain a constant temperature during the test. The loss factor (tan δ) was calculated as the ratio between the energy lost and the energy stored in the test (G''/G').

Determination of Bread Physical Properties

The volume of flat breads was determined according to AACC 10–05.01 method (AACC, 2000) in duplicate. The specific volume of bread was calculated after dividing volume with weight (Eq. 5):

$$\text{Specific bread volume}\left(\text{mLg}^{-1}\right) = \frac{\text{Bread volume (mL)}}{\text{Bread weight (g)}} \tag{5}$$

The height (4 edges of the bread and the center) and width (2 places) of the bread were measured with a caliper to calculate the spread ratio as width/height.

The texture profile (TPA) of the crumb was measured using a texture analyzer (TA1 Texture Analyzer, Ametek Lloyd Instruments Ltd., West Sussex, UK) with a 55 mm diameter aluminum probe. Immediately before the analysis, the bread slices with 12.5 mm thickness were cut into 36 mm diameter pieces. Two pieces stacked together were compressed to 50% of their original height at test speed 2 mm/s and 30 s pause between the first and second compression. The hardness, chewiness, and resilience were evaluated in six replicates using a NexygenPLUS Software (Ametek Lloyd Instruments Ltd., West Sussex, UK).

The *L a b* system was used for determining upper (top) crust color at five points of three bread pieces with a colorimeter (Konica Minolta CM-700d, Osaka, Japan).

Determination of Dietary Fiber Content in Bread

Preparation of flat bread samples for dry matter determination (AACC 44-15A method) was carried out according to AACC 62-05 method. Total dietary fiber (TDF) was determined according to AACC Method 32-05.0 and AOAC Method 985.29 using Total Dietary Fiber Assay Kit (Megazyme, Bray, Ireland). Analyses were done in duplicate.

Statistical Analyses

To assess significant differences between all samples, one-way analysis of variance (ANOVA) with Tukey post hoc test was performed. After excluding control wheat sample, the interactions between flour type and treatment were assessed with two-way ANOVA. ANOVA and Pearson correlation test were performed at a significance level of $p < 0.05$ using Statistica 14 (TIBCO Software Inc., CA, USA).

Results and Discussion

Effect of PEF Treatment on Flour β-Glucanase Activity

The initial activity of β-glucanase was 24% higher in oat than in barley flour. Oat β-glucanase appeared to be consistently inactivated across all tested EFI levels (Fig. 1a). The lowest residual activity was observed after treatment at 12 kV/cm, with only 29.8% of the initial activity retained. In contrast, barley β-glucanase exhibited an activity increase of 22.4% already after treatment at 4.5 kV/cm, while the lowest residual activity of 52.6% was recorded after treatment at 16 kV/cm. These findings are consistent with several previous studies. A similar pattern of increased enzyme activity at lower EFI, followed by a decrease at higher EFI, was reported by Li et al. (2022) and Zhang et al. (2017). In addition, Ohshima et al. (2007) found that horseradish peroxidase at low PEF intensities (< 12 kV/cm) shows a gradual increase in activity, while higher intensities (≥ 12 kV/cm) lead to a reduced activity.

Further on, oat β-glucanase exhibited a substantial decline in activity (76.5%) already after 18 ms of PEF treatment at 12 kV/cm which remained constant even at prolonged treatment time (Fig. 1b). In contrast, barley β-glucanase showed a continuous activity decrease along the treatment time, reaching its maximum reduction of 40.2% after 162 ms at 12 kV/cm (Fig. 1a). Similary, Ohshima et al. (2007) proved that the activity of peroxidase and β-galactosidase decreases with longer treatment time.

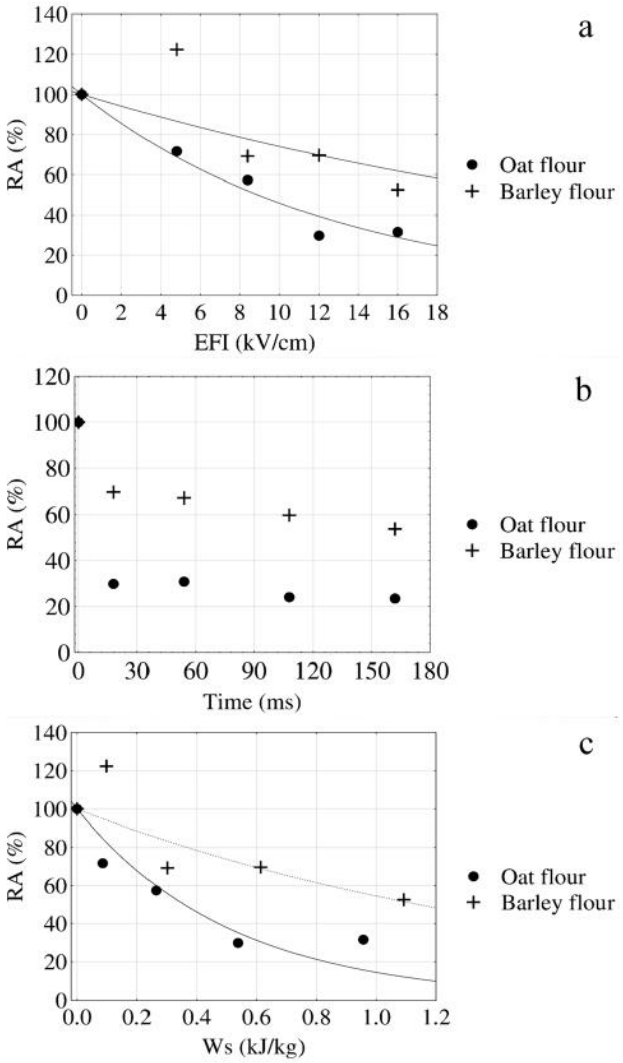

Fig. 1 The residual activity (RA) of oat and barley flour β-glucanase exposed to **a** different EFI at constant treatment time (18 ms), **b** different treatment time (ms) at constant EFI (12 kV/cm), and **c** residual β-glucanase activity in oat and barley flour submitted at different specific energy inputs supplied by high-intensity pulsed electric fields from 4.8 to 16 kV/cm for 18 ms. The values are shown as mean ± standard deviation ($n = 2$)

Consistently with EFI, the effects of different specific energy intakes on the RA of β-glucanase differed between oat and barley flour. With increasing specific energy input, β-glucanase activity of oats was decreasing, whereas an initial activity increase followed by a decrease was observed in barley. The dependence of the RA of oat β-glucanase was well explained by EFI-based exponential decay model (Table 1). Nevertheless, this model did not significantly explain the RA of barley β-glucanase due to the recorded activity increase at low EFI. The Ws-based model significantly explained the dependence of the RA of barley β-glucanase with coefficient of determination of 68%.

Despite the relatively low coefficient of determination, the model shows faster kinetics of the β-glucanase inactivation by PEF in oats compared to barley flour. PEF treatment has some mechanism to activate and inactivate enzymes, and the effective PEF strength differs by the type of enzymes and matrix (Ohshima et al., 2007). The enzyme inactivation by PEF treatment is explained by the impact of high electric field pulses on the three-dimensional structure of the globular protein (Duque et al., 2019). The PEF induces partial unfolding of protein molecules, enhances ionization of sulfhydryl (SH) groups within proteins, and triggers conformational changes and subsequent loss of activity due to challenges in substrate binding at the active site (Fernandez-Diaz et al., 2000). Our study shows that the inactivation of oat β-glucanase is easier than for barley β-glucanase which requires higher EFI, longer treatment time, and Ws. Such an extent and difference in the inactivation between barley and oat β-glucanase were obtained after treatment with high-intensity ultrasound (Grgić et al., 2023). Unlike with ultrasound, the sample heating during PEF treatment was avoided (Table 1).

Considering that after EFI treatment at 16 kV/cm, the activity of oat β-glucanase increased, and the highest inactivation of barley β-glucanase occurred after 162 ms of treatment; further investigation was continued with PEF treatment with EFI of 12 kV/cm and 162 ms (Table 1) of both samples under the same conditions.

Morphological Properties of PEF-Treated Oat and Barley Flour

The SEM images of the untreated and PEF-treated oat and barley flour samples are shown in Fig. 2. Untreated barley flour consisted of irregularly shaped particles with a size of 100–500 µm, which were mainly aggregates of starch, fiber, and proteins (Fig. 2a, b). A relatively large proportion of smaller particles with a size of 10–30 µm, mostly corresponding to individual starch granules, was also observed in barley flour (Fig. 2a). Untreated oat flour consisted predominantly of individual or clustered starch granules, typically 5–100 µm in size, with protein fragments closely adhering to their surface (Fig. 2d, e). In both the untreated oat and barley flour samples, the surface of the individual starch granules appeared uniformly smooth, without cracks or pits. The protein matrix in the untreated barley flour had a slightly wrinkled surface, while the protein fragments in the untreated oat flour exhibited a more granular appearance, but in both samples again without visible cracks or pits on the surface (Fig. 2c, f). The SEM images of the PEF-treated samples showed that the PEF treatment generally had no effect on the size and shape of the flour particles (Fig. 2g, i). In the PEF-treated barley flour samples, morphological changes in the form of small (approx. 100 nm) holes and circular or elliptical pits (100–1000 nm in size) were observed on the surface of the protein matrix (Fig. 2h), most likely caused by a localized high-energy discharge of the electric field. PEF treatment induces unfolding of protein molecules, affecting their secondary and tertiary configuration, which can subsequently lead to interactions and aggregation (Shams et al., 2024). In this study, these changes after the PEF treatment of oat flour were much more discrete and were manifested in the sporadic appearance of shallow pits on the surface of the protein fragments (Fig. 2j). In both PEF-treated samples, no morphological changes were observed on the starch granules even after a detailed SEM examination of the samples.

Total β-Glucan Content in Control and PEF-Treated Oat and Barley Flour

Cereal β-glucan is predominantly a soluble dietary fiber found mainly in the endosperm cell wall and aleurone layer of oats and barley (Shoukat & Sorrentino, 2021). Total β-glucan content was 10.5% higher in untreated oat flour than in barley flour (Table 2). In agreement, Zhang et al. (2019) found 2.65–4.73% of *β*-D-glucan in oat flour. Similarly, β-glucan content in barley grain is reported between 2 and 11% but most often ranges between 4 and 6% (Goudar et al., 2020). After PEF treatment, total *β*-D-glucan content significantly increased (33.5%) compared to untreated samples (Table 2). These results were a consequence of PEF-assisted extraction which increases the rate of mass transfer by electroporation of cell membranes (Kumari et al., 2019). An increased electrical conductivity of oat (20%) and barley (11%) flour (Table 1) after PEF treatment indicated the increase in cell membrane permeability resulting in glucans' better extraction (Nowacka et al., 2019). In addition, the results may be attributed to the inactivation of endogenous enzymes, including β-glucanase by PEF processing, since *β*-D-glucan content correlated negatively with β-glucanase activity ($r = -0.828$, $p = 0.011$). In agreement, Duque et al. (2019) found an increase in *β*-D-glucan content in oat flour (12–20%) after PEF treatment at 4.1–4.3 kV/cm, which they assumed to be related with a possible decrease in β-glucanase activity.

Molecular Weight of Water-Soluble Fractions of Control and PEF-Treated Oat and Barley Flour

The results of GPC analysis of the purified water-soluble polysaccharide fractions F1 (cold water soluble) and F2 (hot water soluble) obtained from control and PEF-treated oat and barley flours are summarized in Table 3. As a rule, the Mw values of F1 were higher than those of F2 fractions. For the control flours, the Mw values of both fractions obtained from oat flour (371,000 and 318,000 g/mol) were higher than Mw of the corresponding fractions from barley flour

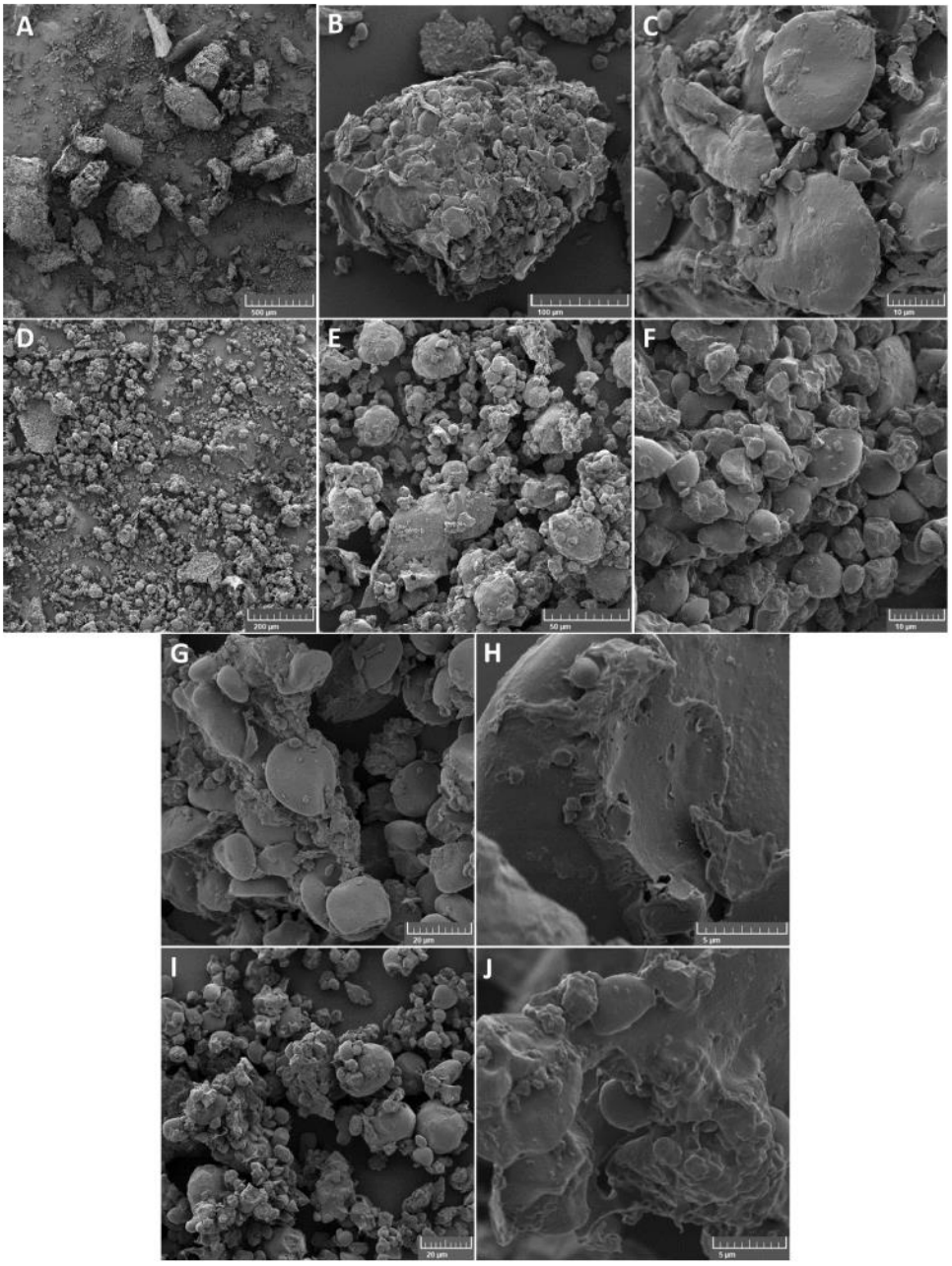

Fig. 2 SEM images of the untreated (**a–f**) and PEF-treated (**g–j**) barley (**a–c**, **g**, **h**) and oat (**d–f**, **i**, **j**) flour samples

(339,000 and 233,000 g/mol). The results are consistent with previous studies describing oat β-D-glucans as having a higher molecular weight than barley β-D-glucans. Liu and White (2011) reported the Mw of oat β-D-glucan ranging from 6.81×10^5 to 7.85×10^5 g/mol, and Karimi et al. (2019) found that the Mw of barley β-D-glucan varies between 0.8×10^4 and 3.77×10^5 g/mol. On the other hand, Zhang et al. (2019) found the average Mw 1.30×10^5 g/mol of oat β-D-glucan and a wide range from 4.05×10^4 to 5.22×10^5 g/mol. This distinction can be attributed to the environment and genotype differences in oats. Higher Mw parts of the fractions are associated with the contribution of β-D-glucan and AX, while the starch and protein residues retained after enzymatic treatments contribute to lower Mw parts. For F1 obtained from oat flour, the main polysaccharide was found to be β-D-glucan (see results of composition and linkage analyses and NMR assignments). Thus, in this case, the PEF treatment leading to a decrease of Mw from 3.71×10^5 to 3.39×10^5 g/mol relates to the partial degradation of this polysaccharide. Previously, the effect of PEF processing on the Mw of various polysaccharides has been studied (Han et al., 2012; Luo et al., 2010; Ma et al., 2012; Maniglia et al., 2021; Rivero-Ramos et al., 2023). For example, Han et al. (2012) demonstrated that PEF treatment causes depolymerization

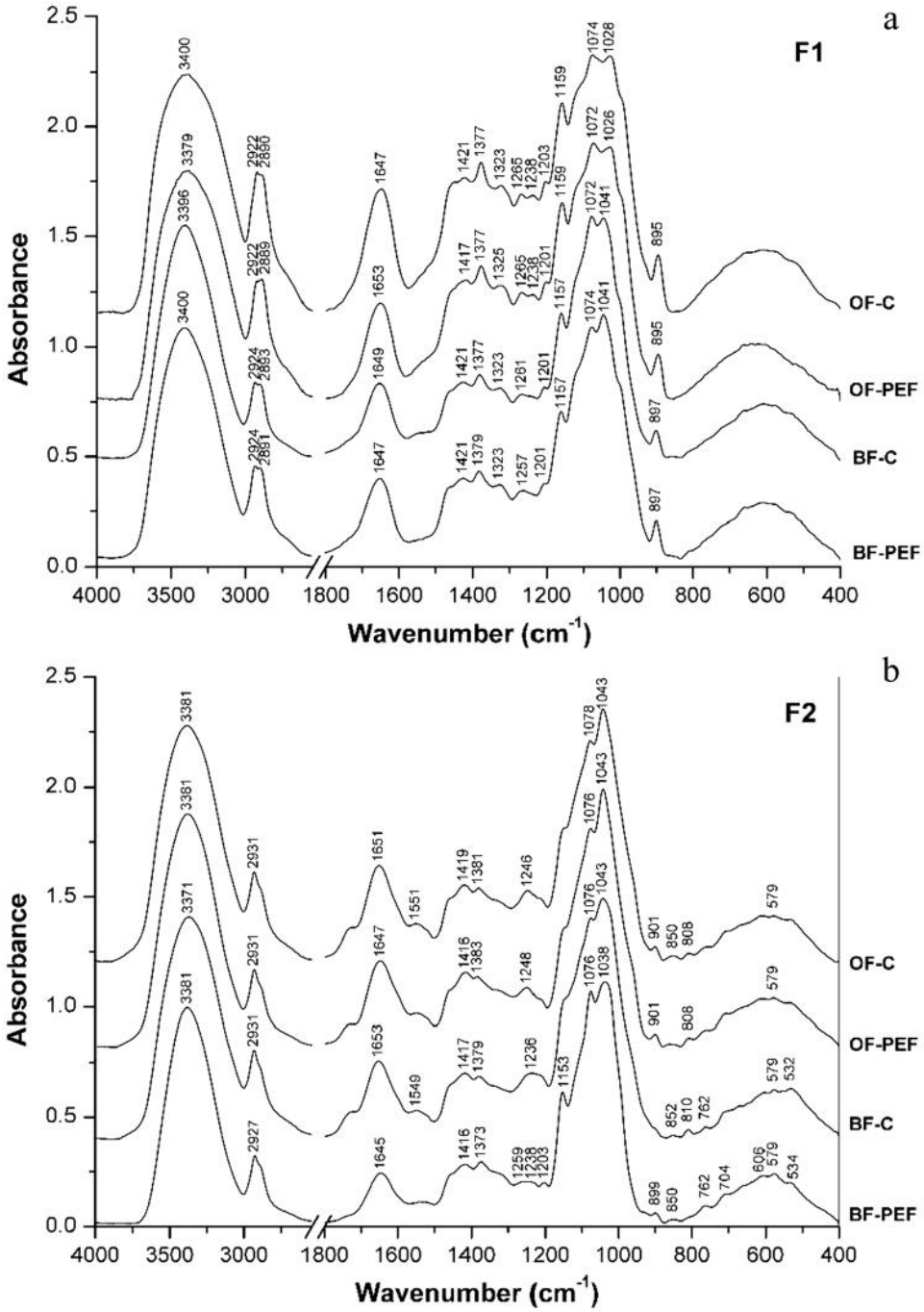

Fig. 3 FTIR spectra of purified polysaccharide fractions F1 (**a**) and F2 (**b**) obtained from oat and barley flour, control and PEF-treated

of corn starch and reduces its Mw. Ma et al. (2012) demonstrated a 9–31% decrease in Mw of sugar beet pectin after the PEF treatment (18–30 kV/cm, 806–2418 µs, 1 kHz). Luo et al. (2010) attributed the Mw decrease of PEF-treated chitosan to the breaks in the glycosidic linkages caused by the hydroxyl radicals ·OH. Yet, no study investigated the Mw of β-glucans. In this study, the opposite effect of PEF treatment was observed for F1 fractions from barley flour, i.e., an increase of Mw from 3.30×10^5 to 3.76×10^5 g/mol that could be due to the higher contribution of AX, while

Table 2 Total β-d-glucan content in the control and PEF-treated oat and barley flour

Flour	β-Glucan (% d.w.)
Oat control	2.81 ± 0.22^{b}
Oat-PEF	3.75 ± 0.14^{ab}
Barley control	3.14 ± 0.02^{b}
Barley-PEF	4.19 ± 0.19^{a}

Values within the same column marked with different letters (a and b) differ significantly according to Tukey's test ($p < 0.05$)

Table 3 The results of GPC analysis of the purified polysaccharide fractions F1 and F2 obtained from the control and PEF-treated oat and barley flour

Fraction	Sample	RV (mL)	M_w ($\times 10^5$ g/mol)	M_n ($\times 10^5$ g/mol)	M_w/M_n	[η] (dL/g)
F1	Oat control	13.22	3.71	3.62	1.02	2.80
	Oat-PEF	13.52	3.39	3.20	1.06	2.00
	Barley control	14.36	3.30	2.99	1.10	4.02
	Barley-PEF	13.84	3.76	3.47	1.09	2.36
F2	Oat control	15.77	3.18	2.80	1.14	1.55
	Oat-PEF	14.30	3.37	1.40	2.40	3.24
	Barley control	16.43	2.33	1.92	1.21	1.09
	Barley-PEF	15.96	3.47	2.92	1.19	0.86

RV retention volume, M_w weight average molecular weight, M_n number average molecular weight, M_w/M_n polydispersity index, *[η]* intrinsic viscosity

the polydispersity Mw/Mn changed slightly and was a little higher for the barley flour products (1.09–1.10) than for the oat products (1.02–1.06) because of more complex nature of the former as a mixture of linear β-d-glucan and branched AX macromolecules. In both samples, the PEF treatment led to significant decrease of intrinsic viscosity [η] from 2.8–4.0 to 2.0–2.35 dL/g. For the F2 fractions, the PEF treatment caused an increase of Mw by 0.20×10^5 g/mol for oat flour and 1.14×10^5 g/mol for barley flour. The effects of PEF on the composition and other GPC parameters of F2 were different for oat and barley flour. The oat product containing maximal contribution of AX (− 55 mol %) demonstrated the highest values of polydispersity (2.40) and [η] (3.24 dL/g), while the barley product having a minimal amount of AX (− 15 mol %) showed the lowest value of [η] (0.86 dL/g).

Monosaccharide Composition and Linkage

The composition of neutral monosaccharides in each fraction is summarized in Table 4. In both fractions of oat and barley flours, glucose prevailed, followed by xylose and arabinose, implying the presence of β-glucans and AX. In addition, the F2 fraction contained a substantial proportion of mannose and galactose, indicating the possible presence of some galactomannans. Glucose was predominant in all fractions, being higher in F1 in oat (94 mol %) than in barley flour (71 mol % in F1), but in F2, it was equally present in both samples.

The influence of PEF processing on glucose content among fractions was adverse; it had no significant effect in the F1 while 18% decreasing effect in F2 of oat flour, whereas 18% decrease in F1 and 52% increase in F2 were found in barley flour (Table 4). This can be related to changes in total β-glucan content found after PEF processing (Table 2). Barley and oat flour are a rich source of AX in addition to β-glucans (Zambrano et al., 2023). Compared to oat flour, the lower glucose content in barley flour was complemented with the higher content of arabinose, xylose, and hence AX in F1, while vice versa was in F2 (Table 4). After PEF treatment of barley flour, the content of arabinose and xylose substantially increased in F1 while it decreased in F2. The PEF treatment of oats led to a 40.6% increase in the AX proportion in the F1 fraction, while their increase in F2 was 10%.

PEF treatment did not significantly affect the Ara/Xyl ratio, but it significantly increased AX content in F1 of both flours, which might be explained by the greater extraction of this polysaccharide. The Ara/Xyl ratio as well as the positively correlated AX content further increased (13% and 10%, respectively) in F2 of oat flour, while Ara/Xyl ratio increased and the proportion of AX decreased (7% and 36%, respectively) in F2 of barley flour. This was confirmed by the negative correlation between these two parameters ($r = -0.987$, $p = 0.013$). These changes in monosaccharides in fractions F1 and F2 after PEF treatment can be attributed to changes in the structure of the polysaccharides, which contribute to greater or lesser water solubility, and to their ability to bind with other polymers (such as hemicellulose) found in plant tissues (Zannini et al., 2022). In general, the higher the Ara/Xyl ratio, the higher the solubility of AX derived from the endosperm (Lazaridou et al., 2008; Zambrano et al., 2023). In contrast, Izydorczyk et al. (2003) demonstrated that insoluble AX from endosperm have higher A/X ratio than soluble AX. However, there is always some variation, usually due to the variety, the germination condition, or the nature of the other polymers in the grain (Zannini et al., 2022).

Table 5 demonstrates the results of methylation analysis for each fraction. Glucose predominating in F1 fractions obtained from control and PEF-treated oat flour is represented mainly by the 1,4-substituted glucosyls (~72—88%) and by fewer amounts of 1,3-linked glucosyls (~7%), which indicates mixed-linkage *β*-D-glucan as expected. A few terminal, 1,4,6- and 1,6-linked glucosyl fragments originated from starch, which were more pronounced for the PEF-treated oat flour, remained in F1 after the enzymatic

Table 4 Molar ratio (%) of monosaccharides and arabinoxylan (AX) content (% of fraction) in the fractions obtained from oat and barley flour before and after PEF treatment

Fraction	Sample	Fuc	Ara	Man	Glc	Gal	Rha	Xyl	AX	Ara/Xyl ratio
F1	Oat control	2.33 ± 0.03^{a}	1.17 ± 0.06^{c}	0.27 ± 0.02^{b}	94.11 ± 0.3^{a}	0.92 ± 0.01^{b}	0.38 ± 0.02^{ab}	1.32 ± 0.02^{c}	2.37 ± 0.03^{d}	0.95 ± 0.04^{a}
	Barley control	2.21 ± 0.14^{a}	10.44 ± 0.49^{b}	0.85 ± 0.04^{a}	70.86 ± 1.16^{b}	1.07 ± 0.11^{a}	0.28 ± 0.01^{a}	14.28 ± 0.4^{b}	22.72 ± 0.87^{b}	0.73 ± 0.01^{ab}
	Oat-PEF	2.57 ± 0.35^{a}	1.98 ± 0.12^{c}	0.31 ± 0.01^{b}	92.24 ± 0.04^{a}	0.22 ± 0.00^{b}	0.35 ± 0.02^{a}	2.33 ± 0.27^{c}	3.99 ± 0.34^{c}	0.85 ± 0.05^{a}
	Barley-PEF	1.23 ± 0.27^{b}	16.02 ± 0.2^{d}	0.47 ± 0.00^{b}	57.86 ± 0.5^{c}	0.41 ± 0.03^{b}	0.20 ± 0.03^{b}	23.80 ± 0.09^{a}	35.41 ± 0.23^{a}	0.67 ± 0.01^{b}
F2	Oat control	0.38 ± 0.09^{a}	19.98 ± 0.36^{b}	10.34 ± 0.01^{b}	32.57 ± 0.69^{c}	14.67 ± 0.05^{c}	0.56 ± 0.09^{b}	21.50 ± 0.09^{a}	49.71 ± 0.44^{b}	0.93 ± 0.01^{c}
	Barley control	0.30 ± 0.00^{ab}	7.79 ± 0.08^{c}	20.77 ± 0.43^{a}	36.83 ± 0.15^{b}	23.62 ± 0.5^{a}	0.87 ± 0.02^{a}	7.08 ± 0.15^{c}	34.34 ± 0.65^{c}	1.1 ± 0.01^{b}
	Oat-PEF	0.20 ± 0.00^{ab}	21.86 ± 0.04^{a}	10.45 ± 0.03^{b}	26.57 ± 0.02^{d}	19.97 ± 0.02^{b}	0.49 ± 0.01^{b}	20.45 ± 0.03^{b}	55.22 ± 0.01^{a}	1.07 ± 0.00^{b}
	Barley-PEF	0.16 ± 0.03^{b}	4.87 ± 0.07^{d}	5.94 ± 0.02^{c}	77.32 ± 0.06^{a}	7.12 ± 0.09^{d}	0.48 ± 0.00^{b}	4.11 ± 0.02^{d}	15.28 ± 0.02^{d}	1.18 ± 0.01^{a}

Values within the same column (for each fraction) marked with different letters (a–d) differ significantly according to Tukey's test ($p < 0.05$)

hydrolysis. Additionally, in this fraction, xylose and galactose fragments were absent in the control. Therefore, the PEF treatment of oat flour supports the co-extraction of some AX and, probably, arabinogalactan together with *β*-D-glucan by cold water.

The corresponding F1 fraction from barley flour contained significantly fewer glucosyls (~45–55%) due to the presence of significant amount of the AX carbohydrates (~42–47%), represented mainly by the non-branched 1,4-linked xylopyranosyls (~20–28%). The mono- and disubstituted xylopyranosyls comprised ~8–11%, while 1,3,4-linked xylosyl fragments were found only in the PEF-modified sample (~4%). The terminal and 1,3-linked anabinosyl fragments represent this sugar (~10–11%), and the latter were found only in the control sample. Therefore, *β*-D-glucan and AX are extracted from barley flour at the comparable amounts, and the PEF treatment of barley flour caused partial degradation of the AX side chains.

In the F2 fraction obtained from control and PEF-treated oat flour, the AX fragments (~46–49.5%) prevailed significantly among those of glucans, and 1,4-linked glycosyl comprised only ~14–15%. The terminal, 1,4,6- and 1,6-linked glycosyls (~15%) confirmed the presence of starch residues, while the 1,3-linked glucosyls from *β*-D-glucan were much less pronounced (~2%). AX is represented mainly by terminal arabinofuranosyls (~12–13%) and 1,4- and 1,2,3,4-linked xylosyls (13–14 and 9–10%). Terminal xylosyls and 1,2- and 1,3-linked arabinosyls comprise disaccharide side chains of oat AX (Pastell et al., 2009). These fractions also contained galactosyl and mannosyl fragments, which could be associated with branched arabinogalactan (AG) and glucomannan (GM). Barley cell wall contains about 3–4% of GM (Bader Ui Ain, 2018), but in this polysaccharide, like in other homo- and heteromannans, mannose is represented by the 1,4-linked units (Voiniciuc, 2022). Commonly, AG is part of the structure of pectins and proteins (Su & Higashiyama, 2018), and AG proteins were found in barley cell walls (Makowska et al., 2017). Cereal AG consist of the (1 → 3)-*β*-D-galactan backbone with the *β*-D-galactosyl side chains bound at the *O*-6 position of some backbone galactoses, which in turn may carry α-L-arabinosyls as well as residues of other sugars (Bader UI Ain, 2018). The PEF treatment on the oat flour slightly influenced the composition and structure of AX and other polysaccharides in F2.

Finally, the F2 fraction from control and PEF-treated barley flour contained significantly more glucosyl fragments (~49–80%) and less AX fragments (~9–16%) than the corresponding fractions obtained from oat flour. However, the F2 fraction from PEF-treated barley flour contains much more glucosyl fragments (~80%) and less all other sugar fragments (~12%) than the corresponding control, which had about equal amounts of glucosyls and the other sugars.

Table 5 Methylation results of the fractions obtained from oat (OF) and barley (BF) flour before (C) and after PEF treatment[a]

Sugar derivative	Linkage	Ratio (mol %)							
		F1				F2			
		OF-C	OF-PEF	BF-C	BF-PEF	OF-C	OF-PEF	BF-C	BF-PEF
2,3,4-Me_3-Rha[b]	Rha*p*-(1→							2.0	
	Total							2.0	
2,3,5-Me_3-Ara	Ara*f*-(1→			5.8	10.3	13.4	12.1	3.6	2.0
3,5-Me_3-Ara	→2)-Ara*f*-(1→					3.3	2.9		
2,5-Me_3-Ara	→3)-Ara*f*-(1→			5.2		4.1	3.5	1.0	
	Total			11.0	10.3	20.8	18.5	4.6	2.0
2,3,4-Me_3-Xyl	Xyl*p*-(1→					5.4	5.4	1.8	1.0
2,3-Me_2-Xyl	→4)-Xyl*p*-(1→		1.2	27.5	20.1	14.1	12.7	6.2	3.8
2-Me-Xyl	→3,4)-Xyl*p*-(1→				3.8				
Xyl	→2,3,4)-Xyl*p*-(1→		1.7	8.4	7.4	9.2	9.7	3.2	1.9
	Total		2.9	35.9	31.2	28.8	27.8	11.2	6.7
2,3,4,6-Me_4-Man	Man*p*-(1→					1.8	2.3	4.0	1.0
2,3,4-Me_3-Man	→2,6)-Man*p*-(1→					4.1	3.8	8.4	2.3
	Total					5.9	6.2	12.3	3.3
2,3,4,6-Me_4-Gal	Gal*p*-(1→					1.5	1.6	2.9	
3,4,6-Me_3-Gal	→2)-Gal*p*-(1→					4.5	4.4	9.4	
2,3,4-Me_3-Gal	→6)-Gal*p*-(1→					1.2	2.1	1.2	
2,4-Me_2-Gal	→3,6)-Gal*p*-(1→		1.8			1.0	1.4		
	Total		1.8			8.1	9.4	13.4	
2,3,4,6-Me_4-Glc	Glc*p*-(1→	2.7	3.1	3.2	2.2	6.4	7.3	8.7	10.3
2,4,6-Me_3-Glc	→3)-Glc*p*-(1→	7.1	7.3	5.7	8.6	2.1	1.9	3.5	7.6
2,3,6-Me_3-Glc	→4)-Glc*p*-(1→	87.6	71.7	34.8	44.6	14.8	14.1	20.9	52.8
2,3,4-Me_3-Glc	→6)-Glc*p*-(1→		8.1			3.8	3.7	8.3	5.1
2,6-Me_2-Glc	→3,4)-Glc*p*-(1→							1.1	
4,6-Me_2-Glc	→4,6)-Glc*p*-(1→		1.3	1.3		4.4	4.2	6.1	4.1
	Total	97.4	91.4	45.0	55.4	31.5	31.2	48.7	79.9

[a]Only fragments whose content exceeded 1 mol % are presented

[b]2,3,4-Me_3-Rha = 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl-rhamnitol, etc.

Therefore, the PEF treatment of barley flour promotes complete extraction of β-D-glucan with hot water, which agrees with the NMR data (see Table 4).

FTIR Spectra of Flours and isolated Polysaccharides

The FTIR spectra of the purified polysaccharide fractions F1 and F2 isolated from oat and barley flour, control and PEF-treated samples, are shown in Fig. 3. The spectra of the standard flour components are shown in Fig. S1 for comparison. In all these spectra, the strong and broad band at 3370–3400 cm^{-1} corresponded to the O–H stretching vibrations in water and hydroxyl groups involved in the intra- and intermolecular hydrogen bonding in the polysaccharide network. The narrow region at 2800–3000 cm^{-1} arose from the C–H stretching vibrations. In cold water extracts F1, two bands with almost equal intensity at 2922–2924 and 2889–2891 cm^{-1} were observed in this region, while in hot water extracts F2, only one band with a low-frequency shoulder at 2927–2931 cm^{-1} was observed. The scissor vibration of water molecules and the amide I vibration in protein residues contributed to the band at 1645–1653 cm^{-1} (Kong & Yu, 2007). Two shoulders observed for some F2 around 1725 and 1549 cm^{-1} were assigned to the C=O stretching and amide II vibrations in uronic acids (Bichara et al., 2016) and remaining proteins (Kong & Yu, 2007), respectively. Intense overlapping bands at 950–1200 cm^{-1} were attributed to C–O–C, C–O, and C–C stretching vibrations in polysaccharides. The broad absorbance at 400–800 cm^{-1} arose from the twisting vibration of water molecules participating in intermolecular hydrogen bonds. The band observed at 895–897 cm^{-1} for F1 and at 899–901 cm^{-1} for F2 was assigned to the C1β–H bending vibration characteristic for β-anomeric configuration

in polysaccharides, i.e., β-D-glucans (Zhang et al., 2018) and β-D-xylans (Kačuráková et al., 1998, 2000), which are found in cereal grains and flour. Weak bands at 850–852 and ~ 762 cm^{-1} observed for F2 originate from C1α–H bending, CH_2 deformation, and C–C stretching vibrations in the remaining starch (Fan et al., 2012; Kačuráková et al., 1998, 2000). For oat flour cold water extracts F1, the bands at 895–897, 1072–1074, 1159, 1201–1203, 1238, 1265, 1377, and 1417–1421 cm^{-1} are characteristic of "mixed linkage" β-D-glucan (Bai et al., 2021; Climova et al., 2021; Fusté et al., 2019; Sourki et al., 2017; Zhao et al., 2020). In the cold water extracts F1 from barley flour, most of the corresponding bands were observed at similar positions. However, the band of β-D-glucan at 1038 cm^{-1} was not pronounced in F1, and the band at 1026–1028 cm^{-1} was observed for oat flour and at 1041 cm^{-1} for barley flour instead due to the contribution of starch residues and AX, respectively (Kačuráková et al., 1999; Mikkelsen et al., 2010; Robert et al., 2005). For PEF-treated barley flour, the band at 1041 cm^{-1} was more pronounced than that of control barley flour, and the control band at 1261 cm^{-1} was shifted to 1257 cm^{-1}. These features confirmed higher amount of AX, having corresponding bands at 1044 and 1253 cm^{-1}. For oat flour hot water extracts F2, the band envelope at 900–1200 cm^{-1} is typical for AX, and the loss of peak multiplicity in this region is characteristic of highly substituted AX (Hromádková et al., 2013). The strong and sharp band at 1043 cm^{-1} and band at 1381–1383 and 899–901 cm^{-1} were attributive for β-D-xylan (Hromádková et al., 2013; Kačuráková et al., 1999; Robert et al., 2005). The weak band at 808–811 cm^{-1} originated from Ara*f* furanoid ring vibration in AX (Kačuráková et al., 1998, 1999). The band observed for all F2 at 1076–1078 cm^{-1} indicates a contribution of β-d-galactan (Hromádková et al., 2013; Kačuráková et al., 1999), while starch residues contribute to this band and several weak bands at 532–534, 579, 704, 762, and 850 cm^{-1} (Mikkelsen et al., 2010). Finally, the spectrum obtained for fraction F2 from PEF-treated barley flour looks like a spectrum of β-D-glucan with α-D-glucan (starch) as a concomitant having characteristic bands of both these polysaccharides (Šandula et al., 1999).

The Composition and Structure of Isolated Polysaccharides by NMR

The 1D and correlation 2D NMR spectroscopy was applied to evaluate the composition and structure of polysaccharides in the purified polysaccharide fractions F1 and F2 obtained from control and PEF-treated oat and barley flours. Figure 4a, b demonstrates the ^{1}H and ^{13}C HMQC spectra observed for these products. Peak decomposition analysis of the ^{1}H NMR spectrum of purified polysaccharide fraction F1 obtained from PEF-treated oat flour is shown as an example in Fig. S2. Table 6 summarizes the assignment of the proton and carbon resonance signals to the monosaccharide units, which are labeled as a–m as in the figures. The assignment was based on the relevant literature (Colleoni-Sirghie et al., 2003; Cui et al., 2000; Guo et al., 2019; Johansson et al., 2000; Pastell et al., 2009; Petersen et al., 2015; Roubroeks et al., 2000; Zhang et al., 2018). Unfortunately, not all signals were well expressed enough to identify all these units.

The signals of β-D-glucan (units a, b, and c) predominate in the HMQC spectra of the F1 fractions obtained from the control and PEF-treated oat flour. The spectrum of the control product also had weak signals from starch residues (unit d). In addition to the β-D-glucan signals mentioned above, the HMQC spectra of the corresponding fractions obtained from barley flour also contained weak signals of AX (units g–k), which were more pronounced for the control sample. In contrast to oat flour, the barley flour used in this study contained a cold water soluble AX fraction, which was found in the F1 extracts. The HMQC spectra of the F2 fractions obtained from the control flour and PEF-treated oat flour showed pronounced signals of AX structures (units g–k) and remaining starch fragments (units d, d′, e, and f), while the signals of β-d-glucan were not observed. The insert shows CH_3 signals in *O*-acetyl groups, indicating that some AX units are esterified with acetic acid. The signals assigned to terminal β-D-xylopyranosyls and 1,2-linked α-l-arabinofuranosyls (units g′ and k) confirmed the presence of 2-*O*-β-D-xylopyranosyl-α-L-arabinofuranosyl side chains in the structure of oat AX (Pastell et al., 2009). The HMQC spectra of the F2 fractions obtained from the control flour and PEF-treated barley flour showed significant differences in the composition of these products. Firstly, the α-L-arabinofuranosyl signals (units i and j) predominated for the control product, while the β-D-xylopyranosyl and β-D-glucosyl signals were not pronounced. Secondly, the β-D-glucosyl signals of MLG (units a, b, and c) were the most intense for the PEF-treated product. Thirdly, the α-d-glucosyl signals of starch residue (units d, d′, e, and f) units were pronounced in both cases. These findings indicate that the PEF treatment of barley flour led to significant changes in the composition of F2 obtained by the hot water extraction.

The relative ratio between the integral areas of the anomeric H1 proton signals at 4.75 ppm (unit a) and 4.53–4.55 ppm (units b and c) represent the ratio between 1,3- and 1,4-linked β-D-glucopyranosyl units in cereal β-D-glucans (Colleoni-Sirghie et al., 2003; Mikkelsen et al., 2013; Roubroeks et al., 2000;). The ^{1}H NMR spectra of the F2 fractions did not have the mentioned signals except that of PEF-treated barley flour, which contained a significant amount of β-D-glucan. In addition, other H1 signals, including those of β-D-xylosyls from AX, complicated the integration in the current spectral region. To avoid this, we used a peak-fitting procedure in the region of H1β signals (4.40–4.85 ppm) and obtained the Voigt components

Table 6 Proton and carbon resonance signal assignments for the main units of the polysaccharidic fractions F1 and F2 obtained from control and PEF-treated oat and barley flour

Unit	Structure	δ (ppm)					
		H1/C1	H2/C2	H3/C3	H4/C/4	H5/C5	H6/C6
a	→3)-*β*-D-Glc*p*-(1→4	4.55	3.54	3.76	3.53	3.54	3.76; 3.92
		102.93	73.55	85.17	68.85	76.34	61.48
b	→4)-*β*-D-Glc*p*-(1→4	4.54	3.38	3.66	3.64	3.65	3.82; 3.98
		102.93	73.72	74.89	79.38	75.56	60.92
c	→4)-*β*-D-Glc*p*-(1→3	4.75	3.42	3.67	3.64	3.63	3.82; 3.98
		103.17	74.01	74.89	79.27	75.56	60.97
d	→4)-α-D-Glc*p*-(1→4	5.36	3.64	3.95	3.64	3.84	3.81; 3.89
		100.58	72.51	74.14	78.20	72.20	61.51
d′	α-D-Glc*p*-(1→4	5.36	3.56	3.69	3.44	3.73	3.81; 3.89
		100.58	72.21	73.91	70.43	73.90	61.51
e	→4,6)-α-D-Glc*p*-(1→4	5.31					
		100.35					
f	→4)-α-D-Glc*p*-(1→6	4.96	3.58				
		98.91					
g	→4)-*β*-D-Xyl*p*-(1→	4.46	3.32	3.58	3.80	3.40; 4.11	
		102.70	73.80	74.53	77.11	63.67	
g′	*β*-D-Xyl*p*-(1→	4.46	3.32	3.46	3.62	3.33; 4.01	
		104.00	73.80	76.38	69.97	65.95	
h	→4)-*β*-D-Xyl*p*-(1→	4.58	3.29				
		102.73	73.80				
h′	→2,3,4)-*β*-D-Xyl*p*-(1→	4.65	3.58	3.87	3.75	3.40; 4.11	
		100.60	74.37	74.72	77.76	63.67	
h″	→2,3,4)-*β*-D-Xyl*p*-(1→	4.68	3.61	3.90	3.75	3.40; 4.11	
		100.60	74.45	74.72	77.76	63.67	
i	α-L-Ara*f*-(1→	5.20	4.12	3.96	4.11	3.72; 3.81	
		109.20	82.45	77.11	85.06	62.03	
i′	α-L-Ara*f*-(1→	5.21	4.14				
		107.51	81.99				
j	α-L-Ara*f*-(1→	5.28	4.15	3.93	4.25	3.72; 3.81	
		108.56	81.87	77.93	85.06	62.03	
k	→2)-α-L-Ara*f*-(1→	5.39	4.29	4.12	4.26	3.72; 3.81	
		107.17	88.7	76.85	85.06	62.03	
l	α-L-Ara*f*-(1→	5.06	4.13	3.94			
		108.27	81.93				
l′	α-L-Ara*f*-(1→	5.12	4.12				
		108.78	81.80				
m		5.22	3.55				
		100.90	76.02				
n		5.06	3.57				
		102.62					

centered at 4.53–4.56 and 4.74–4.76 ppm which corresponded to the 1,4- and 1,3-linked *β*-D-glucopyranosyl units, respectively (Fig. 4). The ratio of 1,4- to 1,3-linkages in *β*-D-glucan were 2.46 (F1, control oat flour), 2.59 (F1, PEF-treated oat flour), 2.53 (F1, control barley flour), 2.44 (F1, PEF-treated barley flour), and 2.33 (F2, PEF-treated barley flour). The corresponding ratio in oat *β*-D-glucans obtained from the resonance signals of the anomeric protons ranged from 2.37 to 2.45 for oat varieties and changed slightly after partial hydrolysis (Colleoni-Sirghie et al., 2003). Mikkelsen et al. (2013) reported the *β*-D-glucan linkage patterns (ratio of *β*-1,4 to *β*-1,3 linkages) calculated from the ^{1}H NMR spectra for barley mutant (2.28), barley control (2.42), and oat (2.48). The values determined for oat and barley glucans were quite similar, while the barley mutant showed a significant difference. In the current study, a slight increase in this

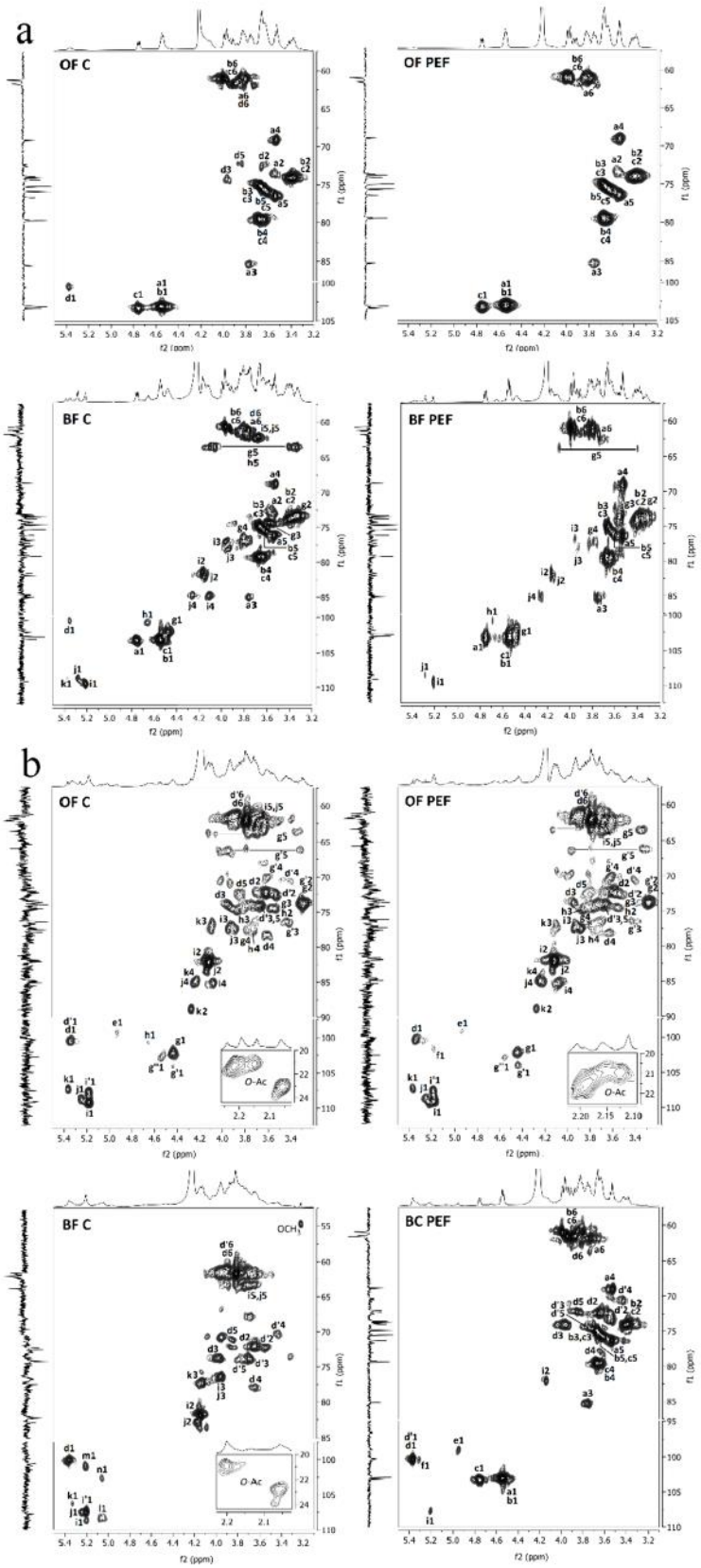

Fig. 4 ^{1}H and ^{13}C HMQC spectra of purified polysaccharide fractions F1 (**a**) and F2 (**b**) obtained from oat and barley flours, control and PEF-treated

value by 0.13 for the PEF-treated oat flour and a simultaneous decrease in Mw could be due to the preferable rupture of the 1,3-glycosidic bonds in oat β-D-glucan. Oppositely, for barley flour, the ratio of 1,4- to 1,3-linkages decreased after the PEF treatment by 0.09 for F1 and by 0.20 for F2, so the cellulose-like fragments could be more sensitive in this case due to the specificity of barley β-D-glucan structure or the influence of other flour components. Even though the changes in this ratio observed for the PEF-treated flours were relatively small, they could indicate a certain sensitivity of cereal β-D-glucans to the PEF treatment. The final effect of this treatment depends on the flour composition and the structural specificity of these polysaccharides, possibly on the distribution of DP3 and DP4 fragments in their chain.

Pasting Properties and Gluten Aggregation of Wheat Flour and Oat/Barley Flour Blend

The pasting profile mirrors real food production processes, aiding in the prediction of the potential industrial (bread making) application of flour since it reflects the starch quality and amylolytic activity of flour. The activity of α-amylase of untreated oat flour (322.04 U/kg) was threefold higher than its activity in barley flour (101.64 U/kg). After PEF treatment, the α-amylase activity of oat flour was reduced by 78%, whereas the amylase activity of barley flour remained unchanged.

Both the flour type and the treatment significantly ($p < 0.05$) affected the pasting properties. Compared to the control wheat sample, the pasting profile of the blend with untreated oat flour was more viscous, whereas the paste containing untreated barley flour was less viscous (Table 7). After adding PEF-treated flours, the MV, CPV, and SV of wheat-oat or wheat-barley paste slightly decreased, but the BV significantly increased compared to samples with untreated flours. The viscosity reduction was unexpected since the α-amylase activity of oat flour was reduced after PEF treatment. The reason could be the depolymerization of starch chains caused by PEF which yields more amylose leaching from the amylopectin (Duque et al., 2019). Jokinen et al. (2023) stated that higher BV with lower peak time, final, and setback viscosity is associated with starch damage and higher amylose content in oat flour. According to Duque et al. (2019), lower values of SV explained the reduced tendency for amylopectin retrogradation in PEF-treated oat samples. Such modification of starch induced by PEF is desirable since retrogradation is the main cause of bread staling (crumb hardening during storage). In this study, the SV values did not decrease significantly, which can be explained by the fact that the MVA analysis was performed on a mixture of wheat and oat/barley flour (for bread dough). We assume that the impact of PEF treatment would be more visible at a higher proportion of treated flour in the blend.

Flour quality can be categorized by the GlutoPeak parameters, where a short PMT and a high BEM indicate high-quality flours, while a longer PMT and lower BEM values indicate weaker flours (Amoriello et al., 2016). The PMT decreased with the addition of untreated oat flour, while it remained unchanged after adding barley flour compared to the control wheat sample (Table 7). Unlike flours with a high gluten content, flours with a high content of dietary fiber (including β-glucans), which have high water absorption capacity, require longer time for the formation of the gluten network (Cao et al., 2023). On the other hand, the unchanged PMT can happen even in the presence of fiber which affects analytical results (Amoriello et al., 2020). An

Table 7 Gluten aggregation, pasting, and viscoelastic properties of control wheat sample and its blend with oat or barley control or PEF-treated flour

Parameter	Wheat control	Oat control	Barley control	Oat-PEF	Barley-PEF
MV (BU)	451.5 ± 5.0^{b}	523.5 ± 12.0^{a}	375.5 ± 7.8^{c}	506.0 ± 5.7^{a}	360.0 ± 5.7^{c}
CPV (BU)	117.8 ± 3.0^{b}	140.1 ± 2.2^{a}	106.5 ± 2.6^{c}	132.7 ± 2.0^{a}	96.1 ± 2.3^{d}
SV (BU)	410.0 ± 18.8^{b}	483.0 ± 8.5^{a}	367.5 ± 10.6^{bc}	463.0 ± 0.1^{a}	333.5 ± 9.2^{c}
BV (BU)	83.0 ± 5.7^{ab}	77.0 ± 6.4^{b}	41.0 ± 1.4^{d}	90.5 ± 0.7^{a}	58.5 ± 0.7^{c}
PMT (s)	82.5 ± 1.4^{a}	65.8 ± 3.2^{b}	87.8 ± 1.8^{a}	33.0 ± 2.1^{c}	63.8 ± 1.8^{b}
BEM (BU)	62.0 ± 0.0^{a}	55.0 ± 2.8^{b}	50.0 ± 1.4^{b}	63.0 ± 1.4^{a}	51.0 ± 1.4^{b}
AM (BU)	24.0 ± 1.4^{bc}	22.5 ± 0.7^{b}	26.5 ± 0.7^{c}	39.0 ± 0.1^{a}	42.0 ± 0.1^{a}
AGGEN (cm^2)	1451.5 ± 8.8^{b}	1229.9 ± 3.6^{c}	1210.9 ± 16.3^{c}	1587.4 ± 0.8^{a}	1444.6 ± 20.6^{b}
Complex viscosity (Pa.s)	102.6 ± 5.1^{d}	157.6 ± 1.8^{b}	121.0 ± 1.7^{c}	218.9 ± 5.1^{a}	118.2 ± 2.6^{c}
τ_{max}	2.02 ± 0.07^{b}	3.54 ± 0.17^{a}	0.49 ± 0.01^{c}	4.15 ± 0.30^{a}	0.42 ± 0.01^{c}

Values within the same row marked with different letters (a–d) differ significantly according to Tukey's test ($p < 0.05$)

MV maximum viscosity, *CPV* cold paste viscosity at end of test, *SV* setback viscosity, *BV* breakdown viscosity, *PMT* peak maximum time, *BEM* maximum torque, *AM* torque before maximum, *AGGEN* gluten aggregation energy, τ_{max} maximum stress tolerated by the sample

additional factor could be the different protein composition, since oats are known for low gluten content, which is lower than in wheat and even lower than in barley (Schalk et al., 2017). Flours with a high protein content and high dough strength build up the gluten network faster and require more energy for effective gluten aggregation compared to lower quality flours (Amoriello et al., 2016). In our study, BEM and AGGEN were also reduced after adding either oat or barley untreated flours (Table 7). Partial replacement of wheat flour with oat or barley flour resulted in a lower gluten concentration and aggregation which indicates a weaker development of gluten network (Wang et al., 2018).

After adding PEF-treated flours, most GlutoPeak parameters changed compared to values obtained after adding untreated oat or barley flour (Table 7). The interaction between flour type and PEF treatment had a significant influence at AGGEN ($p = 0.003$) and somewhat at PMT ($p = 0.053$) and BEM ($p = 0.057$). The PMT of both blends was even shorter after adding PEF-treated flour compared to untreated flours, but the effect was more visible in oat blend. Unlike barley, BEM increased after PEF treatment of oat flour, reaching the value of the control wheat sample. Similary, AM and AGGEN increased after both oat and barley flour were treated in PEF (Table 7). The AGGEN increment was more visible for barley blend than for oat blend. Higher AM and AGGEN values indicate an improved bread making potential of flour (Amoriello et al., 2016; Karaduman et al., 2020). PMT values were inversely correlated with AM ($r = -0.875$, $p = 0.001$). The AM value provides insight into gluten strength before the completion of gluten formation, i.e., higher AM indicates stronger gluten (Karaduman et al., 2020). Our results imply that replacing wheat flour with PEF-treated oat or barley flour has better potential in maintaining the strength of the gluten network than if untreated flours are used. A possible explanation for this is the lower Mw of β-glucans (oat flour) during PEF treatment but also increased proportion of water-extractable AX (Table 7). This was confirmed by the negative correlation between β-glucan Mw and AM and AGGEN of oat flour ($r = 0.990$ or $r = -0.997$, $p \leq 0.01$, respectively) as well as the positive correlation between the content of AX-F1 and AM and AGGEN of barley flour ($r = 0.999$, $p = \leq 0.001$, for both). According to Courtin and Delcour (2002), water-extractable AX due to their high molecular weight form a secondary weaker network, which reenforces the gluten network. It can be concluded that PEF treatment of oat flour in particular had a positive effect on the formation of the gluten network, since a shorter PMT and higher BEM (only for oat), AM, and AGGEN are characteristics of rapid formation of a strong gluten network.

Dough Viscoelastic Properties

The complex viscosity and τ_{max} were dependent on the interaction of flour type and PEF treatment ($p \leq 0.005$). The complex viscosity of the bread dough significantly increased after partial replacement of wheat flour with oat or barley flour (Table 7). The reason for increased viscosity is the high dietary fiber content (including soluble β-D-glucan and arabinoxylan) of these alternative raw materials (Rieder et al., 2012). Unlike barley, the complex viscosity of dough containing oat flour after PEF treatment further increased by 28%. The τ_{max} value increased significantly after adding oat flour untreated or even more if PEF-pretreated. The opposite, τ_{max} drastically reduced after adding barley flour, equally with untreated or treated sample (Table 7). This means that barley samples were softer and consequently could withstand less force without structural deformation, while the oat samples became harder and consequently could withstand more force compared to the control. During PEF treatment, due to extraction, the proportion of soluble AX-F1 of oat flour increased by 40%, while the content of AX-F2 remained unchanged, resulting in a slight increase in the complex viscosity and τ_{max} of the WOF-PEF sample. In barley flour, on the other hand, the proportion of AX-F1 increased by 36%, but at the same time, the proportion of AX-F2 (56%) decreased (Table 4); hence, the complex viscosity and τ_{max} remained unchanged after PEF treatment. The Mw of β-D-glucan is also thought to affect the rheology results, but the reduction in Mw that occurred because of PEF treatment was masked due to the lower β-glucanase activity. Our study shows that PEF technology affects biopolymers and rheological properties of the dough depending on the flour type.

Physical and Nutritional Properties of Flat Bread Made with Oat or Barley Flour

Table 8 shows the nutritional and physical properties of flat breads. Replacing semi-refined wheat flour (30%, w/w) with oat or barley flour in the formulation resulted in flat breads with considerably higher β-D-glucans and total dietary fiber content. Flat breads made from PEF-treated oat and barley flour had higher β-D-glucan content (21–31%) compared to breads with untreated flours due to the inactivation of β-glucanase and improved β-D-glucan extraction during PEF treatment. Flat breads enriched with oat flour could be labeled as a "source of fiber," while those containing barley flour were a "high fiber."

The partial replacement of wheat flour with alternative (untreated) flours led to a significantly ($p < 0.05$) lower (19–24%) specific volume while a bigger spread ratio of flat bread (32%) compared to control wheat bread (Table 8). A similar reduction in specific volume after adding oat flour

Table 8 Nutritive and physical properties of flat breads and polyphenol oxidase (PPO) of flour

Sample	Wheat control	Oat control	Barley control	Oat-PEF	Barley-PEF
Total dietary fiber (g/100 g)	3.12 ± 0.03^{c}	4.79 ± 0.26^{b}	8.08 ± 0.13^{a}	5.12 ± 0.04^{b}	7.73 ± 0.08^{a}
β-glucans (g/100 g d.w.)	0.35 ± 0.01^{d}	2.30 ± 0.05^{b}	1.03 ± 0.01^{a}	2.78 ± 0.07^{c}	1.36 ± 0.03^{c}
Dry matter (g/100 g)	55.37	52.57	55.63	52.45	51.46
Specific volume (mL/g)	3.14 ± 0.03^{a}	2.53 ± 0.02^{b}	2.38 ± 0.00^{bc}	2.25 ± 0.10^{c}	1.82 ± 0.11^{d}
Spread ratio	5.56 ± 0.05^{c}	7.36 ± 0.28^{b}	7.38 ± 0.06^{b}	11.82 ± 0.65^{a}	8.21 ± 0.14^{b}
Hardness (N)	51.56 ± 2.64^{a}	45.68 ± 3.85^{ab}	39.58 ± 3.68^{b}	45.42 ± 4.31^{ab}	35.47 ± 1.93^{b}
Cohesiveness	0.86 ± 0.01^{a}	0.81 ± 0.03^{a}	0.72 ± 0.04^{b}	0.80 ± 0.03^{a}	0.63 ± 0.03^{c}
Resilience	0.85 ± 0.04^{a}	0.80 ± 0.01^{ab}	0.71 ± 0.05^{b}	0.78 ± 0.03^{ab}	0.61 ± 0.02^{c}
Chewiness (Ncm)	15.90 ± 0.68^{a}	14.89 ± 1.33^{a}	9.68 ± 0.25^{b}	14.46 ± 1.72^{a}	7.05 ± 0.93^{b}
L*	53.78 ± 0.53^{ab}	55.03 ± 0.86^{a}	54.17 ± 0.46^{ab}	53.44 ± 0.37^{b}	54.50 ± 0.24^{ab}
a*	1.34 ± 0.12^{b}	1.60 ± 0.27^{ab}	1.51 ± 0.18^{ab}	1.82 ± 0.06^{b}	1.28 ± 0.05^{a}
b*	4.52 ± 0.17^{a}	5.13 ± 0.37^{a}	3.88 ± 0.09^{b}	3.56 ± 0.24^{b}	3.57 ± 0.17^{b}
Δ*E*	-	1.43^{a}	0.42^{d}	1.11^{b}	0.94^{c}
PPO of flour (A_{475nm})	0.115 ± 0.001^{c}	0.126 ± 0.006^{b}	0.15 ± 0.000^{a}	0.154 ± 0.001^{a}	0.112 ± 0.001^{c}

Values within the same row marked with different letters (a–d) differ significantly according to Tukey's test ($p < 0.05$)

to wheat bread was previously demonstrated by Krochmal-Marczak et al. (2010). The reason for this was gluten dilution and the presence of fiber in barley and oat flour. Soluble fiber fractions in interaction with the gluten network make the retention of gasses difficult and, at the same time, make the dough too stiff to incorporate gasses during mixing and fermentation (Andrzej et al., 2019). Further, the water-unextractable AX represent a physical barrier for the formation of gluten network during dough development resulting in a weaker dough structure and lower bread volume (Courtin & Delcour, 2002). In this study, the specific volume of the flat bread was reduced even more, and the spread ratio increased even more if wheat-replacing flours were PEF-treated. Specific volume was influenced by the interaction between flour type and PEF treatment ($p = 0.011$); the reduction was only 11% in oat-containing bread while it decreased by 24% in barley-containing bread. This could be related to changes on barley proteins observed with SEM. Considering the results obtained with GlutoPeak which indicated shortening of PMT after PEF treatment, possibly a shortening of mixing time would be beneficial in preserving a higher bread volume. Another possible explanation is the reduced activity of α-amylase (78%) after oat flour PEF processing. Since this enzyme is necessary for assuring enough sugar for yeast fermentation and carbon dioxide production, amylase or sugar addition might have improved gas production and bread volume.

The crumb hardness, cohesiveness, resilience, and chewiness of barley breads were significantly lower compared to control wheat bread (Table 8). This is consistent with the study of Skendi et al. (2010), who demonstrated that incorporation of *β*-D-glucan isolated from barley leads to a decrease in the hardness of the final product. Such change in texture could be related to lower BEM after partial replacement of wheat flour with oat or barley flour in this study. One possible reason is that *β*-D-glucan can form a gel that has a soft structure, which consequently softens the bread crumb (Andrzej et al., 2019). The PEF treatment of barley flour resulted in a further reduction of cohesiveness and resilience of its bread which could be related to observed changes in protein matrix with SEM.

The interaction between the flour type and PEF treatment significantly ($p < 0.05$) influenced the crust lightness L^*, redness a^*, and yellowness b^*. After the PEF treatment of flour, L^* and b^* were reduced in the case of oat bread. The darkening of oat bread could be related to the increased PPO activity (18%) of oat flour after PEF treatment, which was confirmed by its negative correlation with the L^* parameter ($r = -0.875$, $p = 0.022$). Overall, a small difference in color (dE < 1.5) from the control wheat bread was observed in all samples (Table 8).

Conclusions

This study demonstrated how the effectiveness of pulsed electric field technology in inactivating endogenous β-glucanase enzymes differs between oat and barley flour. Mixed linkage *β*-D-glucans from our oat and barley flour belonged to the MMW group, even after PEF treatment, after which their extractability was improved (33.5%). Therefore, it can be assumed that they have preserved physiological potential. In addition, PEF treatment was found to increase water-extractable arabinoxylans, which, in combination with β-glucans, positively contributed to the reinforcement of the gluten network in bread dough. Consequently, the use of

PEF-treated raw materials in bread making is promising for improving the nutritional and technological properties of bread. However, it is important to note that the bread-making process should be adjusted since PEF technology affects many enzymes and biopolymers of flour. Although PEF technology offers the potential for processing flour with the aim of improving bread quality, further optimization of this treatment approach depending on the flour type is essential to achieve better physical properties of the final product. Future studies should investigate the consumers' acceptability of food containing PEF-pretreated ingredients.

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Data Availability All relevant data are shown in the manuscript. Additional data is available upon request.

Code Availability Not applicable.

Declarations

Competing Interests The authors declare no competing interests.

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Chapter 3

Chapter 3

General discussion

1. Flat bread database
2. Sourdough fermentation kinetics, dough retardation method, oat and barley flat bread properties
3. Pre-processing of oats and barley: effects on enzyme activities, phenolic compounds, non-starch polysaccharides and functional properties of oat and barley flour and bran and dough rheology
4. Nutritional, physical, and sensory properties of oat and barley flat breads made from pre-processed flour and bran

1. Flat bread database

Before developing solutions, it is essential to analyze the challenges that arise in the production of different types of flat bread. *Publication No. 1* presents a detailed study carried out as part of the FlatBreadMine Project, focusing on flat breads in nine Mediterranean countries: Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta, and Spain. It contains extensive data on the different types of flat breads, the ingredients used, the production processes and the nutritional profiles collected through interviews with experts and bakers familiar with flat bread production, as well as through analyses of flat bread products on the Croatian market.

Publication No. 1 contains a database of 143 types of flat breads, including 51 single-layered, 15 double-layered, 66 garnished and 11 fried varieties. Within this assortment, there were a total of 14 different types of Croatian flat breads, of which 7 are garnished, 5 are single-layered and 2 are double-layered, with no fried varieties recorded (*Publication No. 1* – Table 1). Croatian flat breads consist of different types of *peka bread, pogacha, somun, lepinja, poljički soparnik, rudarska greblica, Zlevanka, šlapica, and (zagorski) mlinci,* as well as *focaccia* and *ciabatta* as flat breads originated from Italy but present on the Croatian market. In the production of all Croatian flat breads, refined wheat flour is used as the main flour. Corn flour is the second most common type of flour used, accounting for 21% of all flat breads, followed by whole grain wheat flour and rye flour at 14%, all of which are used in the blend with refined wheat flour. Many Croatian recipes do not contain added lipids, but if is used, sunflower oil predominates, while olive oil and vegetable oil are used to a lesser extent (*Publication No. 1* – Table 3). The use of olive oil usually implies its mixture (50:50) with sunflower oil. On the other hand, fat of animal origin, i.e. lard, is used in the production of certain types of flat bread (*rudarska greblica* and *Zlevanka*). Compressed baker's yeast is the most commonly used leavening agent, accounting for 64% of the total flat bread types. Baking powder and sourdough are used much less frequently (14%), while 21% of flat breads are unleavened (e.g., *poljički soparnik, mlinci, zagorski mlinci*). In addition to oils and fats, garnished flat breads contain a variety of ingredients that contribute to different sensory profiles. These ingredients, which are used to fill or flavor the dough before baking, are crucial to the unique character of each flat bread. In Croatian flat bread production, these additional ingredients can be of both plant and animal origin. Vegetable ingredients such as spices, various vegetables, cereals, and seeds make up

50% of the total, while ingredients of animal origin such as dairy products, eggs, canned fish and meat together make up 56%. Croatian flat breads often contain additional ingredients, as can be seen in varieties such as *pogacha* and *ciabatta* with olives, *poljički soparnik* with mangel and vegetables and *mlinci* with eggs. Most Croatian flat breads can be produced in both ways, *i.e*., they have an artisanal character, but also can be produced at industrial level if this was not already the case. The production process consists of kneading the ingredients (by hand or by machine), bulk fermentation (if present), shaping (usually into circular shape), proofing (controlled temperature and time), and baking which is usually performed at a temperature below 250°C usually using electric deck oven. In case of artisanal production, the baking is performed in the fireplace (named Komin), under hot ashes and embers or on the hearth, under a bell-shaped iron lid (named Peka) which is covered with embers at a very high temperature (>300°C). Around 36% of Croatian flat breads are round in shape, usually with a diameter of between 10 and 40 mm, while the remaining 64% are rectangular or oval in shape. Some Croatian flat breads have national and European quality scheme. *Pogacha z oreji* is awarded the "Intangible Cultural Goods" quality scheme, conferred by the Ministry of Culture of the Republic of Croatia. The "Croatian Quality" label, awarded by the Croatian Chamber of Commerce is associated with *pogacha*, while *poljički soparnik* and *zagorski mlinci* have been awarded the European protected geographical indication.

To assess the nutritional quality of this product, the Croatian flat bread market was considered, where data on the nutritional value of 39 types of gluten-containing Croatian flat bread were collected (*Supplementary* – Figure 2; Garzon et al., 2022). Since refined wheat flour is the main raw material, the main disadvantage of Croatian flat breads is the low dietary fiber content (only 2.9% on average), which makes this product an ideal material for improving the nutritional composition. In addition, the use of sourdough in production is underutilized, although consumer interest in flat breads made with sourdough is increasing. This renewed interest is driven by the perceived improvement in nutritional value and overall quality that sourdough brings to flat breads.

2. Sourdough fermentation kinetics, dough retardation method, oat and barley flat bread properties

The resurgence of sourdough fermentation represents a modern adaptation of traditional biotechnology and offers an efficient way to incorporate non-wheat grains into bread production (Ramos et al., 2021). The fermentation of barley in sourdough has been studied to some extent and has proven successful, while research on the fermentation of oats is limited, mainly because of its low acidification power (Cera et al., 2024; Mariotti et al., 2014; Pejcz et al., 2017; Reidzane et al., 2023). However, all these studies focus on the effects of adding sourdough on the physical, nutritional, and sensory properties of bread, while the acidification kinetics of flour were neglected. The addition of sprouted rye, wheat, maize, barley, and lentil seeds has been shown to stimulate the growth of LAB in rye sourdough and accelerate the acidification kinetics (Diowksz et al., 2014). Like sprouted seeds, bran is a good source of enzymes and high-quality nutrient compounds that can stimulate microbial growth and acidification kinetics during sourdough fermentation (Diowksz et al., 2014). Analysis of the chemical composition of oat and barley flour and bran (*Publication No. 1* – Table 2) showed that bran contained 71-166% higher dietary fiber, 45-165% β-glucans, and 66-189% total phenolic content than flour. Compared to flour, bran contained significantly higher concentrations of all minerals. It contained 147-251% more Fe, 13-60% more Zn, and 27% more Cu. The Mg content varied depending on the type of cereal: in barley, the Mg content in flour was 52% higher than in bran, while in oats it was 69% higher in bran than in flour. Compared to oat flour, barley flour contained a higher content of minerals and β-glucans as well as twice as much dietary fiber and total phenols. However, when comparing bran, oat bran outperformed barley bran for the same components.

The acidification kinetics of OF and BF alone or its mixture with bran as a function of sourdough fermentation conditions dough yield, starter and temperature) are shown in the *Supplementary materials* (Figure 1 and Table 1). The change in pH during sourdough fermentation was well fitted to the Gompertz model, except for the fermentation of OF with LV1 at DY 200 and 22°C. Overall, BF had a higher acidification power than OF (*Supplementary* – Table 1). With increasing DY (from 200 to 300), the acidification rate was slightly lower for OF (31%), while it remained unchanged for BF (*Supplementary* – Figure 1a). By replacing the LV4 starter with LV1, an even higher acidification rate (54%) was achieved in BF under the same fermentation conditions, while it remained unchanged in OF. Increasing the temperature

from 22°C to 30°C shortened the lag time and the time required to reach the maximum acidification rate, while the acidification rate of both BF and OF increased by 104-116% (*Publication No. 2* – Table 3, *Supplementary* - Figure 1b and Table 1). After the addition of bran to the flour, acidification slowed down at 22°C, while it increased at 30°C. Thus, increasing the temperature from 22 to 30°C accelerated the fermentation rate of oat and barley flour/bran sourdough by 12-– 64% (*Supplementary* – Figure 1c), resulted in lower pH (8%) and higher TTA (23-45%) after 24 hours of fermentation with LV1 (*Publication No. 2* - Table 3, *Supplementary* – Table 2). According to Flander et al. (2011), the fermentation temperature has the greatest influence on the acidity of the sourdough (pH, TTA and lactic acid content).

The *Publication No. 2* provides a detailed understanding of the influence of adding OB or BB on the acidification kinetics, total titratable acidity (TTA), microbial viable cell count (CFU) and organic acid concentration in sourdough from OF or BF. The addition of bran to flour (1:3) shortened the lag time and accelerated the acidification rate of the sourdough fermentation. The OF showed a 1.5-fold increase in TTA and a decrease in pH with the addition of OB, while BF showed no significant differences after 24h of sourdough fermentation (*Publication No. 2* – Figure 1 and Table 3). Both oat sourdough samples (flour only and flour-bran mixture) had a 52 % higher acetic acid content than barley sourdoughs (*Publication No. 2* – Table 4). Thus, the fermentation quotient of barley sourdough was about 2 times higher than that of oat sourdough. The addition of bran reduced the lactic acid content of barley sourdough and the fermentation quotient of both types of sourdough. The recommended fermentation quotient is below 5.0, but with large fluctuations from 0.25 to 20 (Arora et al., 2021). Similarly, the addition of sprouted seeds proved to have a stimulating effect on the fermentation process of rye flour, shortening it by 8-16 h and increasing the TTA by 1.25-1.6 times, depending on the dose and temperature (Diowksz et al., 2014).

To reduce the phytate content of bread, a sourdough fermentation is generally used. This traditional process also offers other solutions, such as its suppressive effect (due to its low pH) on enzyme activity in bread making. *Publication No. 2* shows the effects of sourdough addition on the darkening of the dough during retardation process, as well as on the nutritional and physical properties of wheat-oat and barley composite flat breads. A 24-h dough retardation significantly reduced the PA concentration in oat bread by 38% and in barley bread by 27-32% compared to the no-time process (*Publication No. 2* – Table 6). Previous studies have shown that sourdough fermentation successfully reduces the PA content of whole grain bread by 30 to 85% (Leenhardt et al., 2005; Naji-Tabasi et al., 2022; Yildirim & Arici, 2019). Prolonged

fermentation (dough retardation) of doughs containing sourdough also further reduces the PA content (Fang et al., 2023). Certainly, β-glucans are also degraded during prolonged fermentation. The approx. temperature of 30 °C for dough rising favors β-glucanase activity. However, during the retardation process at 0-4 °C, β-glucanase activity is slowed down, resulting in a lower but still significant decrease in β-glucan content in oats (4-9%) and barley (8-28%) in retarded flat bread compared to bread from no-time method (*Publication No. 2* – Table 6). The β-glucans reduction in this study is lower compared to the results in the literature, which can be attributed to the low temperature during retardation and the lower pH of the bread dough due to the addition of sourdough, which together delayed the β-glucanase activity. Flander et al. (2011) and Gamel et al. (2015) observed a reduction (12–23%) in β-glucans when oat sourdough was incorporated into wheat-oat composite bread. As the content of β-glucans in bread has decreased significantly, pre-processing of raw materials is needed to inactivate β-glucanase that catalyzes the β-glucans depolymerization reaction. Still, about 275 g of oat and about 560 g of barley flat bread with 50% sourdough (which corresponds to replacing 30% of wheat flour with OF or BF) could meet the daily requirement of 3 g of β-glucans.

Nevertheless, the addition of sourdough had a positive effect on the color of the dough during retardation, since the activity of PPO, the enzyme that catalyzes oxidative browning, was suppressed at low pH (Liu et al., 2019). Dough samples without sourdough showed a very distinct color change already after 6 h of cold storage (TCD>3), while dough samples with added sourdough (30% and 50% dough weight) were browning slower with the distinct TCD values (1.5<TCD<3) after 24 h of retardation (*Publication No 2.* – Figure 2). Increasing the proportion of oat or barley flour-bran sourdough (from 30% to 50%) in the flat breads resulted in a lower specific volume (*Publication No. 2* – Figure 3), which is in contrasts with the results from Table 6 for the flat breads with OB/BB sourdough, where the amount of sourdough was added to the mixture so that the added bran represented 10% of the weight of the wheat flour. In this case, the addition of 30% or 50% of oat/barley sourdough (dough weight), means the replacement of 18% and 30% of wheat flour with oat or barley flour-bran blend, respectively. Higher content of oat and barley, *i.e.,* increased content of dietary fiber with a high-water binding capacity, limits the water for the formation of the gluten network and has a negative effect on the bread volume (Gill et al., 2002). The reduced pH value of the dough (4.1-4.9) caused by the addition of sourdough increases the solubility of the protein and hinders the formation of new bonds, which leads to a weakening of the wheat gluten. In addition, these acidic conditions favor the proteolysis of gluten and increase the solubility of dietary fibers,

especially arabinoxylan (Arendt et al., 2007). During the retardation process, the amount of gas increases to the extent that the gluten-starch matrix cannot trap it, and there is a significant decrease in bread volume, as shown in *Publication No. 2* (Table 6). The dough shelf life of 48 h of retardation proved to be unacceptable due to excessive darkening of the dough (TCD>3) and reduction in flat bread specific volume (18-30%).

The crumb hardness of flat breads containing 30% or 50% oat sourdough was higher (12–16%) than that of their barley counterparts. Overall, flat breads with 50% sourdough were slightly harder compared to flat breads with 30% sourdough added (4% for barley and 10% for oat breads). These results differ from those in Figure 6, although the reasons for this are like the discrepancies observed in the bread specific volume as described previously. The high content of dietary fiber, especially high molecular weight β-glucan in oat and high AXs content in barley, may interfere with the formation of the starch-protein reaction in wheat flour and consequently lead to a harder crumb structure (Wenjun et al., 2018). The retardation improved the softness of the crumb, although the bread volume was reduced. The 24h-retardation process resulted in very distinct color difference (TCD 2.9-4) of the bread crumb and crust (TCD 4-12) compared to no-time flat breads (*Publication No. 2* – Table 6). However, the reason for this is not only the activity of PPO, but also other enzymes such as α-amylase whose products (reducing sugars) are involved in non-enzymatic browning *i.e.,* Maillard reactions during bread baking (Olaerts et al., 2018). Despite the fact that bread samples had reduced volume (12-18%), and darker crumb and crust color, the storage of dough at low temperature for up to 24h can prolong its shelf-life. Overall, oat flatbreads were characterized by a higher content of β-glucan and phytic acid (PA) and had a lighter color in both the crust and the crumb. In contrast, barley flatbreads had a higher crumb hardness.

3. Pre-processing of oats and barley: effects on enzyme activities, phenolic compounds, and non-starch polysaccharides

The following parts of the thesis include the processing of oat bran (OB) and barley bran (BB) with US (*Publication No. 3*) as well as oat flour (OF) and barley flour (BF) with PEF (*Publication No. 4*) with the aim of minimizing β-glucanase activity, increasing β-glucans extractability, maintaining phenolic content and antioxidant activity while reducing antinutrients.

3.1.The effects of pre-processing on β-glucanase and phytase activity

Cereal-based products containing oats or barley are a potential source of β-glucans, but the production steps lead to their degradation by endogenous β-glucanases (Johansson et al., 2018; Pérez-Quirce et al., 2017). Therefore, it is very important to inhibit β-glucanase activity to maintain the β-glucans content during the production steps and in the final product. Still, the incorporation of OB and BB in food increases antinutrient (phytates) content which can be removed by stimulating endogenous cereal phytase activity (Baumgartner et al., 2018).

In *Publication No. 3*, the effect of US treatment on the endogenous β-glucanase and phytase activity of OB and BB was investigated for the first time. Control OB had 46% higher β-glucanase activity, but 62% lower phytase activity compared to BB. After the US treatment, with the increase of specific energy input (Ws), prolongation of treatment time, and increasing final temperature, the inactivation of β-glucanase showed an increasing trend. The greatest reduction in β-glucanase activity in oats (81.6%) and barley (55.3%) was observed in the treatment with the highest Ws (348 kJ kg^{-1}) and ending temperatures above 70°C (*Publication No. 3* – Figure 1, Table 2). Previous studies have shown that β-glucanase from rice flour and whole grain BF is heat-sensitive and its activity decreases with prolonged exposure to high temperatures during microwave treatment or oven heating (Pérez-Quirce et al., 2016; Rieder et al., 2015a). US treatment leads to changes in the secondary structure of enzymes through three main mechanisms: high temperature, cavitation pressure and free radical formation (Kumari et al., 2018; Y. Sun et al., 2019). The location of β-glucanase in oats and barley is not the same, with barley β-glucanase being further away from the cell membrane and less sensitive to US treatment (Mawson et al., 2011), resulting in a different effect of treatment on enzyme activity. In this study, oat β-glucanase was linearly inactivated after all US treatments with increasing

Ws. In barley, US treatments with lower Ws (87 kJ kg^{-1}) and temperatures below 60 °C resulted in enzyme release and increased activity, while higher Ws and final temperatures resulted in its inactivation (*Publication No. 3* – Figure 1). Milder conditions (lower Ws, shorter time) of US treatment, can lead to a change in enzyme conformation, favoring an enzyme-substrate reaction that stimulates the biological activity of the enzyme (Huang et al., 2017).

Cereal phytases are enzymes responsible for the hydrolysis of phytate and the release of bound minerals (Mandha and Raes, 2023). Various conventional processing techniques of cereals (soaking, fermentation, sprouting, thermal processing and milling) are used to reduce phytic acid and increase the mineral bioavailability (Baumgartner et al., 2018; Guo et al., 2015; Liang et al., 2008). Recent studies have tested the effect of US treatment on phytate reduction from rice bran and finger millet but phytase activity was not investigated (Mohammadi et al., 2021; Yadav et al., 2021). As the activity of endogenous phytase is the most important factor in the breakdown of phytic acid, it is important to monitor the activity of this enzyme (Liang et al., 2008). The temperature of 38-55°C is optimal for the cereal phytase activity (Mandha and Raes, 2023). However, the main trigger for the cereal phytases inactivation is high temperature and prolonged heating (Vashishth et al., 2017). Similar to barley β-glucanase, phytase from both OB and BB was activated after milder US treatments with lower Ws and temperature (87 kJ kg^{-1} and 40-41°C). Still, the lowest phytase activities were recorded after the US treatment with the highest Ws (348 kJ kg^{-1}) and temperatures (72-85°C) (*Publication No. 3* – Figure 1).

The effect of US on enzyme activity was dependent on both the type of enzyme and the sample matrix; phytase activity changed similarly in both samples, while β-glucanase activity depended on its origin.

In *Publication No. 4*, the effectiveness of PEF treatment on the β-glucanase activity of OF and BF was investigated for the first time. Oats had 31% higher β-glucanase activity compared to barley in the case of flour. Moreover, both OF and BF had 33% and 25% lower β-glucanase activity, respectively, in contrast to their bran counterparts. The β-glucanase activity of OF and BF depends on EFI, but mainly on Ws, as it was significantly explained by the first-order kinetic model for both OF and BF (*Publication No. 4* - Table 1). Treatment time had the least influence, as longer PEF treatment time (longer than the total treatment time of 1 min, *i.e.*, effective treatment time of 18 ms) showed a slight continuous decrease in β-glucanase activity, which was finally reduced by a further 7-16 % after 9 min, *i.e.*, 162 ms of PEF treatment. PEF treatment successfully reduced the β-glucanase activity of OF and BF, with the highest inactivation of 76.5% and 52.6%, respectively. Yet, barley β-glucanase was activated after

treatment with the lowest EFI of 4.5 kV cm^{-1} and 52.6% inactivated after treatment with 16 kV cm^{-1}. EFI below 12 kV cm^{-1} can lead to enzyme activation, which is then inactivated by exposure to EFI above 12 kV cm^{-1}, as has been shown for alcalase, horseradish peroxidase and pectinase (Li et al., 2022; Ohshima et al., 2007; Zhang et al., 2017). A similar behavior of β-glucanase from BB is shown in *Publication No. 3*, where a milder US treatment favored enzyme activity. The β-glucanase from oats was inactivated even at low EFI, while the highest activity suppression of 76.5% was achieved after treatment at 12 kV cm^{-1}. In PEF treatment, enzymes are activated or inactivated by high electric field pulses, which influences the protein structure (Ohshima et al., 2007). It leads to partial unfolding of the protein and triggers conformational changes that lead to denaturation and loss of activity (Fernandez-Diaz et al., 2000). *Publications No. 3* and *4* show that oat β-glucanase can be inactivated more easily than barley β-glucanase, which requires a higher EFI, a longer treatment time and a higher Ws value for effective inactivation with US and PEF. However, it is difficult to compare the results obtained with different conventional or innovative techniques, as the heating during PEF treatment is avoided. Still, what can be compared is the Ws input during treatment which was 348 kJ kg^{-1} for the highest inactivation of β-glucanase with US, while it was only in the range of 4.5-5.5 kJ kg^{-1} for PEF treatment to achieve a very similar residual activity.

Comparing the US and PEF technologies, the main difference is the use of a larger quantity of water, i.e. a water suspension with a lower concentration in the case of the US technology and a higher temperature at the end of the treatment (up to 85°C), which in the case of the PEF was at room temperature and varied between 20.0-21.6°C (*Publication No. 3* - Table 2, *Publication No. 4* - Table 1). The high temperature and high-water content during US treatment lead to gelatinization of the starch granules, resulting in an irreversible loss of crystalline structures (Wei et al., 2023). In addition, US treatment can increase the solubility of the starch by breaking up the polymer particles (Ma et al., 2022). Just as the US treatment leads to depolymerization of the biopolymers, this also applies to the PEF treatment. The PEF technology excludes high temperatures, so that gelatinization of the starch is avoided. However, PEF treatment reduces starch molecular weight, the viscosity during gelatinization, gelatinization temperatures and enthalpy, while increasing in vitro digestibility (Achayuthakan et al., 2023; Hong et al., 2020). The effects of US and PEF treatment on other biopolymers such as glucan and AX, which are most important in the case of oats and barley, are similar, which is described in section 3.3.

3.2. The effects of US processing of oat and barley bran on phenolic content, antinutrients and antioxidant activity

This section summarizes the results of the US processing of OB and BB for total phenolic content (TPC), phytic acid (PA), and AO measured by the free radical-scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) using an electron paramagnetic resonance (EPR), or ferric reducing antioxidant (FRAP) assay. *Publication No. 3* shows that the type of bran was the main reason for the differences in TPC, PA and AO. Control OB had higher FRAP antioxidant capacity compared to BB. This discrepancy may be due to the higher TPC and PA content in oats, but also to its particular phytochemicals, such as avenanthramides, which act as powerful antioxidants (Fu et al., 2015). The US can trigger chemical reactions through acoustic cavitation, formation, and decay of small gas bubbles during treatment (Milićević et al., 2021). The resulting free radicals can lead to the breaking of covalent bonds, which can simultaneously improve the extraction of the molecules, but can also cause their damage (Milićević et al., 2021). The process parameters therefore primarily influence the desired result. In *Publication No. 3,* the negative effects of US treatment on TPC, DPPH and FRAP were demonstrated, with the results obtained depending mainly on the Ws. It has been proven that phenolic components are sensitive to temperature, *i.e.,* heating (Antony and Farid, 2022). Therefore, with an increase in Ws followed by a linear increase in treatment time and temperature, there was a significant decrease in TPC and consequently AO (*Publication No. 3* – Table 4). This temperature effect was attenuated by the pulse mode during US treatment, which resulted in 8-15 % lower final temperatures at the same energies, but also by 7-26% higher TPC (*Publication No. 3* – Table 2 and 4). Previous studies (Cui and Zhu, 2020, Habuš et al., 2021c, Yadav et al., 2021, Zhu and Li, 2019) have also shown that the cereal phenolics are sensitive to temperature and duration of US treatment. Still, OB phenolic compounds showed better stability compared to BB.

Phytates, minerals, and phytases differ significantly across various cereal species in their distribution, composition, and how they interact (Mandha and Raes, 2023). In this case too, control OB contain more PA than BB. Just as phenolics are heat-labile, so is PA, which is not only an antinutrient that suppresses the bioavailability of minerals, but also acts as an antioxidant. Therefore, the reduction of PA positively correlated with the reduction of FRAP and TPC. In agreement, Yadav et al. (2021) found that an increase in US amplitude and soaking time resulted in a 67% reduction in phytic acid (PA) content in finger millet samples. Similarly, Mohammadi et al. (2021) observed a decrease in PA content (7-23%) in rice bran when treated

with US at different pH values. They attributed this decrease to both the heat generated during US treatment and the subsequent chemical degradation of PA. *Publication No. 3* confirms these results and emphasizes that PA degradation is not only thermal and chemical, but also enzymatic, which is favored by US-induced endogenous phytase activity. A reduction in PA was observed after all US treatments, with the lowest energy treatments (87 kJ kg^{-1}, 40-41°C) being the result of increased phytase activity. It is hypothesized that in medium energy treatments (217.5 kJ kg^{-1}, 60-67°C) the PA reduction was the result of the combined effect of activated phytase and heat until the time of enzyme inactivation, and then in treatments with the highest Ws input (348 kJ kg^{-1}, 72-85°C), the result of high temperature degradation.

3.3. The effect of US and PEF treatment on the water-extractable NSPs of oat and barley flour and bran

In this section, the effects of PEF treatment (*Publication No. 4*) and US treatment (*Publication No. 3*) on the water-extractable NSPs content and structure of oat and barley flour and bran are presented.

The Mw of β-glucans, monosaccharide composition and FTIR structural analysis of NSPs were investigated for samples treated with selected US treatments. Treatments were selected using the desirability approach based on the results that achieved the highest β-glucanase inactivation and functional properties (water swelling and retention capacity) according to *Publication No. 3*. The overall desirability values for the selected treatments were medium, i.e. 0.529 for OB and 0.627 for BB. The results presented in *Chapter 3 – section 3.3* will be incorporated into a research article with the working title "Ultrasound pretreatment of oat and barley bran contributes to the β-glucans content and technological properties of flat bread with or without sourdough", which is currently under review.

The two main NSPs in oats and barley are β-glucans and AXs (Valoppi et al., 1947). The FTIR spectra with the characteristic bands of these two NSPs (Figure 3, *Publication No. 4* – Figure 3), the composition of the neutral monosaccharides (Table 3, *Publication No. 3* – Table 4), the methylation analysis (*Publication No. 4* – Table 5), and β-D-glucans and AXs signals on the HMQC spectra (*Publication No. 4* – Figure 4) proved this.

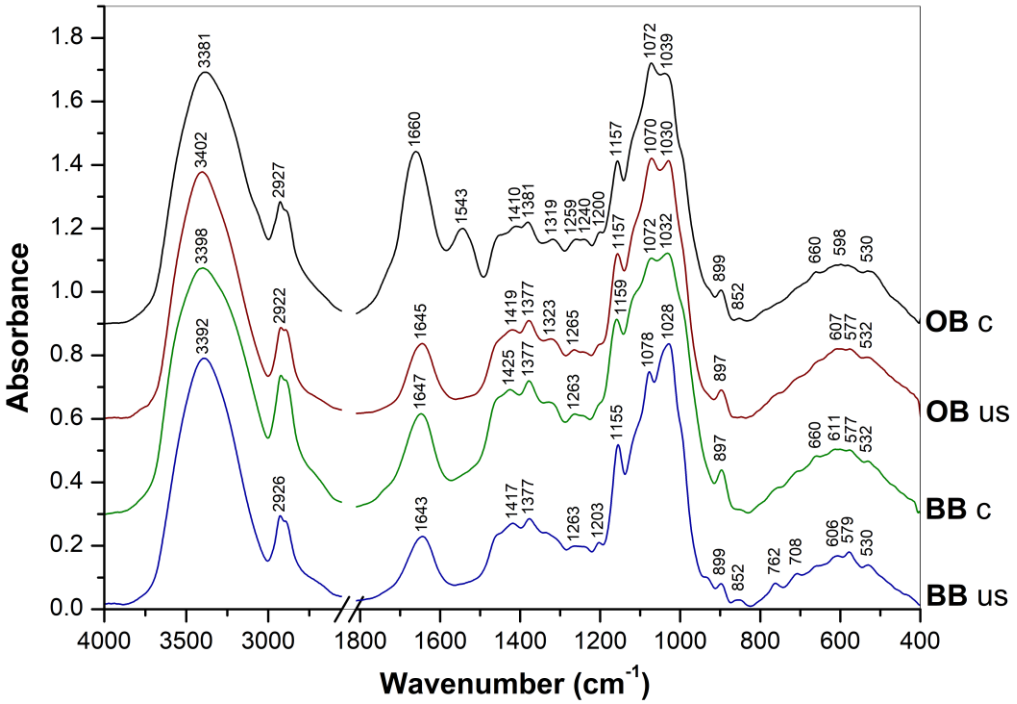


Figure 3. FTIR spectra of the water-soluble polysaccharides isolated from control (C) and ultrasound (US)-pretreated oat and barley bran (OB, BB).

Table 3. Molar ratio (%) of monosaccharides and estimated arabinoxylans content (% of the cold water-extractable fraction, F1) in the fractions obtained from oat (OB) and barley bran (BB) before (C) and after ultrasound pretreatment (US). Results are expressed as mean±standard deviation (n=2)

Sample/sugar	OB-C	BB-C	OB-US	BB-US
Fucose	1.59 ± 0.09^{b}	1.36 ± 0.20^{b}	2.72 ± 0.14^{a}	0.94 ± 0.02^{c}
Arabinose	2.77 ± 0.15^{c}	6.78 ± 0.01^{a}	2.40 ± 0.06^{c}	3.71 ± 0.08^{b}
Mannose	0.46 ± 0.04^{b}	0.73 ± 0.01^{a}	0.52 ± 0.06^{b}	0.41 ± 0.04^{b}
Glucose	91.08 ± 0.77^{a}	80.34 ± 0.29^{b}	87.78 ± 3.97^{a}	89.34 ± 0.85^{a}
Galactose	0.97 ± 0.07^{b}	1.25 ± 0.01^{a}	0.76 ± 0.10^{b}	0.43 ± 0.02^{c}
Rhamnose	0.36 ± 0.01^{a}	0.35 ± 0.09^{a}	0.36 ± 0.02^{a}	0.35 ± 0.01^{a}
Xylose	2.41 ± 0.07^{c}	9.19 ± 0.02^{a}	2.93 ± 0.63^{c}	5.14 ± 0.25^{b}
Total sugars (%)	58.87 ± 0.76^{b}	70.99 ± 2.55^{a}	72.36 ± 1.81^{a}	69.80 ± 2.34^{a}
Arabinoxylans (%)	5.43 ± 0.26^{c}	15.18 ± 0.02^{a}	4.71 ± 0.13^{c}	8.17 ± 0.31^{b}

a–c Values within the same row marked with different letters differ significantly according to Tukey's test ($p < 0.05$).

The cell walls of the endosperm consist primarily of β-glucans, which account for about 70%, with a lower proportion of AXs of about 20%, while AXs predominate in the cell walls of the aleurone cells (67-71%) and β-glucans are present to a lesser extent (26%) (Andriotis et al., 2016; Izydorczyk and Dexter, 2008). Comparing the results from *Publication No. 4* (Table 2) with the results from Table 4, it can be seen that OB had 2.7-fold and BB 1.5-fold higher content of β-glucans than their flour counterparts. The proportion of AXs in the water-extractable fraction was higher in bran than in flour and higher in barley (in both cases) than in oats (Table 3, *Supplementary* – Table 2, *Publication No. 4* – Table 2). It is also important to point out that the proportion of AXs in the fraction obtained by extraction with hot water (F2) was higher than with cold water (F1), which is consistent with the literature (Cyran et al., 2003). The reason for this distribution of AXs and β-glucans, which is on the bran side, is that the content of these NSPs in cereals and edible parts of grain depends on genetic and environmental factors (Izydorczyk and Dexter, 2008). Another important factors that affect the distribution and proportion of NSPs are milling process and grain pearling (debranning). The intensity of debranning process before milling significantly influences the composition, i.e. the AXs content (Jurkaninová et al., 2024). The milling process has a significant influence on the yield of β-D-glucans, as these are distributed asymmetrically in the grain, but are mainly concentrated in the aleurone layer of the endosperm (Jurkaninová et al., 2024). Oat β-glucans generally have a

higher Mw than barley β-glucans (Guleria et al., 2015). In a further comparison of flour and bran, β-glucans had a higher Mw in OF (by 22.8%) and BF (17.4%) than those extracted from bran. Zheng et al. (2011) also proved a higher Mw for β-D-glucans from BF than from BB.

Cereal grains are rarely eaten raw, but mostly in the form of bread, the production of which involves certain processing steps that can have a negative effect on the structure and β-glucan content. In bread making, the most critical phase is fermentation, the conditions of which are ideal for β-glucanase activity, which depolymerizes the β-glucans from the flour or bran, resulting in a lower content in the final product, as presented in *Chapter 3 – section 2*. *Publications No. 3* and *4* show the successful inactivation of endogenous β-glucanase with US and PEF, but the question arises as to how these innovative techniques affect the content and structure of β-D-glucans.

There are a limited number of studies on the effects of US treatment on the yield of extraction of β-glucans from cereals. Benito-Román et al. (2013) and Hematian Sourki et al. (2017) reported an increased extraction yield of β-glucans from BF by 25% and 4%, respectively. The specific energy of ultrasonic cavitation damages the cell walls, which increases the penetration of water and leads to the release of polysaccharides. Nevertheless, prolonged US treatment with high Ws can have the opposite effect, i.e. the degradation of polysaccharides and a decrease in their content (Hematian Sourki et al., 2017). The US treatment of OB (217.5 kJ kg^{-1}) and BB (348 kJ kg^{-1}-P) enhanced β-glucan extractability by 12.3% and 11.5%, respectively, compared to their controls (untreated bran) (Table 4).

Table 4. Total content, solubility, and molecular weight (Mw) of β-glucans in control (C) and US-pretreated oat (OB) and barley (BB) bran.

Sample	OB-C	BB-C	OB-US	BB-US
Total β-glucans (g/100 g d.w.)	7.45 ± 0.27^{b}	4.54 ± 0.02^{d}	8.37 ± 0.34^{a}	5.06 ± 0.12^{c}
Soluble β-glucans (g/100 g d.w.)	4.03 ± 0.02^{b}	1.61 ± 0.06^{c}	6.47 ± 0.11^{a}	3.81 ± 0.18^{b}
Insoluble β-glucans (g/100 g d.w.)	3.42 ± 0.02^{a}	2.93 ± 0.06^{a}	1.89 ± 0.11^{b}	1.25 ± 0.18^{c}
Average Mw (×10^{5} g mol^{-1})	3.02	2.81	2.81	2.20

Results are expressed as mean±standard deviation (n=2)

a–d Values within the same row marked with different letters differ significantly according to Tukey's test ($p < 0.05$).

The Mw of BB β-glucans varies between 1.18×105 and 7.55×105 g mol^{-1} (Zheng et al., 2011) and between 1.19×105 and 2.3×105 g mol^{-1} for OB β-glucans (Åman et al., 2004). Slightly higher results than those listed have been obtained in this research (Table 2), with extraction conditions and measurement methods being some of the factors affecting the results obtained. During US treatment, the effect of cavitation (high shear force) on the sample leads to the degradation of β-glucans and a decrease in their Mw (Benito-Román et al., 2013). The decrease in Mw was positively correlated with the specific energy applied during ultrasonic treatment (Benito-Román et al., 2013). The reduction in Mw of β-glucans in BF after US treatment with Ws between 109.5 kJ kg^{-1} and 1169.5 kJ kg^{-1} ranged between 13% and 43% (Benito-Román et al., 2013). Similar results were reported in other studies, in which the Mw of β-glucans from BF decreased by 13% after only 4.5 min US treatment (Hematian Sourki et al., 2017) and the Mw decreased by 58.6% after 10 min US treatment of *Poria cocos* mushroom (Chen et al., 2015). US treatment of OB and BB resulted in a decrease in the molecular weight of β-glucans by 7% and 21.7%, respectively (Table 2). This difference is the result of the applied Ws and the US treatment time, which was longer for barley (348 kJ kg^{-1}-P, 36 min) than for oats (217.5 kJ kg^{-1}, 7 min). The β-glucans of oats and barley differ in their structure and consequently in a degree of water solubility; OB β-glucans are therefore characterized by a higher solubility (27-51%) compared to BB (18-39%) (Gajdošová et al., 2007; Mikkelsen et al., 2013). The proportion of soluble β-glucans in the total β-glucans of the control OB was 54.1%, while this proportion was lower in the control BB and amounted to 33.5% (Table 4). US treatment of β-glucans leads to polymer degradation and a significant increase in their solubility (Cheng et al., 2010; Duque et al., 2020b), which is of great interest for the bakery industry, especially for bread production, as increased solubility of dietary fibers influences the functional and technological properties of bran, which is explained in more detail in *Chapter 3 – section 2.4*. In the study by (Chen et al., 2015), they observed a remarkable 33.5% increase in the water solubility of β-glucans from *Poria cocos* mushrooms after subjecting it to US treatment with an amplitude of 50-60 % for 10 minutes. Similarly, (Vaitkeviciene et al., 2022) found that US treatment (at 850 kHz, 1.3 W cm^{-2}, for 20 minutes at 40 °C) increased the water-soluble fiber content in rice bran by 17.5 %. This improvement in solubility was attributed to structural changes and a lower Mw of β-glucans (De Vuyst et al., 2017). The effect of US treatment on the solubility of β-glucans depended mainly on the type of bran treated and on the Ws, so that the increase in solubility was different in OB and BB, being 31% and 40%, respectively, compared to their controls (Table 4). In addition, an increased proportion of mannose (24-190%), rhamnose (61-178%) and galactose (16-159%) was found in the F2

(*Supplementary*– Table 2) fraction after US treatment, which indicates an increased solubility of other NSPs such as galactomannans, glucomannans, arabinogalactans and xyloglucans (Bieniek and Buksa, 2023).

There are only a few studies on PEF technology for the extraction of polysaccharides, mainly from mushrooms (Parniakov et al., 2014; Xue and Farid, 2015) or for the extraction of soluble dietary fiber from fruits or legume by-products (Fan et al., 2022; Wang et al., 2023). The effects of PEF technology on the extraction of NSPs, especially β-glucans, have not yet been sufficiently investigated. PEF-assisted extraction of β-glucans from OF and the mushroom *Pleurotus pulmonarius* was successfully performed, increasing the content of β-glucans in ranges of 12-20% and 14-22%, respectively (Duque et al., 2020b; Thikham et al., 2024). The extraction ability of PEF is a consequence of the effect of an external electric field on the plant tissue, which leads to electroporation of cell membranes and promotes the release of intracellular compounds while maintaining the particle size of the raw material (Raso et al., 2016). *Publication No. 4* also confirms earlier studies and shows an increased β-glucans content of 33.5% in PEF-treated OF and BF. There is even less information on the effects of PEF technology on the β-glucans structure. Available studies indicate the effects of PEF treatment on the Mw of various polysaccharides such as corn starch, chitosan, and sugar beet pectin (Han et al., 2012; Luo et al., 2010; Ma et al., 2012), showing a depolymerization and decrease in the Mw (9-31 %) of these polysaccharides. Treatment with PEF caused a decrease in the Mw of β-glucans of OF by 9%, while an increase in Mw β-glucans of BF was recorded by 14%. It is important to emphasize that in the above studies the isolated polysaccharides were treated with PEF, while in *Publication No. 4* the raw material was treated, which may be the explanation for these different results.

The comparison of the effects of PEF and US technology on the β-glucans and AXs of oats and barley shows that the PEF treatment leads to a higher extraction and a smaller change in the Mw of the β-glucans compared to the US treatment. The US treatment also proved to be more aggressive for AXs, which decreased by 46% and 13% in the F1 fraction and by 19% and 15% in the F2 fraction obtained from OB and BB, respectively (Table 3, *Supplementary*– Table 2). In contrast, PEF treatment favored the increase of WE-AX by more than 50%, which was confirmed by the pronounced signals of AXs structures in the HMQC spectra after PEF treatment (*Publication No. 3* – Table 4 and Figure 4). Due to the structural functionality of AXs, the presence of WE-AXs in bread making leads to a better structured matrix in the dough and

ultimately to improved textural properties of the bread (Zhu et al., 2023) (*Chapter 3 – section 2.4 and 4*).

During PEF and US treatment, free radicals are formed, of which the hydroxyl radical -OH is thought to be responsible for breaking the glycosidic bonds, which leads to a reduction in Mw (Ashokkumar et al., 2007; Luo et al., 2010; Zhang et al., 2023). However, the effect of US treatment is based on both mechanical and thermal mechanisms, which have an additional degradative effect on exposed molecules, making it a more aggressive treatment in terms of NSPs (Zhang et al., 2023).

3.4. The effects of pre-processing on flour and bran functional properties and dough rheology

Publications No. 3 and *4* present the application of US and PEF technology in the processing of bran and flour from the perspective of technological quality of oat and barley flat breads.

The OB and BB, whose mean particle size (531 μm and 514 μm, respectively) was larger than that of the flour (231 μm and 295 μm, respectively), were treated with US due to its mechanical effect, which leads to a reduction in particle size and better dispersion of the solids (Zhang et al., 2023), which ultimately benefits the technological and functional properties of the bran. The US technology has found its application in cereal processing, and current research mainly focuses on the direct effects on a single component such as starch and proteins (Ma et al., 2022; Qin et al., 2022; Song et al., 2023; Wang et al., 2020; Zhang et al., 2023; Zhang et al., 2016). The US treatment has significantly improved the hydration properties of buckwheat flour, corn flour, rice flour and whole-grain quinoa flour, such as water retention capacity (WRC) and water swelling (WS) (Harasym et al., 2020; Jalali et al., 2020; Vela et al., 2021, 2023; Zhu and Li, 2019). The treatment of OB and BB with US showed remarkable results, with WRC and WS increased by 70-125% and 110-124%, respectively (*Publication No. 3* – Table 5). The hydration properties of the bran were found to be strongly influenced by the treatment temperature, the Ws and the US treatment duration. These results are in line with research findings by Vela et al. (2021) and Zhu and Li, (2019), which emphasize the crucial role of temperature in the effectiveness of US treatment in hydration properties. Hydration properties depend on the type of cereal, particle size and concentration of protein, starch, and dietary fiber (Elleuch et al., 2011; Godswill Awuchi et al., 2019). In addition, granulation and particle size uniformity play a crucial role in determining the functional and technological performance of flours (Vela et al.,

2021). The observed increase in the hydration properties of bran is attributed to the increased dietary fiber solubility as described in this *Chapter 3* in the *section 2.3*. Furtheron, the physical disruption of the macrostructure of the bran by the mechanical effects of US, altering the particle size distribution and, in particular, increases the proportion of particles in a lower size range (Vela et al., 2021). The accumulation of smaller particles and the subsequent increase in the exposed surface area of starch granules, which leads to the amylose leaching, along with the dissociation of fiber and proteins, increase their water binding sites (Amini et al., 2015; Kaur and Gill, 2019; Qin et al., 2022). As a result, water absorption, retention, and solubility are improved. Sonication of flour or bran have a significant impact on viscoelastic properties of gel (τ_{max}, storage (G') and loss modulus (G"), and complex viscosity). US treatment of rice flour results in a stronger gel that makes them more resistant to structure disruption, which is associated with increased τ_{max}, but lower viscosity (Vela et al., 2021). The viscoelastic properties of the gel of OB and BB depended mainly on Ws, with the parameters τ_{max}, complex viscosity and consistency index (*K', K"*) decreasing linearly with Ws increase (*Publication No. 3* - Table 5). The control BB gel had a higher τ_{max} compared to the OB gel. Nevertheless, the decrease in gel viscosity and τ_{max} caused by US treatment was more pronounced for BB than for OB, suggesting that US-treated BB yields a weaker gel. The explanation lies in the chemical composition of the bran, with OB containing more proteins and water-soluble fibers, particularly higher molecular weight β-glucans, whose mutual interactions form a more stable gel (Liu et al., 2021) than BB (Table 4, *Publication No. 3* - Table 1). The different chemical composition of the cereals affects how US affects the functional properties of the material. Therefore, the differences compared to the results obtained by (Vela et al., 2021) for rice flour are explained by a significantly different chemical composition with a low proportion of β-glucans and a high proportion of insoluble polysaccharides, of which cellulose and hemicellulose are the most abundant (Fernando, 2013), compared to OB and BB. Enhanced hydration properties and reduced complex viscosity resulting from the US treatment synergistically improved the techno-functional properties of OB and BB, contributing positively to the quality of flat breads (*Chapter 3 - Section 4*).

However, due to its potential to improve the functional properties of flour, the processing of oat and barley flour with PEF technology was studied for the first time in *Publication No. 4*. PEF treatment leads to depolymerization of the starch chains and destruction of the amorphous and crystalline structure of the starch (Achayuthakan et al., 2023; Han et al., 2012). Such damage to the starch structure led to an overall decrease in the pasting viscosity profile of PEF-

treated wheat and oat flour (Achayuthakan et al., 2023; Duque et al., 2020b). Further confirmation of the effects of PEF treatment on starch is a higher breakdown viscosity, which together with reduced final and setback viscosity, is associated with damage of the starch granules of the flour (Jokinen et al., 2023). The replacement of wheat flour (30%) with PEF-treated OF or BF resulted in significantly lower peak, cold paste, and setback viscosities compared to blend of wheat flour with the control OF and BF (*Publication No. 4* - Table 7). (Duque et al., 2020b) found that the lower setback viscosity is associated with reduced amylopectin retrogradation of PEF-treated oat flour, which may have a positive effect on the slowing down the bread staling rate. This is confirmed by the results presented in the Table 7.

The effects of PEF treatment on the gluten aggregation properties of flours are not documented in the available literature. However, *Publication No. 4* shows the significant effect of PEF technology on Glutopeak parameters. A short peak maximum time (PMT) and a high aggregation energy (AGGEN) are Glutopeak parameters that indicate high-quality flours (Amoriello et al., 2016). The partial replacement of wheat flour with OF or BF reduced the PMT and AGGEN, indicating a weak development of the gluten network due to the presence of high dietary fiber content, with insoluble fiber having a particularly disruptive effect. These Glutopeak parameters were significantly increased by replacing the control flour with PEF-treated flour even compared to the wheat control (*Publication No. 4 – T*able 7). Numerous studies have shown that WU-AXs have a negative effect on flour quality, while WE-AXs can improve it (Nishitsuji et al., 2020; Saeed et al., 2016). The WE-AXs increase water absorption, affect the ability to bind macromolecules by acting as a "filler" between starch and protein, and form a secondary weaker network, thus supporting the gluten network and contributing to dough stability (Courtin and Delcour, 2002; Zhu et al., 2023). *Publication No. 4* (Table 3 and 4) shows an increased proportion of WE-AXs and a lower Mw of OF β-glucans, which may have contributed to a better development of the gluten network.

Overall, *Publications 3* and *4* describe US and PEF technologies as promising alternative methods for processing flour and bran. These techniques have different effects on their biopolymers and improve four/bran functional properties as well as the rheological properties of the dough.

3.5. The effects of pre-processing on sourdough fermentation of oat and barley flour and bran

To test the efficacy of inactivated β-glucanase with US treatment on the retention of β-glucans during sourdough fermentation, the fermentation of US-treated OB and BB and the acidification rate of these treated materials were investigated (*Unpublished data*).

Comparing the results of acidification rate and TTA from Table 5 and *Publication No. 2* (Figure 1, Tables 3 and 4), OF and BF had a greater acidification power than bran. On the other hand, barley had a higher acidification power than oats in both cases. It is important to emphasize that the sourdough fermentation conditions for flour and bran were different, with the flour being fermented with starter LIVENDO LV1, dough yield (DY) 300, at 30°C for 24 h (*Publication No. 2*), while the bran was fermented with starter LIVENDO LV4, DY 667, at 30°C for 24 h. The pH changes during the 24-h sourdough fermentation were well fitted to the Gompertz model in the case of both the flour and the bran (Figure 4, *Publication No. 2* – Figure 1). The maximum acidification rate (μ_{max}) of OF was only 16% higher than that of OB, while this difference was much more pronounced for BF which μ_{max} was 213% higher than that of BB. Likewise, TTA was 65% higher in OF and 178% in BF in relation to bran.

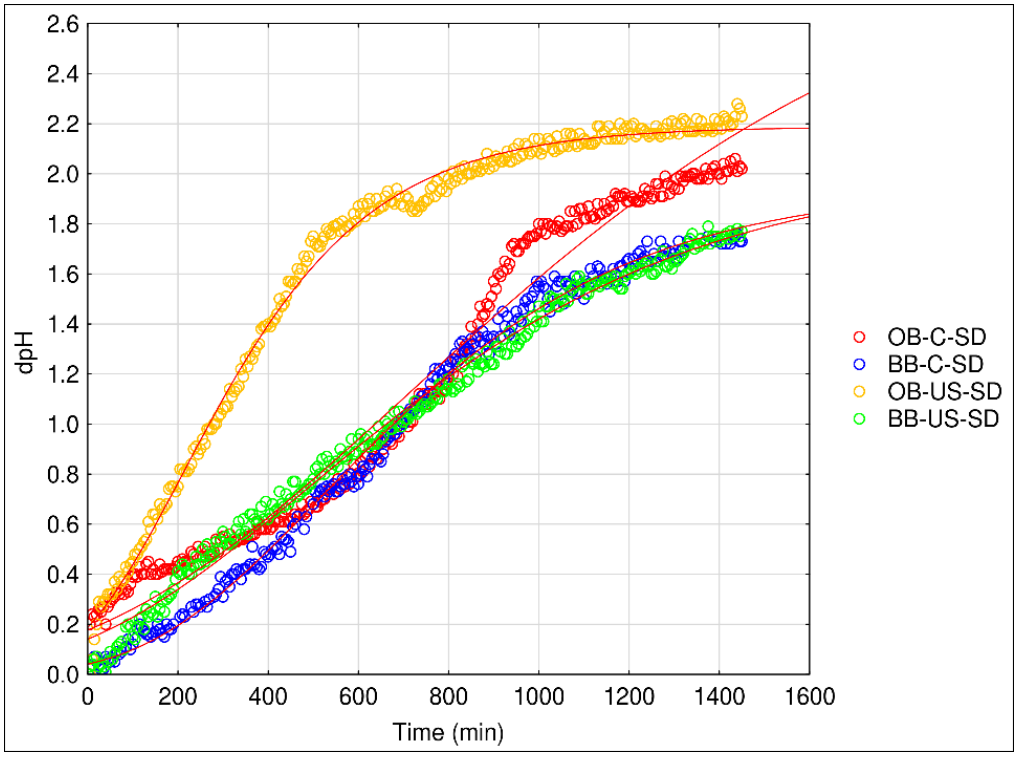


Figure 4. Acidification kinetics fitted to the Gompertz model for control (C) and ultrasonically pretreated (US) oat (OB) and barley (BB) bran.

Table 5. The parameters of acidification kinetics during 24 h of fermentation according to the Gompertz model, colony forming units (CFU) of LAB and yeast, pH value at the end of fermentation, total acidity (TTA), and total β-glucans content of sourdough.

Sample/ parameter	OB-C-SD	BB-C-SD	OB-US-SD	BB-US-SD
μ_{max} (h^{-1})	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.13 ± 0.01^{a}	0.09 ± 0.00^{b}
A (dpH)	2.04 ± 0.02^{b}	1.75 ± 0.02^{c}	2.23 ± 0.03^{a}	1.77 ± 0.01^{c}
R^2	0.996	0.989	0.973	0.996
Time (h)	14.5	18	14.5	23
LAB (CFU/g)	1.63×10^{9}	1.42×10^{8}	1.23×10^{9}	4.35×10^{8}
Yeast (CFU/g)	1.77×10^{6}	1.54×10^{6}	6.15×10^{6}	1.75×10^{6}
pH	3.98 ± 0.13^{a}	3.90 ± 0.04^{a}	3.93 ± 0.16^{a}	3.95 ± 0.08^{a}
TTA (mL 0.1 M NaOH)	3.85 ± 0.42^{b}	2.11 ± 0.15^{c}	5.81 ± 0.28^{a}	2.25 ± 0.31^{c}
β-glucans (g/100 g d.w.)	5.40 ± 0.32^{b}	3.00 ± 0.1^{d}	7.96 ± 0.41^{a}	4.71 ± 0.1^{c}

Results are expressed as mean±standard deviation (n=2)

a–d Values within the same row marked with different letters differ significantly according to Tukey's test ($p < 0.05$)

OB – oat bran; BB – barley bran; C – control; US – ultrasound pretreated; SD – sourdough.

μ_{max} – maximum acidification rate; A – difference in pH (units)

The US technology improves the traditional processing of cereal- and pseudocereal-based foods, including germination, milling, fermentation, and cooking, while improving the functionality of their by-products (Estivi et al., 2022; Fărcaș et al., 2022; Yüksel and Elgün, 2020). In this dissertation, however, the use of US as a pre-treatment prior to sourdough fermentation was investigated for the first time. The results showed that the interaction between the type of bran and the US pre-treatment influences the pH drop and the acidification rate during sourdough fermentation. The US treatment slightly slowed the acidification rate of BB, possibly due to reduced α-amylase activity. Similarly, PEF treatment slowed down the acidification rate of OF (by 12.5%), while it had no effect on BF (Figure 5, Table 3). This was the result of reduced α-amylase activity (by 78%) after PEF treatment of OF (*Publication No. 4*).

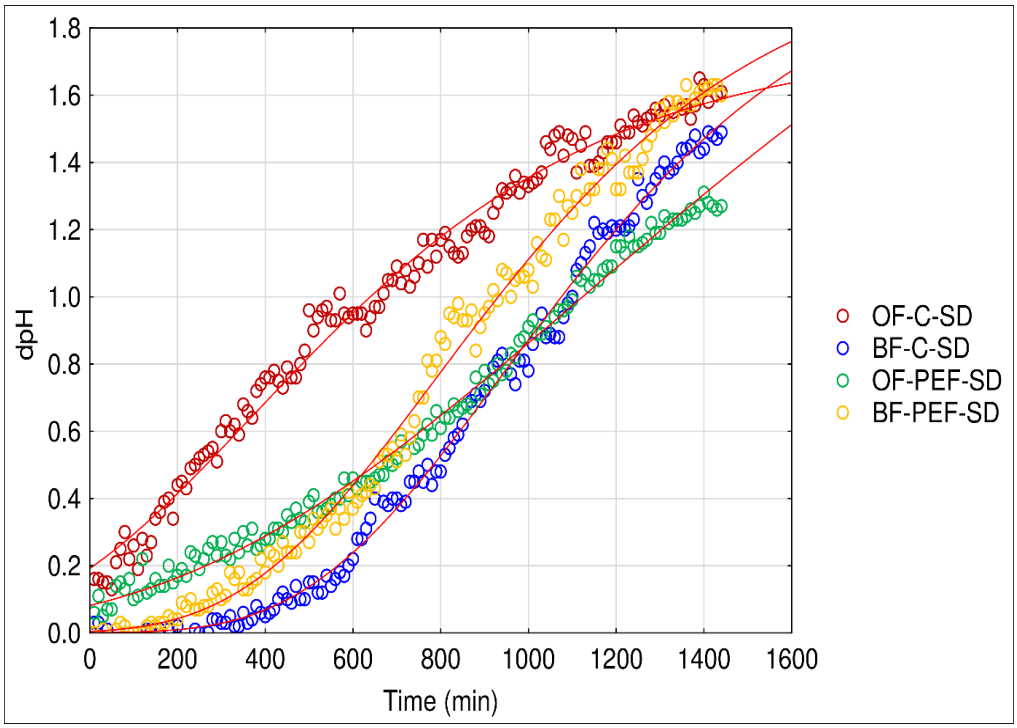


Figure 5. Acidification kinetics fitted to the Gompertz model for control (C) and pulsed electric field pretreated (PEF) oat (OF) and barley (BF) bran.

The optimum sourdough pH for bread making is 4 and is reached in 6 to 24 h at 25°C to 35°C (De Vuyst and Neysens, 2005). The BB required 18 h to reach a stable pH (extended to 23 h after US treatment), while OB required 14.5 h in both cases. Despite lower LAB counts in barley sourdough than in oat sourdough (Table 5), the viable LAB and yeast cell counts at the end of fermentation were typical of mature sourdough (De Vuyst et al., 2017). After US pretreatment, the TTA of the OB sourdough increased by 51% compared to the control and by 158-175% compared to the sourdough from control and US-pretreated BB (Table 5). This increase brought the TTA values close to the values achieved after a longer fermentation time (48 h) as reported by Sahin et al. (2021), although the fermentation time in this dissertation was much shorter due to the US pretreatment. In the control samples, the total β-glucan content in OB and BB was maintained at 66-71% compared to pre-fermentation values, while it was 93-95% in the samples pre-treated with US.

Although control BB had lower β-glucanase activity compared to OB (*Publication No. 3* – Figure 1), prolonged fermentation resulted in greater degradation of barley β-glucans (Table 5), which was favored by ideal sourdough fermentation conditions for β-glucanase activity. The US treatment increased the extractability of β-glucans and decreased β-glucanase activity in

both OB and BB, resulting in a higher total β-glucan content in sourdough compared to those from the control bran (by 47.5% in OB and 56.7% in BB sourdough). Previous studies indicated that the β-glucan content in OB did not change significantly during fermentation with rye sourdough, but the Mw decreased due to endogenous β-glucanase activity (Degutyte-Fomins et al., 2002). Heat or microwave treatment preserved the β-glucan content in oats and oat-based products by inactivating the endogenous β-glucanase (Lu et al., 2019; Pérez-Quirce et al., 2017). To preserve this nutrient from negative changes during fermentation, US treatment has proven to be effective.

3.6. Nutritional, physical, and sensory properties of flat breads with oat and barley ingredients preprocessed with innovative technologies

The influence of innovative (US and PEF) on the nutritional, physical and sensory properties of flat bread are presented in Table 6, Figure 5, and *Publications No. 4.* The addition of OF, BF, OB and/or BB to bread leads to an increased content of β-glucans but also PA.

Replacing 10% of semi-refined wheat flour with OB or BB, native or pretreated, or their sourdough significantly increased the β-D-glucans content of flat breads (Table 6). Enrichment was significantly higher with OB compared to BB, and the same trend was observed after US pretreatment, but relatively lower after sourdough fermentation. The flat breads from US-treated OB and BB had 25-35% and 47-65% higher β-glucans content compared to flat breads from control OB and BB, respectively (Table 6). The most significant enrichment was achieved by replacing wheat flour with US-treated OB (182%), the lowest by sourdough from untreated BB (only 28%). The increase in the content of β-glucans in the flat bread corresponded to that of the raw material, which was confirmed by a strong positive correlation ($r=0.966$, $p=0.03$) between the content of β-glucans in the bread and in the sourdough. Nevertheless, 280-288 g of oat flat bread or 340-390 g of barley flat bread made from US-treated bran (fermented or not) could provide the recommended daily β-glucans intake of 3 g.

The addition of control or US-pretreated OB or BB resulted in a slight increase (8-11%) in the specific volume compared to the wheat control flat bread (Table 6). However, the specific volume of the composite flat breads was affected by both the type of bran ($p=0.049$) and the addition of sourdough ($p<0.01$). These results are consistent with the study by Lee et al. (2020), in which wheat bread enriched with 5-10% wheat bran showed a slightly increased specific volume, while a proportion of more than 15% resulted in a lower specific volume compared to

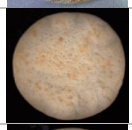
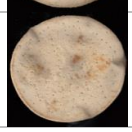
the wheat control. In addition, Tiwari et al. (2013) found that a bread of acceptable quality can be produced when 30% of the wheat flour is replaced by OB, although the specific volume decreases significantly. The specific volume of the bread is influenced by gas retention, which is increased during sourdough fermentation due to the increased solubility of dietary fiber, especially WE-AXs, as emphasized by Verdonck et al. (2023). The favorable ratio of soluble to insoluble fiber in the bran (40% in BB and 50% in OB) (*Publication No. 3* – Table 1) probably contributed to maintaining bread volume. The US treatment leads to an increase in the solubility of biopolymers, i.e. fibers such as β-glucans and AXs (Du et al., 2019). The increased values of WSC and WRC after the US treatment (*Publication No. 3* – Table 5) indicate that the US treatment further improved the ratio of soluble and insoluble fibers, but sourdough fermentation also contributes in the same way. The increased solubility of dietary fiber after the US treatment, already described in this *Chapter 3 – section 2.3*, is evident from the increased content of soluble β-glucans in both bran types (Table 3). The increased specific volume of the flat breads with OB/BB sourdough, where the amount of sourdough was added to the mixture so that the added bran represented 10% of the weight of the wheat flour, contrasts with the results from *Publication No. 2* (Figure 3). In *Publication No. 2* the proportion of 30% and 50% of added oat/barley sourdough (dough weight) means the replacement of 18% and 30% of the wheat flour with oat or barley flour/bran blend, respectively. A higher proportion of oats or barley leads to a higher dietary fiber content, which limits the proper development of the gluten network, as described in *Chapter 3 – section 2*.

In previous studies, US treatment of rice flour resulted in bread with a higher specific volume of 17.6-24% compared to the control with untreated flour, probably due to the partial depolymerization of the starch, which leads to improved fermentation and higher production and retention of CO_2 (Qin et al., 2022; Vela et al., 2021). Previous studies, such as those by De Vuyst et al. (2017) and Martín-Garcia et al. (2023), have highlighted the increased specific volume of sourdough-based bread with bran compared to yeast-control bread. Similarly, (Pontonio et al., 2020) observed a considerable increase in the specific volume of wheat bread by 15-25% when sourdough from barley, wheat or emmer bran was used. In this dissertation, the specific volume of flat breads with sourdough, control or US-pretreated OB and BB increased significantly by 12-23% and thus even exceeded the specific volume of the wheat control bread (Table 6).

The addition of OB slightly darkened the bread color, while BB significantly changed the total color difference (TCD) ($1.5 < TCD < 3$) compared to the control wheat bread. The presence

of pigments in the cereal bran, such as chlorophyll and carotene, contributes to this darkening effect (Hu et al., 2022). Interestingly, OB or BB that underwent US-pretreatment and/or sourdough fermentation resulted in a very distinct TCD (>3). The sourdough flat breads appeared lighter and less yellow compared to the control, possibly due to the fact that the lower sugar content and pH inhibited the activity of PPO, which is responsible for the darkening of the dough. PPO, which is mainly found in cereal bran, catalyzes the oxidation of phenolic compounds, resulting in the brown pigment melanin (Liu et al., 2019; Olaerts et al., 2018). Nevertheless, the color formation of bread during baking is mainly based on Maillard reactions between amino acids and reducing sugars (Olaerts et al., 2018). Sourdough fermentation creates an acidic environment that limits the availability of amino acids and reduces the sugar content, thereby suppressing the Maillard reaction (Limbad et al., 2020). The addition of US-pretreated bran further increased TCD, possibly by reducing α-amylase and/or PPO activity.

Table 6. Physico-chemical properties of composite flat breads depending on the pretreatment compared to the control wheat bread

Appearance	Sample	Dry matter (g/100g)	Total β-glucans (g/100 g d.w.)	Specific volume (cm^3/g)	Spread ratio	Lightness *L**	Yellowness *b**	TCD
	FB-C	60.63 ± 4.42^{a}	0.60 ± 0.01^{d}	2.34 ± 0.20^{b}	18.27 ± 0.72^{bc}	47.06 ± 0.35^{bc}	7.54 ± 0.41^{b}	n.a.
	FB-OB	60.70 ± 3.20^{a}	1.35 ± 0.06^{b}	2.60 ± 0.10^{ab}	20.68 ± 0.23^{ab}	46.69 ± 0.44^{c}	7.13 ± 0.25^{b}	0.44
	FB-OB-US	61.67 ± 5.80^{a}	1.69 ± 0.04^{a}	2.39 ± 0.26^{b}	16.66 ± 0.73^{c}	49.86 ± 2.15^{b}	16.13 ± 0.79^{a}	7.09
	FB-OB-SD	63.31 ± 4.56^{a}	1.22 ± 0.06^{b}	2.75 ± 0.19^{a}	19.31 ± 0.97^{b}	53.54 ± 0.66^{a}	3.10 ± 0.47^{c}	7.88
	FB-OB-US-SD	64.81 ± 5.41^{a}	1.65 ± 0.06^{a}	2.62 ± 0.06^{ab}	22.13 ± 1.34^{a}	55.22 ± 1.42^{a}	3.98 ± 0.84^{c}	8.94
	FB-BB	62.96 ± 7.41^{a}	0.94 ± 0.03^{c}	2.55 ± 0.10^{ab}	20.98 ± 0.57^{ab}	44.95 ± 0.1^{c}	6.22 ± 0.12^{b}	2.38
	FB-BB-US	60.34 ± 6.12^{a}	1.38 ± 0.03^{b}	2.53 ± 0.08^{ab}	16.73 ± 0.59^{c}	46.43 ± 0.92^{c}	14.47 ± 1.22^{a}	9.41
	FB-BB-SD	63.02 ± 6.18^{a}	0.77 ± 0.01^{c}	2.87 ± 0.06^{a}	20.74 ± 0.71^{ab}	54.72 ± 0.14^{a}	3.65 ± 0.03^{c}	8.64
	FB-BB-US-SD	60.87 ± 6.18^{a}	1.27 ± 0.07^{b}	2.85 ± 0.10^{a}	20.18 ± 0.44^{ab}	56.19 ± 1.72^{a}	4.62 ± 1.22^{c}	9.64

Results are expressed as mean±standard deviation (n=3)

FB – flat bread; C – control wheat; BB – barley bran; OB – oat bran; SD – sourdough; US – ultrasound pretreated; TCD – total color difference

n.a – not applicable

a-d Values within the same column marked with different letters differ significantly according to Tukey's test ($p < 0.05$)

Flat bread enriched with control OB and BB exhibited higher hardness (16-64%) and chewiness (28-46%) compared to the control wheat bread (Figure 6). Similarly, Tiwati et al. (2016) demonstrated a significant increase in bread hardness of 303-567% after replacing wheat flour with 50% and 70% OB. On the other hand, US pretreatment and sourdough fermentation have been shown to be techniques that can overcome these problems associated with enriching wheat bread with whole grain flours. As Ma et al. (2022) stated, pregelatinized starch can improve textural properties by reducing the hardness and chewiness of the bread while increasing cohesiveness, as it has a higher water retention capacity and thus improves the overall water content of the bread. In addition, the cavitation that occurs during the US treatment, combined with temperatures of 67°C or 72°C towards the end of the treatment, triggers pregelatinization of the starch. Vela et al. (2023) reported that the bread made with a US treated (20 min, on-off pulse of 80%, 20°C, 22 mm probe diameter) rice flour-water suspension (25%) had significantly lower crumb hardness (23%) and chewiness (16%). Similarly, Jalali et al. (2020) found that US-pregelatinized corn flour significantly reduced (53%) the crumb hardness of gluten-free bread. Cohesiveness was primarily affected by the bran type ($p=0.03$) and the interaction between sourdough and US pretreatment ($p=0.02$). It was significantly higher in barley-containing flat breads than in oat-containing flat breads or after US pretreatment or sourdough fermentation individually. After the addition of sourdough, chewiness decreased by 51-57% in both barley and oat flat bread, while hardness decreased by 18% only in oat flat bread and cohesiveness increased by 8-11 % (Figure 6). These results differ from those in *Publication No. 2* (Figure 3), although the reasons for this are like the discrepancies observed in the bread specific volume. The reduction in the hardness or chewiness of oat flat bread was more pronounced with US pretreatment than with sourdough fermentation. The positive effect of US pretreatment prior to sourdough fermentation was particularly evident in composite wheat-oat flat bread, which showed a 27% reduction in hardness and a 20% reduction in chewiness compared to flat bread made with sourdough from untreated OB. Flat bread with US treated OB or BB exhibited lower hardness (17-48%) and chewiness (52-66%) and higher cohesiveness (16-18%) compared to the wheat control flat bread. This improvement could be due to the increased solubility of β-glucans and the improved water swelling (42-48%) and retention (44-59%) of OB and BB, as previously mentioned (*Publication No. 3* – Table 5).

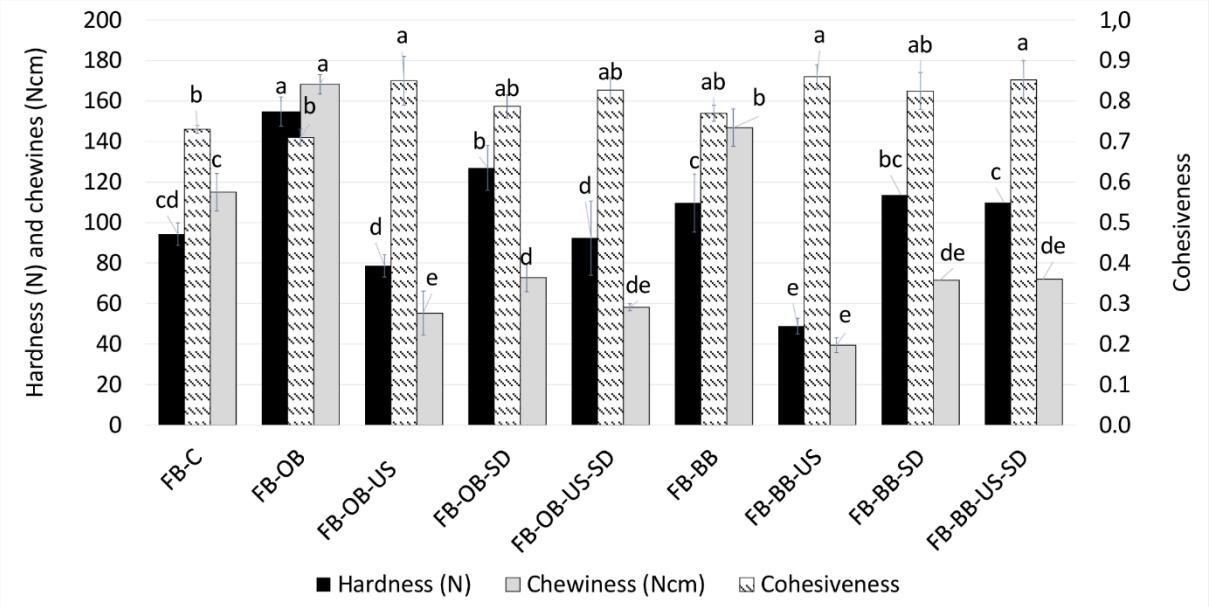


Figure 6. Textural properties of composite flat breads compared to wheat control

FB – flat bread; C – control wheat; BB – barley bran; OB – oat bran; SD – sourdough; US – ultrasound treated; a–e Values marked with different letters differ significantly according to Tukey's test ($p < 0.05$)

The influence of the 30% substitution of semi-refined wheat flour with control and PEF-treated OF and BF was also reflected in the nutritional and physical properties of the bread, which is presented in *Publication No. 4*. It has been shown that the supplementation of wheat flour with OF in a ratio of 5-25% leads to an increase in the proportion of β-glucans in wheat bread by 52-254% and TDF by 48-72% (Astiz et al., 2023), while the addition of OF from the black oat varieties in a ratio of 3-9% leads to an increase in the β-glucans content of wheat bread by 100-143% (Ivanišová et al., 2023). Replacing wheat flour with 40% or 60% of BB also results to a significant increase in β-glucans by 160% and TDF by 67% and 310%, respectively, compared to wheat control bread (Pejcz et al., 2017). These authors have also shown that the addition or replacement of wheat flour with OF or BF results in bread with lower specific volume and impaired textural properties, i.e. higher crumb hardness and chewiness.

Replacing 30% of semi-refined wheat flour with OF resulted in flat breads that showed a remarkable increase in β-glucans content by 557% and TDF by 54%. Replacing wheat flour with BB also resulted in flat breads with significantly increased TDF content (159%) and a considerable, albeit slightly lower, increase in β-glucans content (194%) (*Publication*

No. 4 - Table 8). As a result of the partial inactivation of β-glucanase and the improved extractability of β-glucans, flat breads made from PEF-treated OF and BF showed an additional increase in β-glucans content (21% and 31%, respectively) compared to flat breads made from control flour. Consequently, these flat breads had significantly higher levels of β-glucans, with increases of 694% and 289%, respectively, compared to the wheat control flat bread (*Publication No. 4* – Table 8). In flat breads made from US-pretreated bran or PEF-pretreated flour, the content of β-glucans increased as a result of the extraction of β-glucans, but also of partially inactivated β-glucanase, compared to flat breads made from untreated flour/ bran before bread making, where the content of β-glucans decreased. Consumption of 2 slices of oat flat bread and 3 slices of barley flat bread made from PEF-treated OF and BF can provide a one third of the recommended daily β-glucans intake of 3 g, as was the case with the flat breads made from only 10% OB and BB flour and 90% wheat flour or those made from oat retarded dough with 50% sourdough. The application of the PEF treatment did not result in a significant difference in TDF content. According to the results, flat breads enriched with oat flour could be classified as "source of dietary fiber"," while those with barley flour could be described as "high in dietary fiber".

As already described, substituting 10% of the wheat flour with OB or BB slightly increased the specific volume of the flat breads (Table 6). However, when 30% of the wheat flour was replaced by OF or BF, a decrease in specific volume was observed and a higher spread ration compared to the control wheat flat bread (*Publication No. 4* – Table 8). The specific volume was influenced by the interaction between the type of flour and the application of the PEF treatment. A control wheat flat bread had a higher value, followed by a control oat and barley composite flat bread and a PEF-rated oat and barley composite flat bread (3.14, 2.53, 2.38, 2.25- and 1.82 mL g^{-1}, respectively) (*Supplementary* – Figure 4, *Publication No. 4* – Table 8). The differences in specific volume can be attributed to the increased β-glucan content in these composite flat breads. Since soluble dietary fibers can promote the development of the gluten network and the specific volume of the bread, their presence in high concentrations can have the opposite effect. The increased dietary fiber content of BF and OF dilutes the gluten and impairs its structural integrity. The β-glucan, a soluble fraction of dietary fiber, hinders gas retention by interacting with the gluten network. Its water-binding properties hinder the formation of the gluten network, resulting in an underdeveloped gluten structure and a lower specific volume (Courtin and Delcour, 2002; Gamel et al., 2015; Wang et al., 2018). In addition, both the concentration and the molecular

weight of β-glucans significantly influence the specific volume of the bread (Skendi et al., 2010). In addition to β-glucans, the presence of WU-AXs represents a barrier to the proper development of the gluten network, which in turn leads to a lower bread specific volume (Courtin and Delcour, 2002). The WU-AX ensures that the protein network develops to bind the gas produced during fermentation and ensure sufficient stability for the baking phase (Ahmed and Thomas, 2015). Although the proportion of WE-AX increased significantly by 56% for BF and 68% for OF after PEF treatment (*Publication No. 4* – Table 4), a significantly lower specific volume of the flat breads was observed compared to flat breads from control flour samples. According to the study by Zhang et al. (2023), the specific volume of bread increased by 3.6-4% after the addition of WE-AXs (2.3-3.5%), which ultimately led to a lower crumb hardness, but the addition of a higher AXs concentration of 5% led to the disruption of the dough structure and ultimately to the lower bread volume and higher crumb hardness. It is therefore possible that the increase in WE-AXs after the PEF treatment was too high and ultimately led to a disruption of the gluten network. Another possible explanation for this is the shortened PMT Glutopeak parameter (*Publication No. 4* – Table 7), which indicates the time required for the gluten network to develop. Therefore, a longer mixing time is required after the addition of PEF-treated flour so that the gluten network can develop properly, and the flat bread has a greater specific volume.

The addition of control OF did not lead to any significant changes in the textural properties (crumb hardness, chewiness, cohesiveness, and resilience) of the bread, while the addition of BF significantly reduced these properties compared to the control wheat flat bread. This pattern persisted after the PEF treatment, which further decreased all textural properties of the barley flat bread. Although the replacement of wheat flour with OF or BF was expected to increase hardness and chewiness, minimal changes in the case of OF addition and a decrease in these parameters with the BF addition were recorded (*Publication No. 4* – Table 8). This trend continued after the PEF treatment, which led to a further reduction in all the textural properties of the barley flat bread. Just as the US-pretreated OB and BB improved the textural properties of the flat breads (Table 6), the PEF-treated OF and BF also significantly reduced the textural properties of flat breads (*Publication No. 4* – Table 8). As with US-pretreated bran, such results are attributed to water-soluble fibers, i.e. β-glucans, which form gels with a soft structure and consequently make the bread crumb less hard and chewy (Andrzej et al., 2020). The textural properties during the 96-h storage of oat and barley flat breads at 20 ± 2 °C are shown in the Figure 7 and Table 7. The initial

hardness of control oat flat breads was slightly higher than that of the other flat breads. All flat breads showed a sudden initial decrease in hardness after 24 h of storage, but also an increase after 48 h (Figure 7a). The cohesiveness and resilience of all flat breads decreased continuously, but not significantly, over time (Figure 7b and 7c). The setback viscosity indicates the retrogradation tendency of the amylose in the starch paste and correlates with the texture of different foods (Achayuthakan et al., 2023). The hardening effect of flat breads was more pronounced in flat breads made from control OF and BF, which is evident from the difference in hardness after 96 h of storage (Table 7), also PEF-treated flour had a lower SV compared to the control OF and BF (*Publication No. 4* – Table 7). Therefore, PEF treatment is considered beneficial for improving product quality, as retrogradation usually leads to quality deterioration characterized by syneresis or increased hardness (Achayuthakan et al., 2023). Hardening during the 96-h storage of barley flat bread could be adapted to the Avrami model (Table 7), which was not the case for oat flat breads, especially those made from PEF-treated OF.

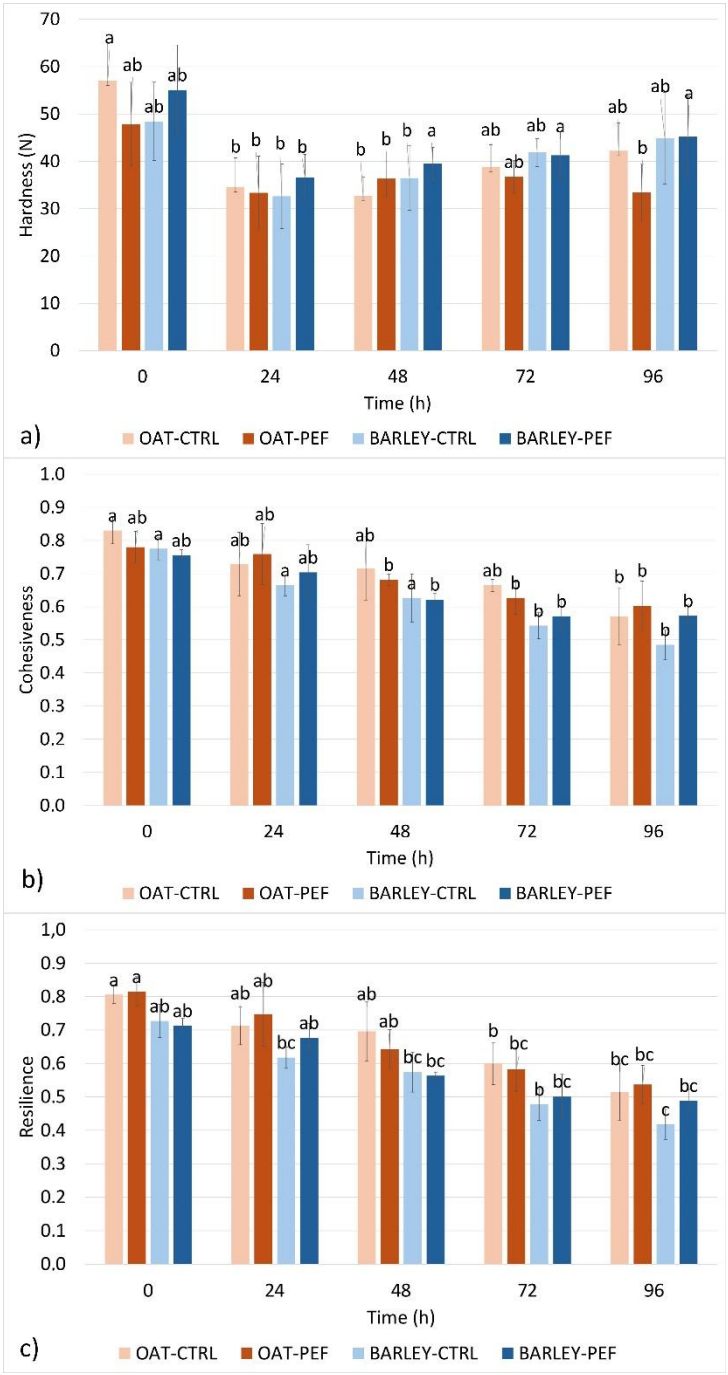


Figure 7. Hardness, cohesiveness, and resilience of oat and barley flat bread during storage at 20 ± 2 °C for 0 – 96 h

Results are expressed as mean±standard deviation (n=3)

a-c Values marked with different letters differ significantly according to Tukey's test ($p < 0.05$).

Table 7. Calculated kinetics parameters from TPA measurements of flat breads made from control and PEF-treated oat and barley flour

Sample	k	n	R^2	p	d(hardness, 96-24h), N	d(hardness, 96-0h), N
Oat-ctrl	<0.001	3.72	0.81	<0.001	7.65	-14.79
Oat-PEF	7.70	19.40	0.00	<0.001	0.05	-14.47
Barley-ctrl	<0.001	3.22	0.99	<0.001	12.21	-3.59
Barley-PEF	<0.001	3.00	0.98	0.09	8.59	-9.91

k – rate constant; n – Avrami exponent; d(hardness) – difference in hardness between 96h and 24h and 0h

Replacing wheat flour with control and PEF-treated OF and BF resulted in minimal differences in flat bread color (TCD<1.5) compared to wheat control flat bread (*Publication No. 4* – Table 8). Sensory analyzes of control flat breads and flat breads with PEF-treated flour, were carried out in the sensory laboratory of the Faculty of Food Technology and Biotechnoloy (PBF), according to ISO 6658:2017. Hedonic acceptability test and ranking according to preferences was conducted on 42 participants (34 women and 8 men, age 21-64), employees of PBF. The procedure described by (Aldughpassi et al., 2021), was used to estimate gluten flat bread. Testers indicated how much they liked or disliked each flat bread sample on a 9-point hedonic scale, also known as a liking scale. The scale ranged from 1 'extremely dislike' to 9 'extremely like'. Flat breads made from PEF-treated OF and BF were equally well accepted according to the sensory hedonic acceptability score (Table 8). The preference ranking test showed that consumers preferred oat over barley flat breads and PEF-treated oat flat breads over its control, while there was no difference in preference for barley flat breads (Figure 8). Thes results indicate that PEF-treated flours can be used to produce flat breads without a significant difference compared to the control flat breads.

Table 8. Scores for individual sensory parameters and overall acceptability of flat breads

Sample	Appearance	Odor	Flavor	Texture	Overall
Oat control	7.64 ± 1.53^a	7.31 ± 1.73^a	7.33 ± 1.86^a	7.55 ± 1.56^a	7.38 ± 1.64^a
Barley control	7.12 ± 1.56^a	6.76 ± 1.78^a	6.57 ± 1.68^a	6.90 ± 1.66^a	6.90 ± 1.49^a
Oat-PEF	7.81 ± 1.44^a	7.45 ± 1.55^a	7.76 ± 1.64^a	7.86 ± 1.56^a	7.74 ± 1.56^a
Barley-PEF	6.93 ± 1.74^a	6.81 ± 1.35^a	7.10 ± 1.56^a	7.36 ± 1.50^a	7.12 ± 1.47^a

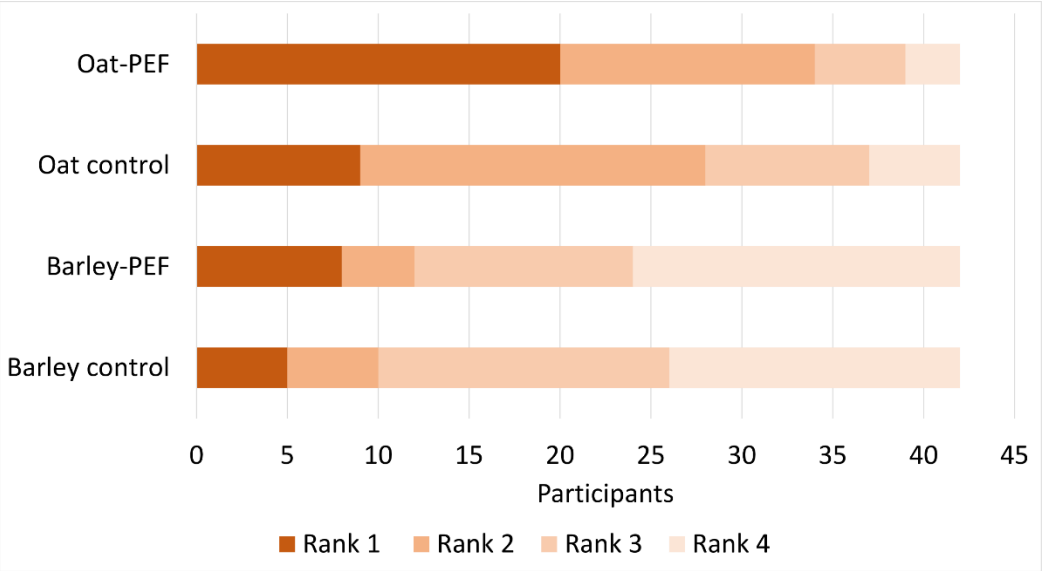


Figure 8. Ranking of flat breads by preference.

Chapter 4

Conclusions and prospects

- Among Croatian flat bread products, single-layer flat bread predominates, with the various types of *pogacha* being the most common type. Refined wheat flour is used as the main ingredient, which is why Croatian flat breads have a low fiber content (only 2.9 g/100g on average). Other flours used are corn and rye, whereas oat- or barley-containing breads are not present at the Croatian market. Most of the Croatian flat breads are yeast-leavened. Croatian flat breads have received various national and European quality recognitions such as European protected geographical indication (*poljički soparnik* and *zagorski mlinci)* an "Intangible Cultural Good" (*Pogacha* z *oreji*), or the "Croatian Quality" label (*pogacha*).
- The majority of Croatian consumers (56%) prefer to eat conventional bread, with 37% of them consuming bread every day. The majority of consumers (70-94%) is interested in flat bread with improved nutritive value, mostly enriched in fiber (β-glucans), and the majority (62-67%) is interested in flat bread enriched with oats or barley.
- Bran contains significantly higher levels of dietary fiber (71-166%), β-glucans (45-165%), total phenolic content (66-189%), and all minerals (10-65%) compared to flour. Barley flour is richer in minerals and β-glucans than oat flour and has twice the dietary fiber and phenolic content. However, the opposite is true for bran.Enriching wheat flat bread with oat and barley flour or bran has a positive effect on the nutritional value, but has a detrimental effect on the physical properties. The partial substitution of wheat flour with oat and barley bran considerably diminishes the textural properties of flat bread by increasing its hardness (16-64%) and chewiness (28-46%). On the other hand, the partial replacement of wheat flour with oat and barley flour leads to a significant reduction in the specific volume (19-24%) and an increase in the crumb hardness (11-23%) of the flat bread. These shortcomings can be improved by using traditional (sourdough fermentation) and innovative (US and PEF) techniques.
- The OF has a low acidifying capacity when fermented with a commercial starter. Partial replacement of OF with OB (in a ratio of 3:1) increases (46%) the acidification rate of its sourdough. The higher addition (50% vs. 30% of dough weight) of oat or barley sourdough contributes to a higher β-glucan content in composite flat bread. The delayed fermentation (retardation at 2 °C, 24h) reduces the content of phytic acid (27-38%), but also promotes the degradation of β-glucans (4-28%). Yet, the lower sourdough addition (30% vs. 50% of dough weight) results in a more desirable bread volume and texture.

- Sourdough as a clean label ingredient in bread production successfully slows down (36-79%) the enzymatic darkening of the dough during the retardation process and prolongs its shelf life at temperatures of 0-4 °C.
- The US treatment of oat or barley water-bran suspensions (15%) with a higher Ws (217.5 kJ kg^{-1} and 348 kJ kg^{-1}) leads to longer treatment times (7.8-13.4 min) and higher final temperatures (65-85°C). Conversely, the pulsation mode extends the treatment time but lowers the final temperatures with the same Ws. The setting of the Ws and the pulsation mode during US treatment has a significant influence on the enzyme and antioxidant activities, the PA concentration, the TPC as well as the rheological and hydration properties of OB and BB.
- The highest Ws during US treatment (348 kJ kg^{-1}) causes the most significant reduction in β-glucanase activity (by 82% and 55%) and increase in hydration properties *i.e.*, WS (by 42-48 %) and WRC (by 44-59 %) of OB and BB. Medium Ws (217.5 kJ kg^{-1}) in pulsating mode effectively reduces PA content (by 17% and 39%), while the lowest specific energy input (87 kJ kg^{-1}) activates endogenous phytase in OB and BB (by 40-44%). Elevated temperatures (above 85°C) developed during US treatment have a detrimental effect on the TPC and antioxidant activity (DPPH and FRAP).
- The US treatment of OB and BB increases the total β-glucan content by 12%, water solubility by 31-40% while reduces its Mw by 7-22%. As a pre-treatment before sourdough fermentation, US increases the acidification power of OB, but has the opposite effect on BB. Replacing 10% of the wheat flour with US pretreated and/or fermented OB or BB improves the specific volume, color, and textural properties of the flat bread compared to the wheat control flat bread and flat bread with 10% of untreated OB or BB. Flat breads with US-treated (fermented or non-fermented) OB and BB have a 25-65% higher content of β-glucans compared to flat breads with untreated OB and BB or even 112-182% compared to common wheat bread.
- Hence, US pretreatment of OB and BB can be recommended to reduce the content of antinutrients, to successfully inactivate β-glucanase and prevent β-glucans degradation during fermentation and bread making steps, while enable the production of bread with higher specific volume and improved crumb texture.
- The inactivation of β-glucanase of BF and OF by PEF technology depends on EFI and the Ws, with the highest inactivation (by 40% and 77%, respectively) can be achieved at 12 kV cm^{-1}, 162 ms and 4.5-5.5 kJ kg^{-1}. PEF treatment improves the extractability of

β-glucans by 33.5% with minimal changes in Mw, but also increases the WE-AXs content (56-68%), which together help to strengthen the gluten network of the dough.

- Compared to untreated flours, the addition of PEF-pretreated OF or BF increases β-glucans content (21-32%) of flat bread while does not affect consumers acceptance. In addition, it slows down the hardening of the flat breads during the storage at 20°C for 96 h. Hence, the use of PEF-treated flours in bread making is promising for improving the nutritive value of flat bread; yet it is very important to adapt the bread making process to preserve product technological quality.
- The use of sourdough as a traditional method of bread making is simple and improves bread quality but has a negative effect on the content of β-glucans. In contrast, innovative techniques such as US and PEF increase β-glucans content in processed ingredients and preserve it during bread making but require expensive equipment and a better understanding of process parameters. To achieve the enzyme activity reduction of the PEF technique, the US technique requires higher energy input, longer treatment times, higher temperatures and lower sample-water suspension concentrations. Although the US process consumes more energy and has a lower production capacity, the aggressive cavitation and mechanical effects change the structure of biopolymers and reduce the particle size, which improves the functional properties of the bran for the bakery industry.

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Autobiography

Autobiography

Tomislava Grgić is a research assistant working in the research and higher education since 2021. She holds a Bachelor's degree in Food Technology and a Master's degree in Food Engineering from the Faculty of Food Technology and Biotechnology, University of Zagreb. She is finishing her Ph.D. study at the Faculty of Food Technology and Biotechnology, University of Zagreb, in the field of Food Technology. She is employed as a research assistant in the project Flat Bread of Mediterranean area; INnovation & Emerging process & technology, which is part of the PRIMA programme, an Art.185 initiative supported and funded under Horizon 2020, the European Union's Framework Programme for Research and Innovation. Her research is focused on the enhancement of nutritional and functional properties of flat bread. It involves application of different non-thermal technologies on oat and barley flour and bran, and monitoring their impact on enzymatic and antioxidant activity, dietary fiber, and non-starch polysaccharides (β-glucans and arabinoxylans), and different functional properties. In combination with fermentation, she uses these non-thermal technologies in making a flatbread with improved shelf-life, texture, and consumer acceptability. During her Ph.D study, she spent 3 months at the University of Chemistry and Technology (Prague, Czech Republic) within the Central European Exchange Program (CEEPUS). Until now, she co-authored 5 scientific papers in journals indexed in Web of Science/Current Contents Connect (59 citations; h-index is 4) and 1 scientific paper indexed in PubMed Central. She has participated in international congresses where she presented her research results and published conference posters, all in the area of cereal chemistry and technology. She has participated in the supervision of two diploma theses.

List of authors publications

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Supplementary materials

Table 1. Total acidity (TTA, mL 0.1 M NaOH), pH value at the end of 24 h fermentation, and the acidification kinetics according to Gompertz model depending on the sourdough fermentation conditions

Substrate/ starter	DY	Temp (°C)	pH	TTA (mL 0.1 M NaOH)	μ_{max} (h^{-1})	A (dpH)	λ (h)	T_i (h)	*p*-Value	R^2
Oat flour / LV4	200	22	4.47	n.d.	0.081	1.97	4.24	13.1	<0.001	0.997
Oat flour / LV4	300	22	4.84	6.88	0.056	1.55	5.48	15.6	<0.001	0.994
Barley flour / LV4	200	22	4.27	n.d.	0.099	3.53	9.39	22.4	<0.001	0.995
Barley flour / LV4	300	22	4.11	9.60	0.103	2.05	8.25	15.6	<0.001	0.990
Oat flour / LV1	300	22	5.03	3.50	0.057	0.98	7.42	56.4	0.304	0.907
Barley flour / LV1	300	22	4.19	8.00	0.159	1.38	10.38	13.6	<0.001	0.986
Oat flour&bran/ LV1	300	22	3.98	6.85	0.103	2.94	0.98	11.5	<0.001	0.989
Barley flour&bran/ LV1	300	22	3.97	6.78	0.288	1.76	19.27	44.7	<0.001	0.936

DY – dough yield; TTA – total titratable acidity; μ_{max} – maximum acidification rate; A – difference in pH (units); λ – lag phase; T_i – time to reach μ_{max}

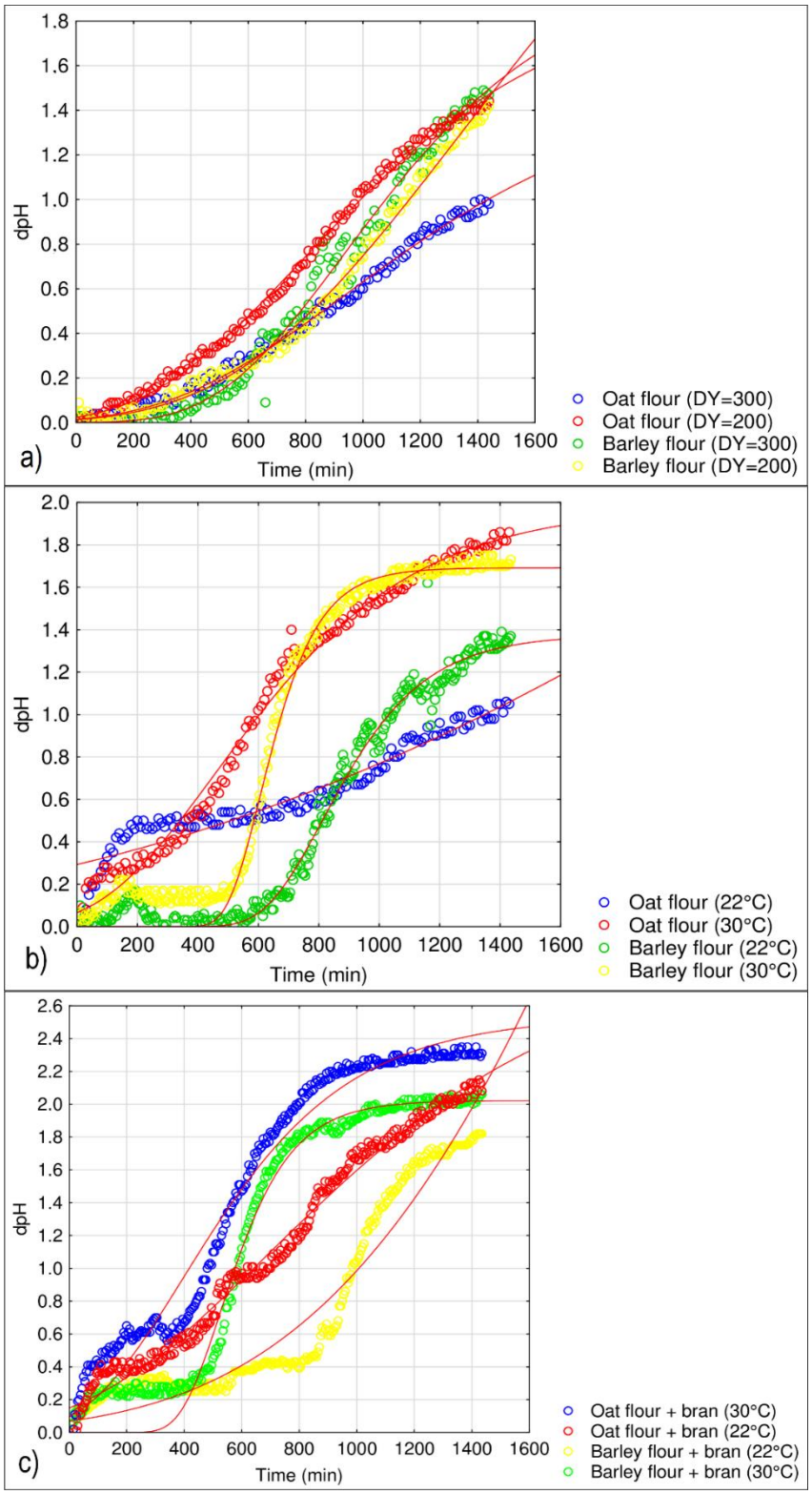

Figure 1. Acidification kinetics fitted to the Gompertz model a) for oat and barley flour depending on dough yield (DY=200 or 300, LV4 starter, 22°C); b) for oat and barley flour at two different temperatures (DY=300, LV1); c) for mixture of oat/barley flour and bran at two different temperatures (DY=300, LV1 starter). DY – dough yield

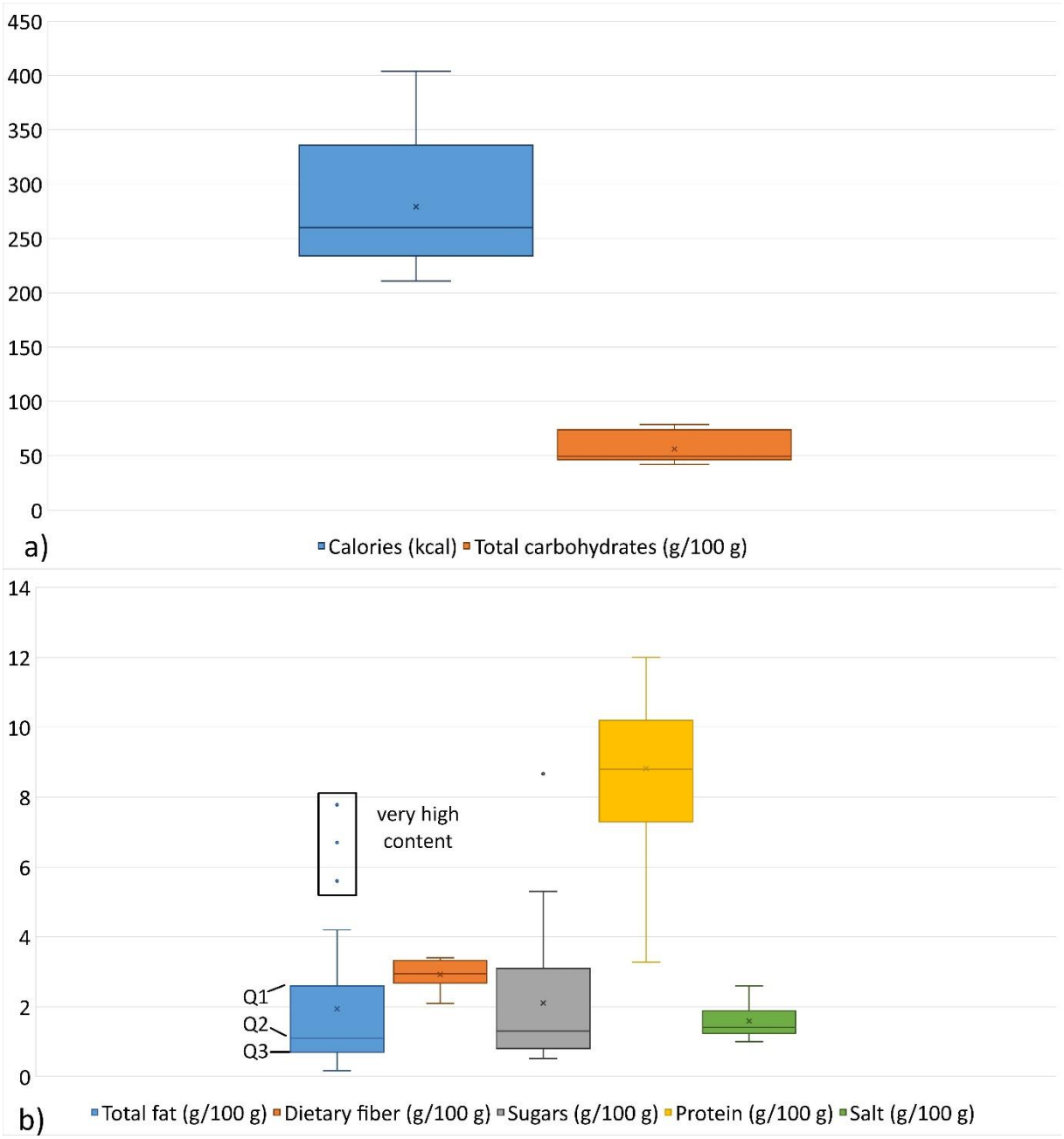

Figure 2. Average nutritional composition of 39 types of gluten-containing flat breads on the Croatian market. a) calories and total carbohydrates b) total fat, dietary fiber, sugars, protein, and salt. The median of the dataset indicates the midpoint of the nutritional composition of the flat breads. A classification is proposed according to the average nutritional composition: low nutrition component content (content ≤ Q1), intermediate (Q1 < content < Q3), and high nutrition component content (≥ Q3) or very high content (> Q3)

Table 2. Molar ratio (%) of monosaccharides and estimated AXs content (% of the hot water-extractable fraction, F2) in the fractions obtained from oat (OB) and barley bran (BB) before (C) and after ultrasound pretreatment (US)

Sample/sugar	OB-C	BB-C	OB-US	BB-US
Fucose	0.11 ± 0.01^{c}	0.55 ± 0.21^{a}	0.23 ± 0.01^{b}	0.24 ± 0.01^{b}
Arabinose	24.01 ± 0.23^{a}	21.28 ± 1.34^{a}	22.03 ± 0.37^{a}	10.81 ± 0.09^{b}
Mannose	6.94 ± 0.00^{c}	8.05 ± 0.58^{bc}	8.59 ± 0.25^{b}	23.69 ± 0.07^{a}
Glucose	27.94 ± 0.18^{b}	39.57 ± 1.68^{a}	29.11 ± 0.66^{b}	25.62 ± 0.02^{b}
Galactose	13.13 ± 0.09^{c}	11.38 ± 0.96^{c}	15.26 ± 0.06^{b}	29.43 ± 0.04^{a}
Rhamnose	0.46 ± 0.02^{c}	0.50 ± 0.01^{c}	0.74 ± 0.03^{b}	1.39 ± 0.02^{a}
Xylose	27.40 ± 0.31^{a}	19.16 ± 1.24^{c}	24.05 ± 0.62^{b}	8.81 ± 0.06^{d}
Total sugars (%)	53.91 ± 0.67^{b}	60.00 ± 2.26^{a}	55.00 ± 0.47^{ab}	50.88 ± 0.15^{b}
Arabinoxylans (%)	57.06 ± 0.15^{a}	45.83 ± 0.28^{c}	54.28 ± 0.28^{b}	43.76 ± 0.10^{d}

Results are expressed as mean±standard deviation (n=2)

a–c Values within the same row marked with different letters differ significantly according to Tukey's test ($p < 0.05$)

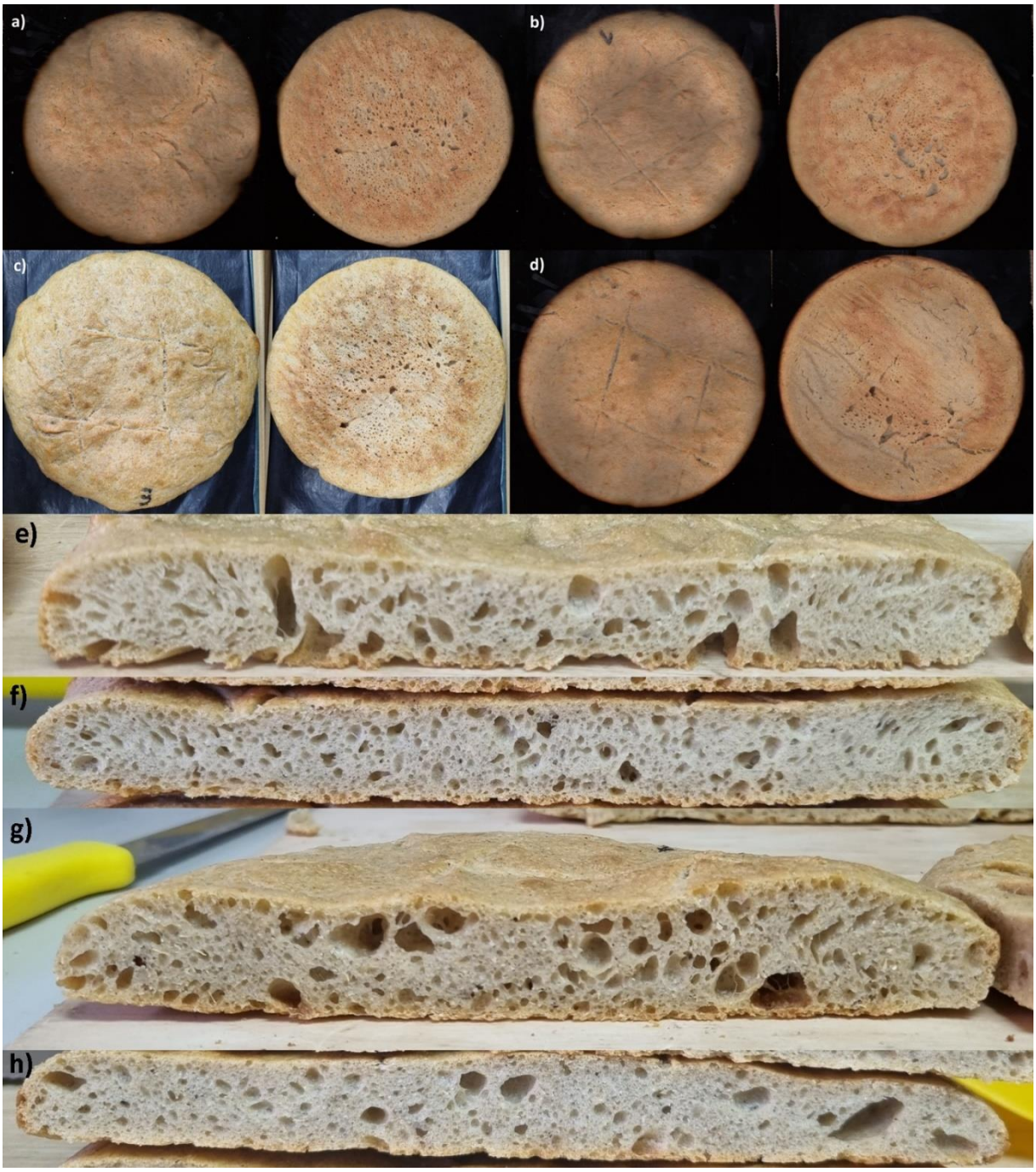

Figure 3. Appearance of gluten flat breads made with the addition of untreated (a, e) and PEF-treated (b, f) oat flour, and with the addition of untreated (c, g) and PEF-treated barley flour (d, h)

Figure 4. Appearance of gluten flat breads made with no-time process with 30% and 50% of oat and barley sourdough (a, e and c, g, respectively) and made with retardation process (24 h) with 30% and 50% of oat and barley sourdough (b, f and d, h, respectively