Investigating and optimizing bread-making properties of whole wheat flour

by

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B.S., Jiangnan University, China, 2015
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AN ABSTRACT OF A DISSERTATION

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Department of Grain Science and Industry
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Abstract

Whole wheat flour (WWF) is a valuable source of dietary fiber with potential health benefits, making it important for sustainable baking product production. This research aims to optimize the production of high-quality whole wheat bread by investigating the impact of bran particle size, assessing correlations between white flour (WF) and WWF properties, understanding the chemical composition of WWF, and exploring the potential uses of wheat bran extracts. The specific objectives are as follows: 1) Evaluate the effect of bran particle size on the rheology properties and baking quality of WWF from four different varieties; 2) Compare and corelate the bread-making properties of WF and WWF from 64 different genotypes; 3) Investigate the influence of wheat bran chemical composition, lipolytic enzyme activities, and phenolic components and dough rheology and baking properties through correlation analysis; 4) Explore the effects of wheat bran extract on gluten structure, dough rheological, and bread characteristics; 5) Characterize the antioxidant potential and anti-aging effects of xylo-oligosaccharides and peptide hydrolysates extracted from hard and soft wheat brans.

The results showed that medium to coarse bran size was more suitable for bread production, while fine bran size was associated with lower specific loaf volume, darker crumb color, and larger hardness value for most wheat varieties. Larger volumes were observed for white bread with higher flour protein content. However, this trend was not consistently observed for WWF. Mixolab tests revealed that WWF had higher water absorption compared to white flour. Strong correlations were observed between WF and WWF for protein content, flour water absorption, pasting properties, and mixing time, while bread volumes and water retention capacity were weakly correlated. Bran lipid content was correlated with mixing peak time, water absorption, water retention capacities, antioxidant capacity, and certain Mixograph properties. Besides, negative correlations were found

between mixing peak time and ABTS scavenging capacity, as well as between bran lipoxygenase activity and bread volume. Furthermore, the addition of wheat bran extract (WBE) significantly increased bread volume and enhanced dough properties, including strength, viscosities, and modulus. Scanning electron microscopy analysis revealed the promotion of protein and starch aggregation within the dough by WBE. Xylo-oligosaccharides and peptide hydrolysates derived from hard and soft wheat brans demonstrated significant antioxidant potential and inhibitory effects against collagenase, elastase, and hyaluronidase. Notably, hard wheat bran xylo-oligosaccharide (HXOS) exhibited the highest hyaluronidase inhibition activity (49.9%) at a concentration of 0.5 mg/mL. HXOS comprised 76.8% xylose and arabinose as the major monomeric sugar units, which were significantly higher than those in soft wheat bran xylo-oligosaccharide (SXOS). In terms of peptide hydrolysates, the molecular weight distribution of peptides hydrolysates derived from hard wheat bran (HP) and soft bran (SP) ranged from 1.06 to 17 kDa, with glutamic acid, aspartic acid, and arginine being the most abundant amino acid residues in the peptides. HP contained higher levels of essential and non-essential amino acids, as well as hydrophobic amino acids. Furthermore, HP demonstrated higher total phenolic contents, DPPH and ABTS scavenging activities compared to SP. The total content of hydrophobic amino acids was 163.90 mg/g in HP and 134.50 mg/g in SP. The higher content of hydrophobic amino acids in HP suggests an enhanced potential antioxidants activity and improved interaction with free radicals, thereby offering protection against oxidative damage.

This study provides a comprehensive characterization of whole wheat flour, elucidating its composition and bioactive properties. The findings contribute to the understanding of wheat bran's complex nature and its impact on bread quality, while also highlighting the potential of wheat bran extracts for enhancing the nutritional value of bread.

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Table of Contents

List of Figures

List of Tables

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Chapter 1 - Literature review: exploring the chemical composition, antioxidant potential, and bread quality effects of the nutritional powerhouse: wheat bran

Abstract

Wheat occupies a preeminent status as one of the paramount crops globally. Wheat bran, a prized by-product derived from the wheat milling process, represents an abundant reservoir of essential nutrients, dietary fiber, vitamins, minerals, and bioactive compounds, synergistically contributing to commendable antioxidant and anti-inflammatory properties. In this chapter, we undertake a comprehensive analysis of the proximate compositions of both hard red winter wheat and wheat bran, drawing on previous research to unveil the exceptional potential of wheat bran as a nutritional powerhouse.

Subsequently, this chapter delves into the protein content of wheat bran, emphasizing its potential as a plant protein source with a nutritionally balanced amino acid composition. Bioprocessing with enzymes and fermentation can be used to enhance protein hydrolysis, solubilization, and digestibility, thereby adding value to wheat bran as a protein-rich ingredient. Furthermore, the comprehensive composition of dietary fiber, including soluble and insoluble fractions, is discussed, along with its diverse physiological activities in promoting bowel health, reducing blood cholesterol, and mitigating the risk of heart disease, diabetes, colon cancer, and obesity. Additionally, the rich mineral content of wheat bran and its significance in maintaining body metabolism and homeostasis, along with the strategies to enhance mineral bioavailability in wheat bran, such as hydrothermal treatment, particle size reduction, and fermentation, are explored. The antioxidant potential of bioactive peptides and arabinoxylan derived from wheat bran is extensively studied, revealing their diverse physiological activities, including immunomodulating, antihypertensive, osteoprotective, and antimicrobial properties. The influence of processing techniques on the release of bioactive compounds is examined, paving the way for further exploration and utilization of these valuable compounds in functional food and nutraceutical applications. Finally, the study delves into breadmaking, addressing the challenges related to incorporating fiber-rich wheat bran into whole wheat bread by utilizing the treatments including pretreatments such as dry and wet preheating and fermentation, and enzymatic treatments such as transglutaminase, glucose oxidase, and amylolytic enzymes.

Overall, this chapter provides a comprehensive understanding of the chemical composition of wheat bran and its influence on bread quality. The findings contribute valuable insights to the fields of nutrition, functional food research, and food product development, offering opportunities to create nutritionally enriched and healthier wheat-based products with potential benefits for consumer health and well-being.

Key words: wheat bran; chemical composition; antioxidant potential; bread.

1.1 Introduction

Wheat ranks third among US field crops in terms of both planted acreage and production. Over the past 10 years, hard red wheat has emerged as a dominant force, contributing to more than half of the annual wheat production. In particular, the hard red winter wheat production, has showcased a remarkable average of approximately 700 million bushels per year (**Figure 1**), as reported by the United States Department of Agriculture National Agricultural Statistics Service (USDA, 2023). The consistent estimation of higher stocks for hard red winter wheat among the wheat classes underlines its contributed prominence, reaffirming its robust demand and resilience in the market. The popularity of hard red wheat can be attributed to a number of factors. For one, it is a high- demand quality wheat variety that is well-suited to many different types of baking, especially bread.

Whole wheat bread, for its nutritional value and wholesome appeal, has gained immerse popularity in recent years as health-conscious consumers seek healthier food options. Incorporating wheat and non-wheat whole grain in bread delivered functional ingredients that possess health benefits, such as increasing the dietary fiber, phytochemicals relevant for gut microbiota and overall health benefits (Y. Wang, Jian, Salonen, Dong, & Yang, 2023). The health benefits of whole wheat bread largely depend on the characteristic of the bran, a critical component of whole wheat flour. Bran, comprising the outer layer of the wheat kernel, including pericarp, testa, and aleurone layer, which makes up 14-19% of the grain, houses essential nutrients and bioactive compounds, such as polyphenols (Cui, Wu, & Ding, 2013). The bioactive compounds present in wheat bran are in relation to their antioxidant and anti-inflammatory activities. It holds the potential to be used as a functional ingredient or sustainable source of phenolics for whole grain-based foods. Most phenolics in wheat bran exist in their bound form and naturally present with carbohydrates linked to their hydroxyl or aromatic carbon moieties (Velderrain-Rodríguez et al., 2014). Thus, the wheat bran is often pre-processed by chemical or physical treatment to obtain a more desirable sensory and higher nutritional value before commercial use in the food market. This chapter summarized current studies on 1) Chemical composition of wheat bran, flour, and bread; 2) Antioxidant potential of bioactive peptide and arabinoxylan in wheat bran; 3) The impact of fiber and enzymes on bread quality. This chapter provides fundamental knowledge for wheat bran phytochemical profiles and functional improved for baking applications.

1.2. Chemical composition of wheat

The proximate compositions of hard red winter wheat, wheat bran, white flour, and bread formulated from either white or whole wheat flour are presented in **Table 1**. Among them, wheat bran provided higher levels of dietary fiber (42.8%), fat (4.25%), ash (5.79%), essential minerals and B vitamins in comparison to wheat, wheat flour, and bread (**Table 1**). Nonetheless, a concern with bran lies in its relatively high lipid content, which may cause rancidity. To solve these issues, bran is subjected to a heat-treatment process, aimed at thwarting enzymatic fat oxidations (Van Wayenbergh et al., 2023).

1.2.1. Protein of wheat bran

Wheat bran contains on average 15.6% protein and holds as a potential plant protein source. The distribution of protein within the wheat bran is not uniform, with aleurone layer showing the highest prevalence at 23 %, followed by the outer layer (5.1%) and intermediate layer (5.7%) (Shewry et al., 2009). Notably, the protein content of wheat bran not only surpasses that of the white flour but also exhibits a nutritionally balanced amino acid composition (Y. Li et al., 2023).

Amino acid analysis of wheat bran protein has revealed a profusion of essential amino acids, including Glu, Pro, Arg, and Leu, with molecular weights ranging from 97 to 12 kDa (Arte, Huang, Nordlund, & Katina, 2019). The protein composition of three distinct bran fractions in bread wheat reveals intriguing functionalities. The outer layer has enzymes that fortify tissue strength and provide protection against pathogens, while the intermediate layer comprises a diverse proteome, including enzyme inhibitors aimed at countering pathogenic enzymes. On the other hand, the aleurone layer features proteins predominantly involved in metabolism processes. Defense enzymes, inhibitors, collectively contribute to distinct lines of defense for the embryo and nutrient-rich endosperm (Jerkovic et al., 2010). Bioprocessing was reported to alter the protein composition and increase peptide concentration of wheat bran (Coda, Rizzello, Curiel, Poutanen, & Katina, 2014). Bioprocessing, facilitated by enzymes like xylanase, b-glucanase, phytase, acidic protease, cellulase, endoglucanase enzymes and lactic acid fermentation enhanced the protein hydrolysis, solubilization, and digestibility (Arte et al., 2019). Notably, the increased proportions of β-sheet and β-turn secondary structure content were significantly reduced after bioprocessing (Arte et al., 2019). In addition, enzymes like cellulose, hemicellulose, and pectinase have been applied to modify the bran cell wall structure, effectively facilitating the release and solubilization of proteins from wheat bran through biochemical modification involving microbes (Arte et al., 2019; Nordlund, Katina, Aura, & Poutanen, 2013).

1.2.2. Dietary fiber of bran

The comprehensive composition of dietary fiber encompasses components, including crude fiber, resistant starch, non-starch polysaccharide (NSP) like cellulose, hemicellulose, β-glucan, pectin, gum, mucilage, oligosaccharide, and lignin (Korczak & Slavin, 2020). Scientific evidence supports the diverse physiological activities induced by dietary fiber, including increased bowel health, reduced blood cholesterol, and a decreased risk of heart disease, diabetes, colon cancer, and obesity (Waddell & Orfila, 2022). Classification based on the solubility in water results in two main types of dietary fiber: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) (K. Wang, Li, Wang, Liu, & Ni, 2021). SDF comprises mainly inulin, β-glucans, pectin, gums, and mucilage, while IDF consists primarily of cellulose, hemicelluloses, lignin, and resistant starch (Saini et al., 2023). Among different wheat samples, wheat bran contains high total dietary fiber at 42.8g/100g, while wheat flour lags with 3g/100g of total dietary fiber (**Table 1**). Hard red winter wheat presents total dietary fiber of 12.2g/100g, and whole wheat flour contained 10.6g/100g of total dietary fiber. Wheat bran contains significant levels of arabinoxylan (22-30%), cellulose (6.5-9.9%), and β-D-glucan (2.2-2.6%) (Rosa-Sibakov, Poutanen, & Micard, 2015). Arabinoxylan, a critical component in wheat bran, possesses a structural backbone comprising β-(1,4)-linked xylose residues, which are further substituted with arabinose residues at the C(O)-2 and/or C(O)-3 positions (**Figure 2**). Additionally, phenolic acids, notably ferulic acid and *p*-coumaric acids, can be esterified on the C(O)-5 position of arabinose units (Ishii, 1997). Previous research has highlighted the positive impact of ferulic acids on improving the antioxidant capacity of arabinoxylan, a crucial aspect in minimizing oxidative stress (Hromádková, Paulsen, Polovka, Košťálová, & Ebringerová, 2013; Zeyu Zhang, Yang, & Zhao, 2022). Such insights provide a deeper understanding of the potential health benefits associated with dietary fiber, specifically wheat arabinoxylan, in promoting overall well-being and mitigating oxidative damage.

1.2.3. Minerals of bran

Minerals play crucial roles in maintaining body metabolism and homeostasis, insufficient intake of these essential nutrients can lead to an increased occurrence of disease symptoms (Gharibzahedi & Jafari, 2017). Wheat bran stands out as a rich source of Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K). The distribution of minerals in cereals is concentrated in the outer layers, particularly in the aleurone cells, which also serve as a key reservoir of dietary fiber and phytic acid (Frølich, 1990). However, in wheat, approximately 80% of its phosphorus (P) exists in the form of phytate, which can form complexes with minerals such as Ca, Zinc, and Mg (Brouns, Hemery, Price, & Anson, 2012). This phytate content in whole grains has been associated with potentially reducing mineral bioavailability due to the strong mineral chelating properties. Besides, various treatments like rinsing, soaking or boiling may lead to some minerals being lost (McClements, 2023). Nevertheless, short chain fatty acids produced during fermentation solubilized the mineral complexes and led to increased absorption of minerals (Kumar, Sinha, Makkar, & Becker, 2010). This fermentation process provides an alternative pathway for optimizing mineral utilization in the body. Moreover, the potential negative effects of phytate in binding minerals can be counterbalanced by the substantial mineral content found in wheat, along with their robust antioxidant properties. To enhance the mineral bioavailability, various strategies have been employed to decrease phytic acid levels in wheat bran, including hydrothermal treatment, particle size reduction, malting, soaking, and fermentation (Majzoobi, Pashangeh, Farahnaky, Eskandari, & Jamalian, 2014).

1.2.4. B Vitamins of bran

Wheat bran is also a remarkable source of essential B vitamins, particularly thiamin, niacin, vitamin B6 and folate (**Table 1**). Wheat bran contains 13.6mg/100g Niacin, 1.3mg/100g Vitamin B6, and 0.523mg/100g Thiamin. In contrast, white flour exhibited lower levels, with 1.59 mg/100g Niacin, 0.085/100g Vitamin B6, and 0.298mg/100g Thiamin. In light of the nutritional importanance, the United States has taken regulatory action to fortify enriched grain products with folic acid (Administration, 1996). Research has been devoted to exploring the potential benefits of folic acid in reducing the risk of neural tube defects (NTD) and congenital heart defects (CHD) (Christensen et al., 2009; Zheng & Cantley, 2019). Furthermore, microwaving, steam, extrusion, superheated steam, fermentation and enzymatic treatments have been found to significantly enhance folic acid contents while concurrently reducing Thiamin contents (Ye et al., 2021). Moreover, the nixtamalization of wheat bran could improve niacin bioavailability and positively influence bread volume (Argun et al., 2023; Shahidi, 1997).

1.3. Antioxidant potential of wheat bran

In recent years, the exploration of natural sources for bioactive compounds with potential health benefits has gained significant attention in nutrition and functional food research. Among the promising candidates are bioactive proteins, peptides, and arabinoxylan derived from wheat bran. Wheat bran, a by-product of wheat milling, has attracted considerable interest due to its abundant availability and cost-effectiveness. This section delves into the intriguing world of bioactive proteins, peptides, and arabinoxylan derived from wheat bran, unveiling their diverse physiological activities and antioxidant capacities, and understanding the factors influencing the antioxidant capacity of arabinoxylan, such as degree of substitution and molecular weight, adds valuable insights to the overall bioactive potential.

1.3.1. Bioactive protein and peptides

The effects of bioactive proteins and peptides have been investigated through in vitro and animal trials, revealing a spectrum benefits, including immunomodulating, antihypertensive, osteoprotective, antioxidative and antimicrobial (Möller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008). These compounds have garnered significant interest following the discovery that they are enzymatically formed during the passage of food proteins through the gastrointestinal tract or generated through fermentation during processing. Studies have extensively explored the isolation of peptides with antioxidant activities derived from wheat germ, utilizing various techniques such as alkali extraction, microwave or ultrasonic assisted alkali extraction, subcritical water extraction, enzymatic extraction, and reverse micelle extraction (Liu, Chen, Shao, Wang, & Zhan, 2017; C. Wang et al., 2022; Weng et al., 2021; Zhi hui Zhang et al., 2023). However, the information regarding bioactive peptides extracted from wheat bran remains limited. Protein hydrolysates obtained through pepsin or pancreatin gastrointestinal digestion of wheat bran have displayed promising antioxidant capacity, with DPPH radical scavenging capacity ranging from 8.3 to 9.0 umol TE/g, depending on the particle size of the bran (Y. Li et al., 2023). On the other hand, superfine milling has been observed to enhance the hydrolysis of wheat bran protein into smaller peptides, leading to increased antioxidant activity. Certain amino acids, such as arginine, tyrosine, and phenylalanine, are likely to contribute to this antioxidant effect (You, Zhao, Regenstein, & Ren, 2010). Regarding molecular weight, the fraction of wheat bran protein hydrolysate with a molecular weight of less than 1kDa exhibited the highest renin-inhibitory and oxygen radical antioxidant activities compared to the 1-3kDa fractions, suggesting its potential as a source of antihypertensive agents (Zou, Wang, Wang, Aluko, & He, 2020). Interestingly, it was found that wheat bran globulin proteins, rather than peptides, inhibited mushroom tyrosinase, reducing its diphenolase activity (Ortíz-Estrada et al., 2012). These findings shed light on the diverse bioactive potential of peptides derived from wheat bran, paving the way for further exploration and utilization of these valuable compounds in various functional food and nutraceutical applications.

1.3.2. Antioxidant potential of arabinoxylan in wheat bran

Arabinoxylan serves as an important reservoir of antioxidant phenolic compounds, including alkylresorcinols and phenolic acids. Among these compounds, ferulic acid predominantly exists in bound form, attached to arabinoxylan. Various processing technologies, such as thermal, alkaline, enzymatic, ultrasonic treatments, and fermentation, have been explored to enhance the release of ferulic acid and other bound phenolic (Aguedo, Fougnies, Dermience, & Richel, 2014; Moore, Cheng, Su, & Yu, 2006; Pihlajaniemi et al., 2020; Yuan, Wang, & Yao, 2005). For instance, alkaline pretreatment with potassium hydroxide (KOH) followed by enzymatic hydrolysis has been shown to promote the yield of oligomeric arabinoxylan and enhance antioxidant release (Pihlajaniemi et al., 2020). However, graded ethanol precipitation did not exhibit significant improvement in the antioxidant capacity of arabinoxylan oligosaccharides (Malunga & Beta, 2015). Recent studies revealed that the degree of substitution and the molecular weight, determining the antioxidant potential of arabinoxylan. Lower degree of substitution resulted in a higher antioxidant capability of arabinoxylan (Chen et al., 2021). Similarly, the water extractable arabinoxylan fractions with endoxylanase with higher degree of substitution demonstrated higher antioxidant capacity (Malunga, Izydorczyk, & Beta, 2017).

1.4. Breadmaking

Bread has long been a popular staple food, but with increasing demand for nutritional options and the mechanization of baking processing, there is a need to enhance the viscoelastic of the dough, and textual properties of whole wheat bread. The exceptional gas-holding power and viscoelasticity of wheat flour are attributed to its gluten proteins. However, when incorporating fiber, particularly from wheat bran, into whole wheat bread, challenges arise, such as reduced bread volume compared to counterpart made with white flour. However, various pretreatments of wheat bran and the addition of enzymes have shown promising in enhancing bread quality.

1.4.1. Effect of fiber on whole wheat bread

Whole wheat bread exhibited lower volume compared to white flour counterparts (C. Li, Tilley, Chen, Siliveru, & Li, 2023). Various factors influence bread volume, including the dilution of gluten content in whole wheat flour, the particle size of the bran, and the type of bran used (Ding et al., 2023). Pretreatments of wheat bran, such as dry and wet preheating (roasting, boiling, or autoclaving), extrusion, fermentation, and germination, has shown promise in improving the bread quality (Gómez, Jiménez, Ruiz, & Oliete, 2011; Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006). A kneading method of adding wheat bran after mixing the white flour with water has been shown to improve whole wheat bread volume and texture (Sun et al., 2023). Shifts in gluten structure from β-turns to β-sheets with bran inclusion may contribute to the lower quality in whole wheat bread (Bock, 2019). The impact of particle size is evident, as 20% bran addition decreased bread volume while superfine bran (D_{50}=11.3um) showed no significant compared to whole wheat bread made with coarse bran (D_{50} = 362.3um) (Lin et al., 2022). Soluble oat fiber containing 70% oat b-glucan at 12-15% replacement resulted in darker color, lower specific volume and porosity (Ortiz de Erive, He, Wang, & Chen, 2020). However, the negative effect can be mitigated by increasing water content by 20% (Ortiz de Erive et al., 2020). On the other hand, incorporating 0.5-2% water-extractable arabinoxylan from wheat bran partially inhibited gluten polymerization, leading to improved loaf volume and texture in steamed bread (P. Wang et al., 2019). This enhancement was particularly pronounced for arabinoxylan with lower molar mass and higher degree of substitution (P. Wang et al., 2019). The water-extractable arabinoxylan functions similarly to gluten, stabilizing gas cells during the fermentation process and enhancing the uniformity crumb structure (Courtin & Delcour, 2002). While water-unextractable arabinoxylan typically exhibit a negative impact on breadmaking, hydrolysis of water-unextractable arabinoxylan during breadmaking process releases additional water-extractable arabinoxylan that could potentially contribute to improved dough and bread quality (Nishitsuji, Whitney, Nakamura, Hayakawa, & Simsek, 2020).

1.4.2. Enzymes and improved bread quality

Gluten cross-linking enzymes play an important role in current baking processes (Gerrard, 2002). Transglutaminase, through ε-N-(γ-glutamyl) lysine cross-links and disulfide bridges formation, enhanced water availability of whole wheat dough, resulting a stronger gluten network and improved bread quality (Autio et al., 2005; Larré et al., 2000; Motoki & Seguro, 1998; M. Wang et al., 2020). Glucose oxidase promotes oxidative reactions, leading to covalent bonds within gluten structure, contributing to improving bread quality (Gujral & Rosell, 2004; Tilley et al., 2001). Xylanase improved the quality of the bread by increasing its volume and shelf life by degrading the arabinoxylan in dough(Shah, Shah, & Madamwar, 2006). Xylanase alone or in combination with other enzymes decreased water absorption, improved the gluten network and quality of the whole wheat bread. Combinations such as xylanase, glucose oxidase, and peroxidase have been reported effectively improved the bread quality (Caballero, Gómez, & Rosell, 2007). Phytase degraded the phytate acid in bran provided desirable properties such as increased bread volume (Haros, Rosell, & Benedito, 2001). The incorporation of laccase enhances gluten quality and induces changes in dough rheological properties, resulting in a slight reduction in dough extensibility (Primo-Martin et al.,2003), increased dough consistency, and accelerated dough breakdown during mixing (Labat, Morel, & Rouau, 2001). This enzymatic addition has also been associated with increased bread volume and reduced hardness (Molina et al., 2023). Amylolytic enzymes have been also proposed as active contributors towards fresh bread quality and staling behavior (Bollaín, Angioloni, & Collar, 2005) during storage. Furthermore, wheat bran peroxidase has the capacity to form covalent disulfide and dityrosine cross-links within glutenin subunits, making it a valuble dough improver (Manu & Prasada Rao, 2011).

1.5. Conclusion

This chapter has summarized the chemical composition of wheat bran, flour, and bread, highlighting their rich nutritional profile, including essential nutrients, dietary fiber, vitamins, minerals, and bioactive compounds. Wheat bran, as a valuable by-product derived from the wheat milling process, stands out as a significant source of dietary fiber, protein, minerals, and B vitamins. Despite its remarkable nutritional benefits, challenges such as the relatively high lipid content leading to potential rancidity have been addressed through heat-treatment processes.

This chapter has also explored the antioxidant potential of bioactive peptide and arabinoxylan present in wheat bran, shedding light on their contributions to overall health and well-being. These compounds have exhibited diverse physiological activities, including antioxidative, immunomodulating, antihypertensive, and antimicrobial properties. The antioxidant capacity of arabinoxylan was found to be influenced by factors such as degree of substitution and molecular weight, providing opportunities for further optimization and utilization in functional food and nutraceutical applications.

Additionally, the impact of fiber and enzymes on bread quality were studied. Bread, when incorporating wheat bran, can face challenges such as reduced volume compared to white flour bread. However, various pretreatments of wheat bran and the addition of enzymes have shown promise in improving bread quality. Enzymes such as transglutaminase, glucose oxidase, laccase, and amylolytic enzymes have been utilized to enhance gluten structure, leading to improved bread volume, texture, and freshness during storage.

Overall, this comprehensive exploration of the chemical composition of wheat bran and its impact on bread quality contributes valuable information to the field of nutrition and functional food research. The findings from this study pave the way for the development of nutritionally enriched and healthier wheat-based products, which could positively impact consumer health and well-being. Furthermore, the insights gained from the antioxidant potential of wheat bran's bioactive compounds open new avenues for functional food development and provide opportunities for the food industry to innovate and meet the growing demand for nutritious and health-promoting products.

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Table 1.1. Proximate compositions of wheat bran, whole wheat flour, wheat flour and commercial whole wheat bread (USDA, 2019; USDA, 2020).

Proximate compositions	Wheat bran	Hard red winter wheat	Whole wheat flour, unenriched	Wheat flour (unenriched, unbleached)	Whole wheat bread	White flour bread
Proximate						
Water (g/100g)	9.89	13.1	9.44	11.1	38.7	39.6
Energy (kcal/100g	216	327	370	362	254	238
Protein (g/100g)	15.6	12.6	15.1	12	12.3	10.7
Total fat (g/100g)	4.25	1.54	2.73	1.7	3.55	2.15
Ash (g/100g)	5.79	1.57	1.56	0.56	2.36	3.69
Carbohydrate (g/100g)	64.5	71.2	71.2	74.6	43.1	43.9
Total dietary fiber (g/100g)	42.8	12.2	10.6	3	6	9.2
Minerals						
Calcium (mg/100g)	73	29	38	22	163	684
Iron (mg/100g)	10.6	3.19	3.86	1.18	2.56	4.89
Magnesium (Mg) (mg/100g)	611	126	136	36.1	76.6	26
Phosphorus (P) (mg/100g)	1010	288	352	134	212	103
Potassium (K) (mg/100g)	1180	363	376	150	250	127
Sodium (N) (mg/100g)	2	2	3	2	450	*478*
Zinc (Zn) (mg/100g)	7.27	2.65	3.24	1.15	1.76	0.95
Copper (Cu) (mg/100g)	0.998	0.434	0.452	0.212	0.226	0.149
Manganese (Mn) (mg/100g)	11.5	3.98	3.56	0.819	2.18	0.634
Selenium (Se) (μg/100g)	77.6	70.7	23.6	20.1	25.8	16.1

Molybdenum (Mo) (µg/100g)	NS*	NS	58.5	42.6	NS	NS
B Vitamins						
Thiamin (mg/100g)	0.523	0.383	0.504	0.298	0.391	0.51
Riboflavin (mg/100g)	0.577	0.115	0.128	0	0.166	0.225
Niacin (mg/100g)	13.6	5.46	5.55	1.59	4.43	4.46
Vitamin B-6 (mg/100g)	1.3	0.954	0.268	0.085	0.216	0.08
Folate (Total) (µg/100g)	79	38	39	23	42	127

Table 1.2. Influence of enzymes and fiber on bread quality.

Enzyme	Mechanism	Function	References
Amylase	Produces Maltooligosaccharides	Increased whole grain wheat bread volume	(Bae, Lee, Yoo, & Lee, 2014)
a-Amylase	Catalyzes the hydrolysis of α-1, 4-glycosidic linkages into starch molecules	Anti-staling agent, improved whole wheat bread volume	(L. Zhang et al., 2019)
Xylanase	Randomly attacks arabinoxylan backbone	Decreased water absorption, improved whole wheat bread volume	(Shah, Shah, & Madamwar, 2006)
Phytase	Releases of calcium-ions from calcium-phytate complexes promoted by phytase activity, promotes endogenous a-amylase	Increased whole wheat bread volume	(Haros et al., 2001)
Lipoxygenase	Catalyzes the deoxygenation of polyunsaturated fatty acids	Improved whiteness of whole wheat bread	(Shi et al., 2020)
Transglutami nase	Forms ε-N-(γ-glutamyl) lysine cross-links and disulfide bridge	Enhances gluten network and bread quality.	(Larré et al., 2000; Motoki & Seguro, 1998; M. Wang et al., 2020)
Glucose oxidase	Promotes oxidative reactions, forms covalent bonds within gluten structure	Improves bread quality	(Gujral & CM Rosell -, 2004; Tilley et al., 2001; Xiao et al., 2021)
Laccase	Oxidative Cross-linking enzymes generate covalent bonds in and between food biopolymers	Enhances gluten quality, increases bread volume	(Molina et al., 2023; Primo-Martin et al., 2003)
Lipase	Decreases galactolipids and phospholipids and concomitant increased free fatty acid	Increased bread volume and stability of dough, reduced or retard staling, develop flavor	(Gerits, Pareyt, & Delcour, 2014)
Amylase and lipase	Amylose-lipid complexes formation	Preserved softness of bread during storage	(León, Duran, & Benedito De Barber, 2002)

Combination of a-amylase, xylanase and cellulase	Degrades cell wall components	Decreased Increased dough extensibility	(W. Liu, Brennan, Serventi, & Brennan, 2017)
Combination of peroxidase, glucose oxidase, and xylanase	/	Decreased water absorption, not affect the volume	(Pescador-Piedra et al., 2009)
Hemicellulose, glucose oxidase, α-amylase	/	Synergistic effects with other enzymes, enhances bread quality	(Caballero et al., 2007)
Wheat bran Peroxidase	Forms covalent disulfide and dityrosine cross-links within glutenin subunits,	Dough improver	(Manu & Prasada Rao, 2011)

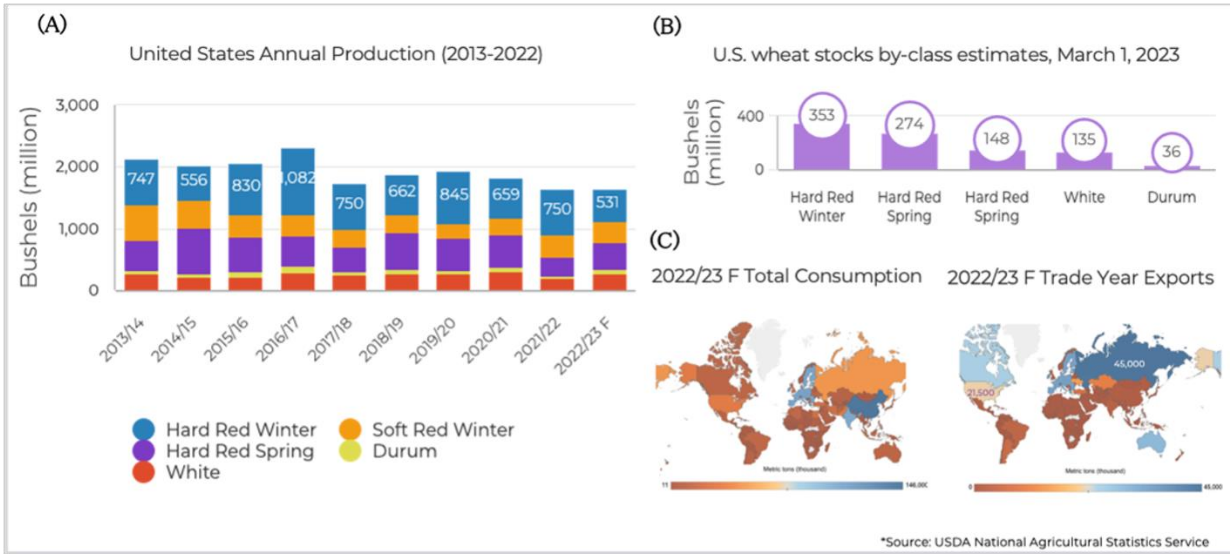

Figure 1.1. United states annual production (2013-2022) (A), US wheat stocks by-class estimates (B), and 2022/23 total consumption and trade year exports (C). F denotes forecast year. (USDA-NASS, 2023).

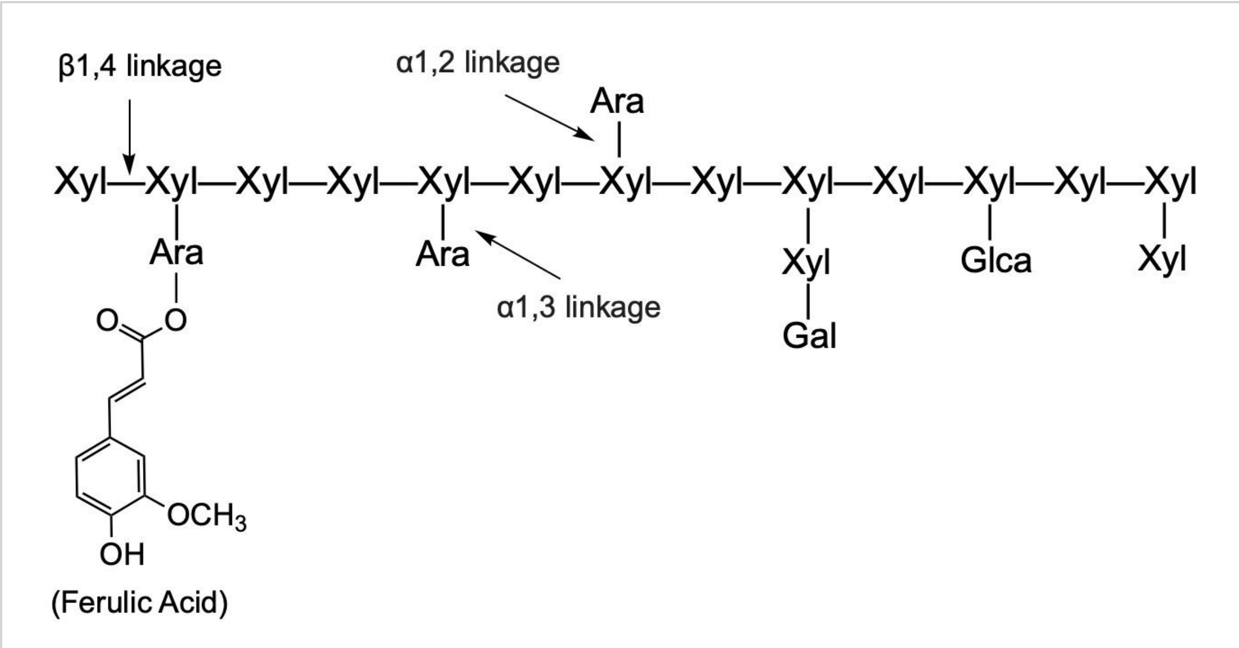

Figure 1.2. Structure of wheat arabinoxylan.

Chapter 2 - Effect of bran particle size on rheology properties and baking quality of whole wheat flour from four different varieties [1]

Abstract

The potential health benefits of consuming whole grain foods are increasingly attracting customers' interest. The effect of bran particle size on whole wheat flour (WWF) bread-making performance was investigated using four different varieties with protein levels of 9–15 g/100g. Five different bran particle size ranges from 590.5 to 159.7 μm were obtained using different milling methods. Mixolab tests revealed that white flour had a lower water absorption (56.2–59.2 g/100g) than the corresponding whole wheat flours (63.2–67.0 g/100g). Larger specific loaf volume was observed for white bread with a relatively higher flour protein content, but this was not always associated with the trend for the WWF. For most wheat varieties, the bread from WWF with the fine bran had the lowest specific loaf volume, darker crumb color, and larger hardness value than bread made with white flour or with WWF of other particle sizes. Overall, WWF with medium to coarse bran size was more suitable for bread production. The findings of this study contribute to the standardization of whole flour bran particle sizes for quality assessment and effective industrial production.

Keywords: Whole wheat flour Bran particle size Dough rheology Bread Texture

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[1]Li, Cheng, Michael Tilley, Richard Chen, Kaliramesh Siliveru, and Yonghui Li. "Effect of bran particle size on rheology properties and baking quality of whole wheat flour from four different varieties." *LWT* 175 (2023): 114504.

2.1. Introduction

The Cereals and Grains Association (AACC) provided a general definition for whole grain back in 1999, as "after refining and reconstitution process, the kernels of the whole grains shall present intact caryopsis in the same relative proportion components as that of the original one". Consuming whole grain foods can reduce the risk of developing metabolic syndrome that can result in type II diabetes, cardiovascular diseases, obesity, and several other diseases (Seal & Brownlee, 2015; Tullio, Gasperi, Catani, & Savini, 2021; Zhang et al., 2021). The benefits of whole grains are related to the functional components widely existing in the bran and germ, mainly dietary fibers and phytochemicals including phenolic compounds (Călinoiu & Vodnar, 2018; J. Liu, Yu, & Wu, 2020). It is still not very clear which specific components in whole grains responsible for the reduced risk of a specific disease (Călinoiu & Vodnar, 2018). Most likely the combination of different compounds and their synergistic effects provide the health promoting effects of a diet rich in whole grains. With the consumption of whole wheat bread, lower glycemic response and increased satiety were reported compared to consuming white bread (Kristensen et al., 2010). Moreover, whole wheat flour (WWF) has significantly higher antioxidant capacity than refined flour, and the bread-making process could enhance the release of bioactive compounds and their bio-accessibility and bioavailability (Anson et al., 2011; Tian, Wang, et al., 2022; Yu & Nanguet, 2013).

Due to the multiple health and nutritional benefits of whole wheat products, many previous efforts have been made to understand and optimize the quality parameters of whole wheat flour and bread (Gao et al., 2022; Sun, Wu, Koksel, Xie, & Fang, 2023; Tian, Chen, Tilley, & Li, 2021). Compared to white bread, making high-quality whole-wheat bread can be more challenging, due to the complex flour composition and presence of brans and germs that impact gluten development and dough rheology (Heiniö et al., 2016; Ma et al., 2022). In addition, there are multiple barriers for the wider consumption of whole wheat products, such as less desirable quality attributes, consumer taste preferences, cost, and convenience (Kuznesof et al., 2012). Furthermore, whole wheat bread also has disadvantages when compared to white bread because of the hard and gritty texture, risk of rancidity, bitter taste, lower loaf volume, and color changes (Sanz-Penella, Laparra, Sanz, & Haros, 2012). The bitter taste may be due to the presence of various phytochemicals and proteolysis of albumins and globulins in wheat flour and the presence of phenolics, as well as the pinellic acid and other oxidation products of linoleic acid (Cong, Schwartz, Tello, Simons, & Peterson, 2021; Jiang & Peterson, 2013).

Bran particle size is one of the major factors affecting the bread-making quality of whole wheat flour. Literature reports related to the effect of bran particle size on dough and baking performances has been somewhat contradictory (Coda et al., 2014; Lin et al., 2022; N. Wang, Hou, & Dubat, 2017). It is largely unknown how the particle size of bran in whole wheat flour influences its technological properties of the dough and baking characteristics. Some researchers claimed that bread made from flour containing brans with smaller particle size had better crumb grain and larger specific volume (N. Wang et al., 2017), while others found that whole wheat bread made with finer particles had lower loaf volume (Lin et al., 2020). Coda et al. found that optimum intermediate bran particle size (170 μm), among the investigated range among of 50–750 μm, resulted in mild flavor and highest loaf volume (Coda et al., 2014). Because conclusions from previous studies are inconsistent, and many prior studies were based only on very few (e.g., two or three) bran particle size ranges from a single wheat variety, this research was aimed to have a more comprehensive and systematic study using multiple wheat varieties with a wider range of bran particle sizes. Our results would bridge the knowledge gap between bran particle size and whole wheat flour bread-making properties.

2.2. Materials and methods

2.2.1. Wheat samples and whole wheat flour preparation

Four different varieties of hard red winter wheat with protein content ranged from 8.48 to 14.61 g/100g were obtained from a local seed company, namely Zenda, Tatanka, Ag Gallant, and Turkey Red. Proximate composition analysis (protein, moisture, fat, ash, and total carbohydrate) was carried out according to AACC approved methods (AACC International, 2010). Total carbohydrate content was determined by subtracting protein, crude fat, ash, and moisture content from 100. Prior to milling, the wheat kernels were tempered in tempering bins and allowed overnight equilibration to achieve 15 g/100g moisture content. The wheat kernels were then milled using a Quadrumat Senior laboratory mill (C.W. Brabender, South Hackensack, NJ, USA) for straight-grade flour and bran (shorts were collected together in the bran), following AACC Approved Method 26–50.01(AACC International, 2010). The bran from each of the varieties was further ground using a Perten laboratory mill 3303 (PerkinElmer, Waltham, MA, USA), Wiley mill Thomas Model 4 with 2 mm screen (Thomas Scientific, Swedesboro, NJ, USA), and through a pin mill (model UT-03, Bauermeister, Inc., Memphis, TN, USA) with different passes (1, 3, and 6 passes), respectively, to obtain five reground brans with different particle size ranges, namely coarse (C), coarse to medium (CM), medium (M), medium to fine (MF), and fine (F). Whole wheat flour (WWF) was prepared by combining each of reground brans with the corresponding straight-grade flour (white flour, WF) at the original ratio for each variety based on the roller milling results. The flowchart of milling and whole wheat flour preparation is shown in Fig. 1.

2.2.2. Particle size analysis

The particle size of the reground brans was determined by sieving 100.0 g of sample using a Tyler Ro-tap shaker machine with 12 sieve stacks (W.S. Tyler, Mentor, OH, USA) for 10 min. The sieve sizes were US No. 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, and 270. The weight retained on each sieve was expressed as w/w g/100 g. Mean particle size (MPS, dgw) of the bran was calculated using the formula as Eq. (1) following the ASABE method (ASABE, 2008):

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

Where d_{gw}: geometric mean diameter of particles by mass, µm.

W_i=mass on sieve i, gram

d_i=nominal sieve aperture size of the i^{th} sieve, µm

2.2.3. Mixolab

The dough mixing characteristics of the flours were evaluated using a Mixolab analyzer (Chopin Technologies, Villeneuve-la-Garenne, France) according to the AACC Approved Method 54–60.01. 75 g (14 g/100g moisture base) flour was used for each test under the standard 'Chopin+' protocol. The target consistency (C1) was set at 1.1 Nm. The dough was first mixed at 30 °C for 8 min, then heated for 15 min at the rate of 4 K/min until the mixer temperature reached 90 °C. After that, the dough was held at 90 °C for 7 min and cooled to 50 °C at a rate of 4 K/min and finally held at 50 °C for 5 min. Each flour was analyzed twice at the optimal water absorbance level. Some important parameters collected from the test are listed below: Water absorption: amount of water necessary to reach C1; Amplitude: width of curve to C1; Dough development time: time required to C1; Stability: mixing resistance of dough; C1: maximum torque during mixing; C2: protein weakening based on mechanical work and temperature increase; C3: maximum torque during the heating stage, expressing the rate of starch gelatinization; C4: minimum torque during the heating period, indicating the stability of the hot gel formed; C5: torque after cooling at 50 °C, representing starch retrogradation during the cooling stage; C3–C2: starch gelatinization rate; C5–C4: starch retrogradation at cooling stage.

2.2.4. Bread baking

Bread was prepared according to the AACC Approved Method 10–10.03, without adding any of the optional ingredients. The basic formula included:100 g (14 g/100g moisture base) flour, 6 g sucrose, 1.5 g salt, 3 g shortening, 0.2 g malt, and 2.0 g yeast (Bellarise Red, PAK Group Pasadena, CA, USA), and optimum water absorption and mixing time determined based on Mixograph test for each flour. The 90-min fermentation and 33-min proofing times were applied, and the dough was baked at 215 °C for 24 min in a reel oven (National Manufacturing Co., Lincoln, NE, USA). Two loaves of bread were made from each flour type. After 2 h of cooling at ambient temperature, loaf weight was measured, and loaf volume was determined according to the rapeseed displacement method (AACC Approved Method 10-05.01). Bread was then sliced to 15 mm thickness per slice and sealed in polyethylene bags for further analyses.

2.2.5. C-cell image analysis

A C-Cell Bread Imaging System (Calibre Control International Ltd., Appleton, Warrington, UK) was used to analyze two middle slices of bread according to AACC Approved

Method 10–18.01 (AACC, 2010) The parameters obtained included the number of cells per slice area, area of cells, cell wall thickness, and cell diameter.

2.2.6. Bread crumb color properties

Bread crumb color was measured using a digital precise colorimeter (Cielab, Xitian Machine Equipment Co., Ltd, Huizhou, Guangzhou, China) to obtain L*, a*, and b* values. Triplicate analysis was conducted using the center slice from each loaf.

2.2.7. Texture profile analysis

The texture properties of the bread were analyzed using a TA-XT2 Texture Analyzer (Stable Micro System, Godalming, Surrey, UK) with a 25-mm diameter cylinder probe. Four slices (15 mm thick) from the center of each loaf were analyzed. Each slice was subjected to a 50% strain at a pre-test and test speed of 1.0 mm/s, trigger force of 5 g. Texture exponent software was then used to analyze the data. Bread texture quality parameters, including hardness, springiness, gumminess, and chewiness were collected.

2.2.8. Bread acrylamide analysis

The analysis of bread acrylamide was measured according to the method reported by Shen et al. (Shen, Chen, & Li, 2019) with a minor modification. Each lyophilized bread sample (2 ± 0.001 g) was accurately weighed and dispersed in distilled water (20 mL) in a tube and then placed in a water bath for 1 h at 80 °C with 180 rpm oscillation. Then the tube containing the sample was centrifuged at 2320 g for 30 min. An SPE cartridge was activated with methanol and water, followed by applying the supernatant, afterwards the residue was eluted with water (2 mL). Then, the elute was collected and mixed with potassium bromide (6 g) and saturated bromine water solution (4 mL) for derivatization. The mixture was kept at 4 °C overnight, and the excess bromine was decomposed by adding 1 mol/L sodium thiosulfate solution. Excess sodium sulfate (3 g) was then added to remove any water residue in the extracts to obtain colorless solution. Ethyl acetate (4 mL) was used to extract the acrylamide with shaking for 5 min. The extract was centrifuged at 2320 g for 10 min, and the supernatant was collected. The final extracted solution was filtered through a 0.2 μm microfilter (Thermo Scientific Titan3™) before GS-MS analysis. Each sample was extracted twice.

The extract was injected into a GC-MS QP2010-SE system (Shimadzu Co., Kyoto, Japan) in spitless mode at 200 °C. The flow rate of the carrier gas was kept at 1 ml/min with ZB-WAXplus capillary column (30 m × 0.25 mm i.d., 0.25 μm; Phenomenex, Torrance, CA, USA). The temperature was set from 85 to 250 °C with a rate of 20 K/min, followed by holding for 10 min. The ion source was set at 180 °C, and the electron impact mass spectra were obtained at 70 eV. Selected ion monitoring mode was conducted to characterize (m/z 106, 108, 150, 152) and quantify (m/z 152) brominated acrylamide. The concentration was calculated from the calibration curve obtained by plotting the peak area against acrylamide standards and presented as μg/kg lyophilized bread. The limit of quantification for acrylamide was 10 μg/kg.

2.2.9. Statistical analysis

The results were reported as mean ± standard deviation (SD). Statistical analysis of the results was performed in SAS Studio 3.7 (SAS Institute Inc., Cary, NC, USA) using one-way analysis of variance (ANOVA). $P < 0.05$ was considered significant by Duncan's test. Principal component analysis (PCA) was carried out using XLSTAT™ software (Addinsoft Inc, New York, NY, USA).

2.3. Results and discussion

2.3.1. Particle size of wheat brans

Proximate compositions of the wheat samples are shown in Table 1. Protein content of the four wheat varieties ranged from 8.48 to 14.61 g/100g. Turkey red had the highest protein and ash content, and the lowest total carbohydrate content among all the tested varieties. The mean particle size values of coarse (C), coarse to medium (CM), medium (M), medium to fine (MF), and fine (F) brans from the four different hard wheat are presented in Fig. 2. The bran particle size ranges were selected in this study to encompass five different sizes, considering that in practice wheat grains can be processed by different milling technologies, resulting in various particle sizes. Coarse wheat bran, obtained through Perten mill had a mean particle size of 438.3–590.4 μm across all the four varieties. A mean particle size of 368.0–395.9 μm was obtained by Wiley mill using a 2 mm sieve, resulting in the coarse-medium bran. Pin milling with 1, 3, 6 times passes resulted in medium bran, medium to fine bran, and fine bran with a mean particle size of 315.3–367.7 μm, 224.8–273.7 μm, and 156.7–187.1 μm, respectively for the four wheat varieties. The average mean particle size of CM, M, MF, and F brans decreased by 26%, 33%, 53%, and 67%, respectively, compared to the C. The difference of bran particle size for different varieties from the same milling method could be due to the kernel characteristics, such as kernel hardness, bran composition, and milling work efficiency (Tian et al., 2022). This disparity became less obvious with more grinding as particle size decreased. Particle size distribution of whole wheat flour is the most straightforward and comprehensive indicator impacting flour physico-chemical and functional properties (Drakos et al., 2017; Zi et al., 2019). The quality of wheat, sifter sieve opening, mill's flow, type of roll, and their settings all affect the particle size distribution generated during milling (Siliveru, Ambrose, & Vadlani, 2017).

2.3.2. Influences of bran particle size on rheological properties of whole wheat flour

The moisture content of white flour from all the wheat types was around 14–15 g/100g (dry basis, db). After further grinding of the bran and recompositing with respective white flour into whole wheat flour (WWF) significantly decreased moisture content ($p < 0.05$) with decreased bran particle size for the WWF. All the wheat grains were tempered to 15 g/100g moisture content prior to the milling, which led to the consistent moisture of the resultant white flour. Different milling methods of the bran imposed additional shear and heat during bran grinding, thus causing different levels of moisture evaporation and resulting in different bran, and varied WWF moisture content.

A typical Mixolab curve can be divided into two phases. The first phase (C1–C2) displays the protein characteristics during dough mixing, and the second stage (C3–C5) demonstrates the pasting properties of starch in dough when subjected to mechanical stress and temperature changes. As shown in Table 2, the water absorption of WWFs with different particle size brans increased by around 7–8 g/100g compared to the corresponding white flour under the condition of C1 = 1.10 ± 0.05 Nm, due to the greater water absorption capacity of arabinoxylans, pentosans and other fibers in the bran (Arif, Ahmed, Chaudhry, & Hasnain, 2018; Xiong, Zhang, Niu, & Zhao, 2017). Among the WWFs, the optimal water absorption varied among different bran particle sizes and different wheat types. For Zenda variety, water absorption of the WWF with coarse bran (mean particle size of 550 μm) had slightly lower water absorption compared to other bran sizes (mean particle size of 396 µm and lower), while for Tatanka variety, the WWF with coarse bran had higher water absorption than the other treatments. The variation in water absorption for different wheat varieties and bran particle sizes could be related to the intrinsic characteristics of the wheat genotypes, surface area of the bran particles, bran compositions, and interactions with water during hydration (Penella, Collar, & Haros, 2008). In addition, with more intensive grinding of the bran during particle size reduction, increasing breakdown of starch granules can occur, resulting in more damaged starch from the bran after milling, and therefore increasing water absorption (Q. Wang, Li, & Zheng, 2020). Onipe, Beswa, and Jideani (2017) reported that when the particle size of wheat bran was reduced from coarse to medium, water absorption increased from 66.03% to 67.30%, but then decreased to 65.87% for the finer bran (Onipe et al., 2017).

Mixolab dough stability revealed the resistance of the dough to prolonged shearing during mixing. No significant differences in stability values between white flour and WWF were found for any of the other wheat varieties, except for Zenda, where dough stability was significantly higher for the WWF containing fine bran compared to the white flour or the WWFs containing other particle sizes of bran. Previous studies reported increased stability for WWF with the reduction of particle size (Lin et al., 2020; T. Liu, Hou, Lee, Marquart, & Dubat, 2016; N. Wang et al., 2017). It is possible that finer bran has less destructive influence on WWF dough formation and could increase dough's resistance during kneading.

No statistical differences were observed for amplitude except for Tatanka and Zenda. Dough development time is the time between the first addition of water and when the dough reaches its peak. There was no significant difference in development time for each wheat variety. This result was consistent with a study where WWF containing fine bran (less than 125 µm) had little effect on development time based on Farinograph (Niu, Hou, Wang, & Chen, 2014). Contrary to the above report, others reported a longer development time of WWF than white flour, and reduced dough development time with smaller bran particles, possibly due to the differences in hydration rate and interactions of gluten proteins with brans of different sizes (Penella et al., 2008; Xu, Xu, Wang, & Zhou, 2018). Wheat bran contains certain active substances (e.g., arabinoxylan, pentosan, enzymes) that could modify its effect on gluten network development and structure (Noort et al., 2010, Si et al., 2021).

The C3–C2 viscosity value (paste viscosity from starch gelatinization under mixing and heating constraints) of the white flour varied among different wheat varieties. Within all wheat varieties, the changes in C3–C2 values were not significant with the decrease of the particle size except for Tatanka. WWFs with coarse and coarse to medium particles had larger viscosity values than other sizes and the white flour. A previous study found no significant difference in viscosity between hard wheat winter WWFs with different particle sizes (T. Liu et al., 2016). The C5–C4 value indicates starch retrogradation. The C5–C4 values were mostly similar or lower for WWF compared to the corresponding white flour. Especially for Tatanka, the retrogradation value of the WWFs was only around one third of that for the white flour, implying a much stronger retrogradation potential for the white flour. In white flour, the retrogradation process is determined by the starch-protein complexes with varied sizes and thermal stabilities that are produced by gluten proteins with different HMW-GSs at 80–85 °C (X. Wang, Peng, Appels, Tian, & Zou, 2022). Compared to white flour, the fibers in WWF have a higher capacity to absorb water, hence making water molecules less available for starch retrogradation, which may account for the differences between white flour and WWF in C5–C4 values. This reduced starch retrogradation in WWF systems could be beneficial for delaying the hardening of baked breads during storage.

2.3.3. Bread volume

The specific volume of the bread is presented in Table 3, and bread slice pictures are shown in Fig. 3. Among all the white breads, breads with relatively higher protein contents, for example, AG-Gallant and Turkey Red (with protein content of 12.54 and 14.61 g/100 g, respectively) had larger specific volume compared to Tatanka and Zenda (with protein contents of 9.97 and 8.48 g/100 g, respectively). The whole wheat breads had a significantly lower specific volume than their corresponding white breads prepared from each wheat variety. Despite that Noort (2010) observed a similar volume in whole wheat breads with coarse bran and white bread (Noort et al., 2010). Other researchers observed a similar trend as ours that there was a lower specific volume of whole wheat breads with 8–24% bran supplementation compared to their white bread's counterparts (Alzuwaid, Pleming, Fellows, Laddomada, & Sissons, 2021; Hemdane et al., 2018; Packkia-Doss, Chevallier, Pare, & Le-Bail, 2019). The different observation by Noort (Noort et al., 2010) may be attributed to the relatively small bread volume range that was recorded. The specific volumes of whole wheat breads in our study ranged from 2.54 to 4.00 cm^{3}/g and white breads from 4.42 to 5.87 cm^3/g, while the specific volume of white breads in that study (Noort et al., 2010) was lower than 3.4 cm^3/g, which eliminated the differences between whole and white breads.

The negative effects of brans on loaf volume were aggravated when the particle size of bran was reduced for most varieties. Peak specific volume values were found in WWF breads made with bran with large or medium particle size range, and this was also dependent on the wheat variety. For all wheat varieties, the lowest specific volumes were found in WWF breads made with fine bran (the smallest particle size bran in our study) except for Tatanka. These results demonstrated the significance of using five particle size ranges to determine the effect of the particle size and optimum particle size of the bran may exist for specific wheat variety. Lai and others attributed this detrimental effect of bran addition on bread making to the dilution of gluten proteins (Lai, Hoseney, & Davis, 1989). They found that the extent of decrease in volume matched the decrease expected from the dilution of gluten proteins. However, the effect appears to be contentious since different trends were observed on the increased dough extension resistance with bran addition (Han, Ma, Li, Zheng, & Wang, 2018). It was confirmed by Magnetic Resonance Imaging that the decreased particle size of WWF (50.21 μm) would greatly increase the water absorption of arabinoxylan (AX) gels, which resulted in water migration from the gluten network to the AX gels and resulted in inferior quality of whole wheat bread (Li et al., 2012). The additional negative effect from brans was probably related to the complex mechanism of the bran-gluten interactions, which also depend on properties of fiber such as composition, molecular weight, and the degree of polymerization. Upon the inclusion of wheat brans in dough, researchers also observed a loss of large molecular weight protein, a decreased S–S content, and increased dominance of β-sheet and β-turn conformations, as well as fewer aggregates (Han, Ma, Li, Zheng, & Wang, 2019).

2.3.4. Color and texture properties of breads

The L*, a*, and b* color values of whole wheat bread and white bread crumb are shown in Table 3. Compared to white breads, bran incorporation reduced the lightness (lower L* value) and increased the reddish and yellow (higher a* and b* values) of the WWF bread crumb, especially with finer bran, the a* and b* values became even larger. Generally, breads made with fine particle size bran had the lowest lightness, largest reddish and yellow value for all wheat varieties. During baking, Maillard reaction and caramelization are the main reasons for the formation of the bread crumb color. The variation in WWF bread color may be related to the different levels of pigmented compounds, and the finer particle size bran enhances these reactions, resulting in a darker color of the crumb. The main elements for these reactions are amino acid residues, reducing sugars during baking further contribute to the brown color. Similarly, a compact crumb resulting in lower lightness with a simultaneous increase in redness and yellowness was reported (Protonotariou, Stergiou, Christaki, & Mandala, 2020). Furthermore, increased browning and Maillard reactions due to more sugars provided by damaged starch contributed to crumb color, which has a significant impact on the sensory quality.

Bread texture was affected by bran addition, with significantly increased bread hardness, gumminess, and chewiness for the WWF breads compared to the white breads (Table 4). The white breads had good external characteristics, soft and resilient crumbs, and evenly distributed cell structure. The TPA values of white bread slices showed hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, and chewiness in the range of 134.49–273.52 g, 40.48–46.42%, 0.80–0.85%, 164.95–181.57%, 113.50–217.43 g, and 206.01–358.37 g, respectively. Compared to white breads, WWF breads had larger values in hardness, gumminess, and chewiness. Resilience and cohesiveness values decreased in breads containing brans, and whole wheat bread with finer bran had the lowest resilience. In our study, whole wheat bread with medium or coarse bran had lower hardness values compared to that with the fine bran. Further sensory study should be conducted to assess consumer's preference to the bread textures from different bran particle sizes. In previous studies, whole wheat bread made from WWF with an average particle size of 96.99 μm had better baking quality than breads made from WWF of two other particle sizes of 50.21 and 235.40 μm (Li et al., 2012). Better product quality was also obtained using medium particle size WWF for producing noodle, tortillas, and cracker (T. Liu et al., 2016; Niu et al., 2014;

N. Wang, Hou, Kweon, & Lee, 2016). Therefore, there might be an optimal range of WWF particle sizes for each type of product.

The cells per slice area, area of cells, wall thickness, and cell diameters determined are shown in Table 5. C-Cell analysis of the slices revealed that the white bread slices had small and uniform pores compared to the whole wheat breads. For the dough made from white flour, leavened gas could be well retained and expanded in the continuous viscoelastic gluten network during fermentation and baking. However, for the WWFs containing bran particles, the gas retention capacity was greatly limited due to the interruption of the gluten network by the particles, resulting in smaller leavened dough and loaf volume (Hassan, Alkareem, & Mustafa, 2008). For most of the varieties except for Turkey Red, bread produced from WWF with coarse to medium and medium bran size had relatively fewer cells per slice area and larger cell diameters than the other whole wheat bread from the same variety. It has been reported that small pores and high number of cells per slice area are more desirable when producing high-quality bread (Iuliana, Georgeta, Sorina, & Iuliana, 2012). Among all bran particle sizes, the breads with fine bran had the largest number of cells per slice area, smallest cell area and smallest cell diameter.

Principal component analysis further demonstrated the associations among some important parameters (bran particle size, dough development time, bread specific volume, and hardness of the bread) for the tested samples (Fig. 4). Eigenvalues 1 and 2 from the PCA biplot explained 86.59% of the variability in the data. Dough and bread parameters from the white flours, WWFs with coarse/medium brans (C, CM, M), WWFs with medium/fine brans (M, MF, and F), and even different varieties are distinguishable in the plot, further confirming the previous discussions.

2.3.5. Effect of bran particle size on acrylamide formation in bread

The analytical quantification of acrylamide formation in bread was carried out using GC-MS, and the results obtained for white bread and WWF bread containing various particle sizes of bran are presented in Fig. 5. The linearity of the standard calibration curve ranged from 0.5 to 500 μg/L with a calculated correlation coefficient value of R2 > 0.999. The retention time of the derivative 2,3-dibromopropionamide was 12.89 min, which was confirmed by mass spectrometry. The acrylamide contents in the white bread ranged from 27.9 to 32.2 μg/kg, and the WWF bread generally had a higher level of acrylamide which ranged from 23 to 40.1 μg/kg depending on the wheat variety. Compared to white bread, the WWF bread had a relatively higher or similar acrylamide concentration, and the lowest acrylamide contents appeared, not statistically different, in the whole wheat breads with the medium particle size bran for most of the studied wheat varieties (except for Tatanka). A previous study reported that acrylamide concentration in bread crisp samples with whole wheat represented a higher level than samples with white wheat (Capuano et al., 2009). Acrylamide is subjected to a partial degradation at high temperature during baking, and the formulation of the bread influences the development of Maillard reaction and browning development. Acrylamide is formed during baking through the Maillard reaction during food processing. The formation of acrylamide in bread is dominated by reducing sugars and free asparagine. It was reported that acrylamide in bread is mainly in the crust, and the content of acrylamide increased with baking time and temperature, which is attributed to a high rate of water loss from the surface of the bread (Boyacı Gündüz & Cengiz, 2015). Various particle size of bran added to the breads produces high nutritional value breads because of the high content of dietary fiber and mineral, while there are no significant changes in WWF breads acrylamide accumulation based on bran particle size.

2.4. Conclusion

This research indicates that the size of the bran particles had an impact on the dough characteristics, bread volume, and texture properties of whole wheat bread. The Mixolab results showed that whole wheat flour presented greater water absorption, similar development time, and reduced starch retrogradation than white flour. Whole wheat flour with medium and medium to coarse size bran produced relatively soft bread with larger loaf specific volume. Results also revealed that using most fine bran had a negative influence on the hardness, chewiness, and color properties (darker color) of the bread. Moreover, larger specific loaf volume was observed for white bread with a relatively higher protein content in the flour, but this was not always associated with the trend for the whole wheat flour. The whole wheat bread had a similar or slightly higher acrylamide concentration than the white bread regardless of the variation in bran particle size. Our results demonstrated that reducing wheat bran to medium particle sizes (315–363 μm) is effective in improving the bread-making quality of whole wheat flour. The findings of this study contribute to the standardization of whole flour bran particle sizes for quality assessment and effective industrial production. Future work should evaluate more wheat varieties to understand the associations between bread-making properties of white and whole wheat flours. In addition, it would be interesting to evaluate consumer's preference on the whole wheat breads with different bran particle sizes through sensory studies and antioxidant activity and bioavailability of the bioactive compounds in such bread products.

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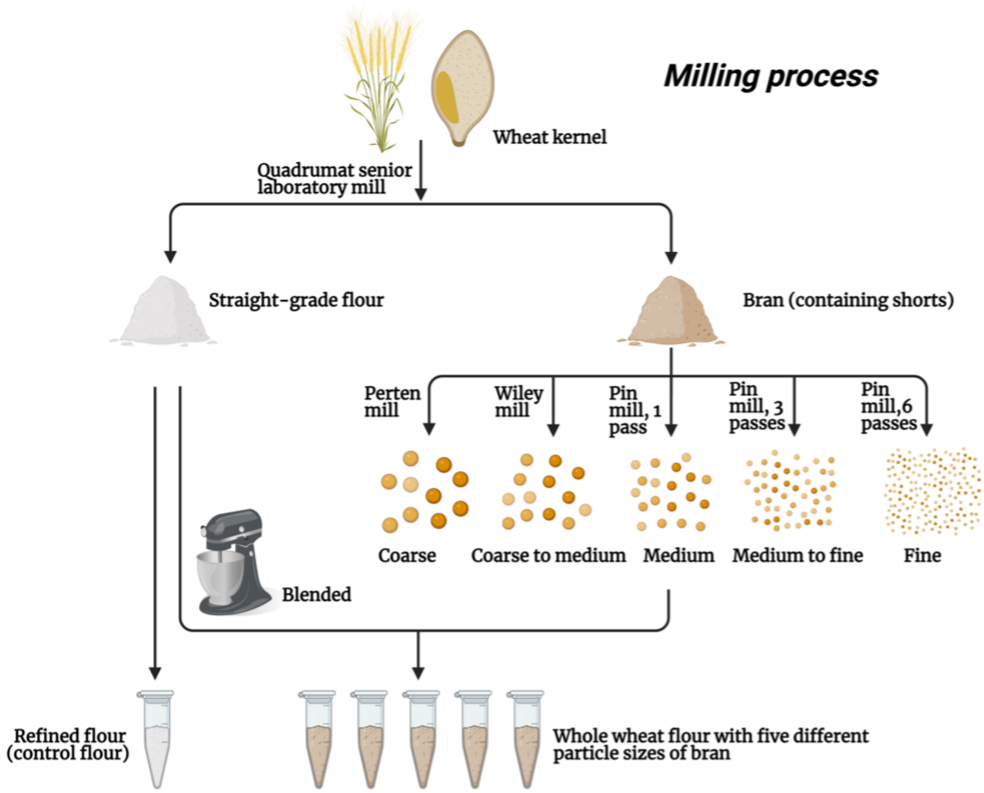

Figure 2.1. Flow chart for preparing reconstituted whole wheat flour.

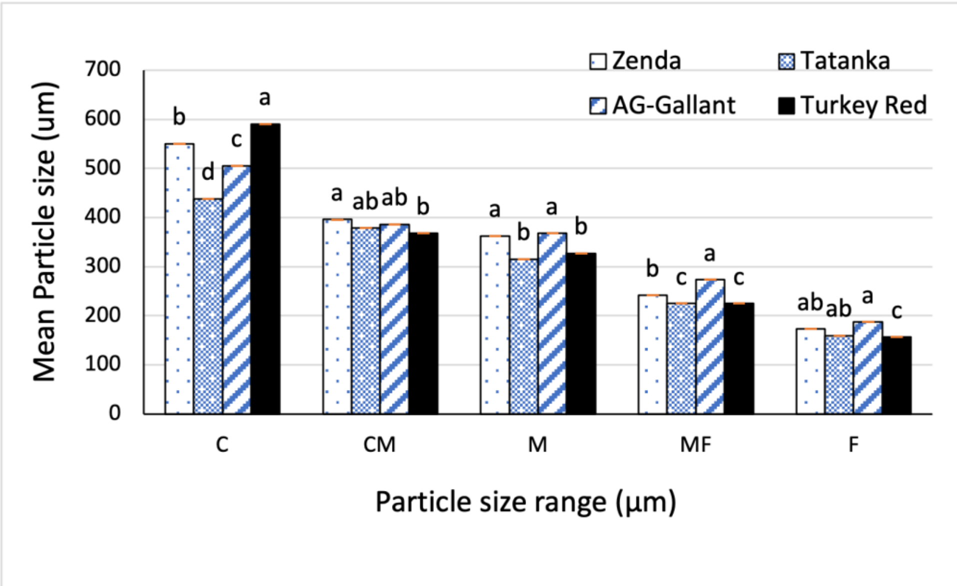

Figure 2.2. Mean particle size of different wheat brans.

C, coarse; CM, coarse to medium; M, medium; MF, medium to fine; F, fine. Bars with different letters of the same particle size range differ significantly ($p < 0.05$).

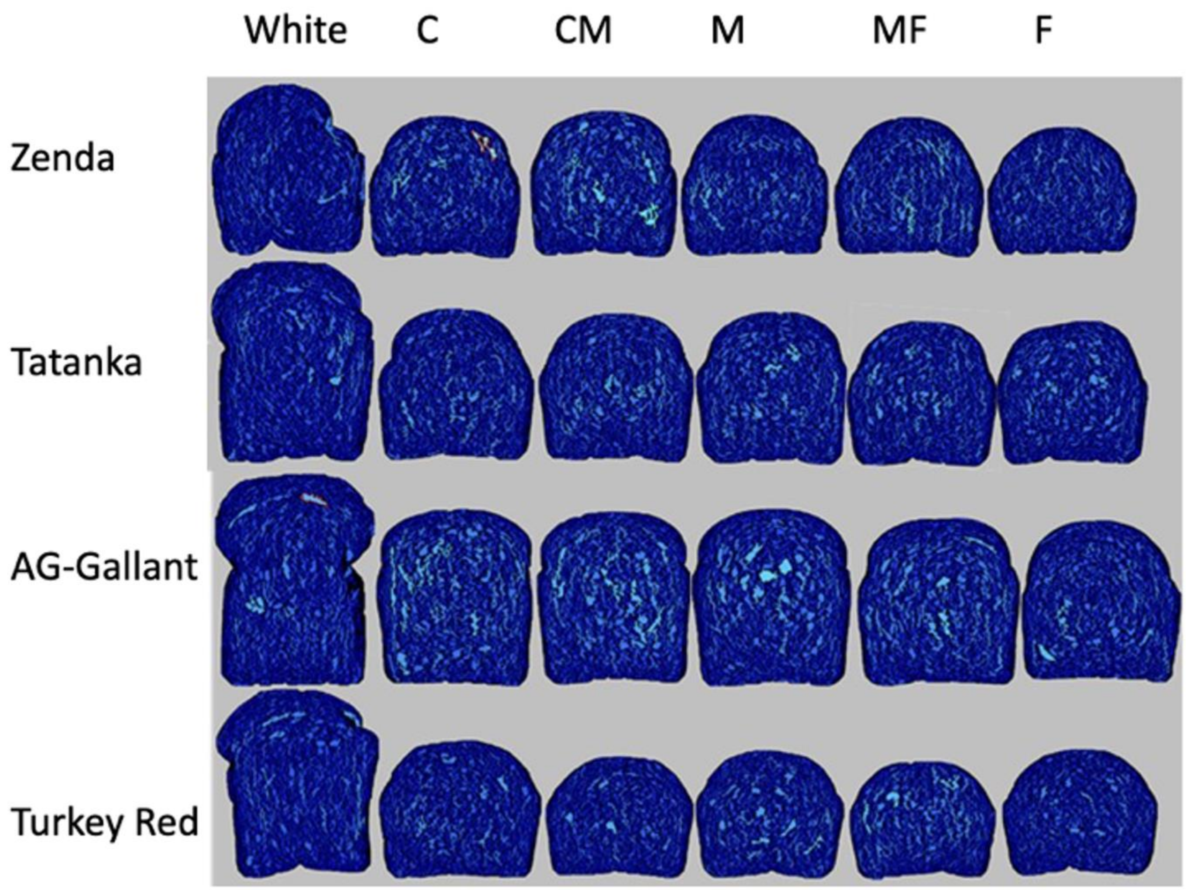

Figure 2.3. Bread slice images from C-Cell.

C: coarse, CM: coarse to medium, M: medium, MF: medium to fine, F: fine.

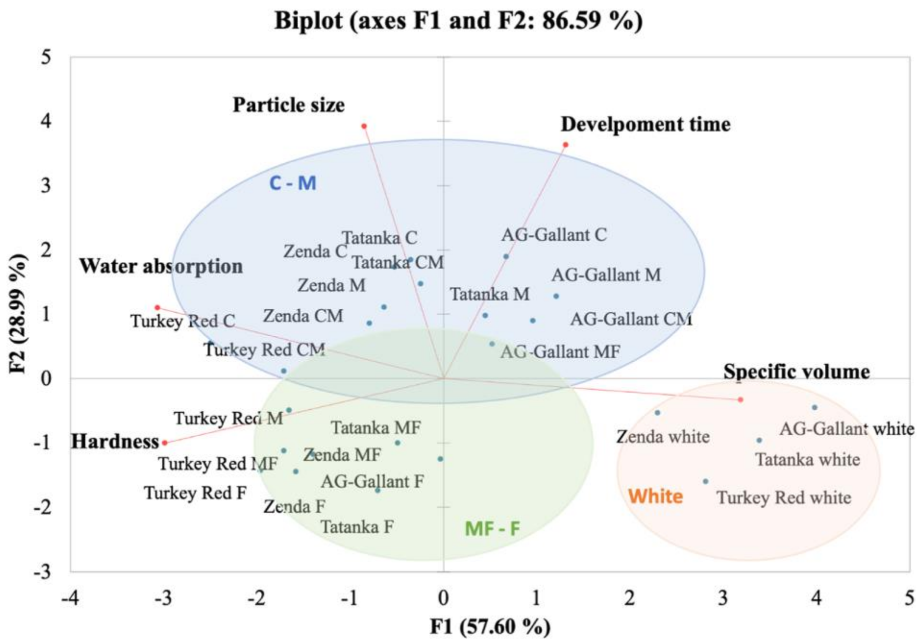

Figure 2.4. Principal components analysis (PCA) biplot describing the selected parameters of the brans, flours, and breads.
(C: coarse, CM: coarse to medium, M: medium, MF: medium to fine, F: fine, white: white flour/bread.)

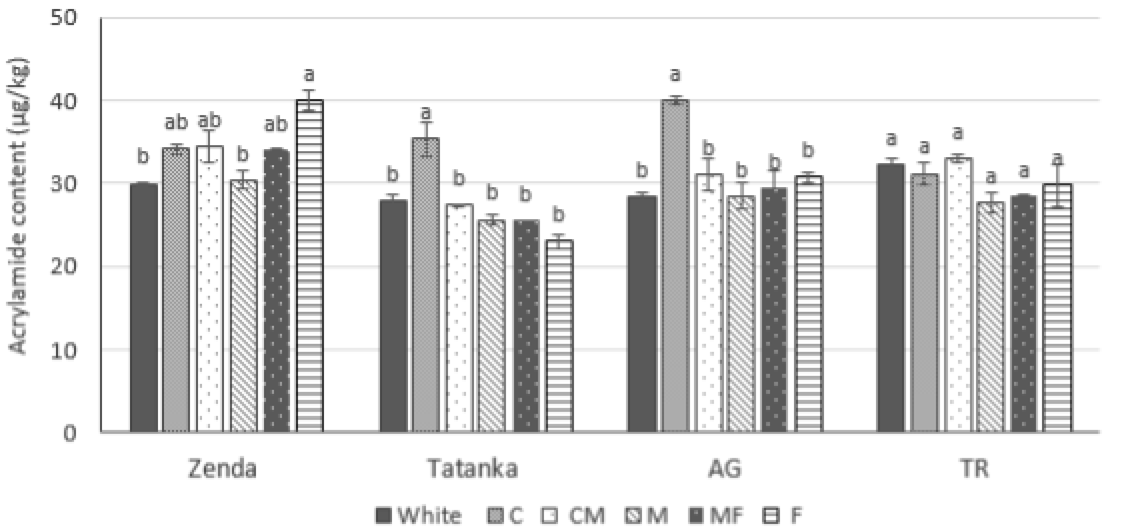

Figure 2.5. Acrylamide content of white and whole wheat breads from different wheat varieties. (C: coarse, CM: coarse to medium, M: medium, MF: medium to fine, F: fine). Values with different letters differ significantly ($p < 0.05$).

Table 2.1. Moisture content and Mixolab parameters of white flour and whole wheat flours.

Sample	Moisture (%)	Water absorption (%)	Amplitude (Nm)	Stability (min)	Development time (min)	Viscosity (C3-C2) (Nm)	Retrogradation (C5-C4) (Nm)
Zenda white	14.18±0.01a	59.20±0.00c	0.09±0.00ab	9.50±0.28b	8.00±0.00a	0.96±0.00a	0.88±0.04a
Zenda C	13.86±0.00ab	65.90±0.42b	0.07±0.00b	10.10±0.57ab	7.79±0.09a	1.56±0.01a	0.71±0.03abc
Zenda CM	14.00±0.00ab	66.60±0.00ab	0.08±0.00ab	9.05±0.35b	7.52±0.33a	1.51±0.01a	0.72±0.02ab
Zenda M	13.72±0.00b	66.95±0.07a	0.08±0.01ab	9.55±0.49b	6.72±1.82a	1.56±0.00a	0.67±0.07bc
Zenda MF	13.08±0.00c	66.85±0.07a	0.10±0.00a	10.10±0.14ab	5.89±0.12a	1.56±0.00a	0.84±0.05ab
Zenda F	12.50±0.00d	66.55±0.07ab	0.09±0.00ab	11.15±0.21a	5.45±0.92a	0.88±0.93a	0.53±0.05c
Tatanka white	14.07±0.04a	57.00±0.00c	0.80±0.24a	9.40±0.00a	7.48±0.08a	0.57±0.20b	2.50±0.03a
Tatanka C	13.69±0.00ab	65.25±0.35a	0.06±0.01b	9.70±0.00a	8.78±0.42a	1.38±0.02a	0.77±0.02b
Tatanka CM	13.92±0.00a	64.95±0.35ab	0.09±0.02b	10.15±0.07a	7.66±1.57a	1.37±0.01a	0.80±0.03b
Tatanka M	13.24±0.00bc	63.50±0.00b	0.07±0.00b	9.65±0.49a	9.08±0.06a	0.37±0.03b	0.79±0.03b
Tatanka MF	12.90±0.00cd	64.25±0.35ab	0.10±0.01b	9.95±0.07a	6.75±0.10a	0.30±0.15b	0.77±0.05b
Tatanka F	12.39±0.00d	63.55±0.78b	0.10±0.00b	10.50±0.57a	6.99±0.69a	0.23±0.11b	0.74±0.06b
AG white	14.53±0.06a	56.20±0.00e	0.23±0.24a	10.55±0.49a	9.01±0.51a	0.87±0.07a	0.90±0.05a
AG C	13.74±0.00b	64.00±0.00b	0.08±0.01a	10.60±0.14a	9.32±0.64a	0.48±0.20a	0.71±0.01b
AG CM	14.06±0.00b	63.50±0.00c	0.08±0.01a	10.80±0.00a	8.82±0.66a	0.91±0.80a	0.69±0.01b
AG M	13.71±0.00b	63.20±0.00d	0.07±0.02a	10.40±0.00a	9.41±0.37a	0.69±0.01a	0.81±0.05ab
AG MF	13.27±0.00c	64.40±0.00a	0.09±0.01a	10.40±0.42a	8.85±0.31a	0.41±0.02a	0.69±0.03b
AG F	12.59±0.00d	63.50±0.00c	0.08±0.00a	9.90±0.57a	7.47±0.85a	0.87±0.20a	0.81±0.06ab
TR white	14.78±0.00a	58.85±0.49c	0.06±0.01a	9.65±0.21a	6.34±0.02a	0.91±0.02a	0.94±0.00a
TR C	14.52±0.00a	66.00±0.00ab	0.08±0.01a	10.30±0.57a	6.69±0.62a	1.11±0.34a	0.82±0.07a
TR CM	14.30±0.00a	66.50±0.00a	0.24±0.23a	10.40±0.42a	7.51±0.30a	1.07±0.01a	1.67±1.12a
TR M	13.75±0.00b	66.45±0.07a	0.08±0.02a	9.45±0.49a	6.95±0.39a	0.85±0.42a	0.74±0.04a
TR MF	13.20±0.00c	66.20±0.00ab	0.09±0.01a	9.65±0.49a	6.72±0.14a	1.23±0.18a	0.81±0.05a
TR F	12.60±0.00d	65.50±0.00b	0.09±0.00a	10.35±0.63a	7.25±0.18a	1.00±0.19a	0.83±0.00a

Note: Samples with different letters in the same column differ significantly ($p < 0.05$).C: coarse, CM: coarse C: coarse, CM: coarse to medium, M: medium, MF: medium to fine, F: fine.

Table 2.2. Specific volume and color properties of different wheat breads produced from whole wheat flour and white flour.

Bread	Specific volume (cm^3/g)	L*	a*	b*
Zenda white	4.42±0.02 a	65.2±0.58 a	1.28±0.06 e	10.31±0.46 d
Zenda C	3.08±0.11 b	52.06±0.96 b	2.8±0.32 d	17.02±0.33 c
Zenda CM	3.02±0.12 b	50.88±0.72 bc	3.9±0.35 bc	18.45±0.59 b
Zenda M	3.00±0.08 b	51.98±1.20 b	3.63±0.27 c	18.45±0.70 bc
Zenda MF	2.85±0.01 b	49.62±0.55 c	4.58±0.41 ab	20.74±0.72 a
Zenda F	2.62±0.01 b	51.16±0.38 bc	5.03±0.19 a	21.69±0.38 a
Tatanka white	5.43±0.08 a	63.28±0.86 a	1.01±0.16 c	11.46±0.84 c
Tatanka C	2.96±0.05 c	49.28±0.59 ab	3.68±0.27 b	19.77±0.50 b
Tatanka CM	3.12±0.06 c	47.52±0.40 c	3.78±0.43 b	20.34±0.74 b
Tatanka M	3.41±0.00 b	49.93±0.45 b	3.36±0.13 b	19.39±0.35 b
Tatanka MF	3.08±0.06 c	48.12±1.08 bc	4.44±0.17 a	21.73±0.22 a
Tatanka F	3.12±0.03 c	48.20±0.81 bc	4.70±0.18 a	22.36±0.19 a
AG white	5.87±0.02 a	63.85±1.19 a	1.19±0.05 c	8.32±0.26 d
AG C	3.86±0.01 b	51.11±0.75 b	2.55±0.66 b	16.53±1.42 c
AG CM	3.90±0.00 b	51.75±0.82 b	2.42±0.38 b	16.78±0.52 c
AG M	4.00±0.02 b	47.32±0.58 bc	3.36±0.15 ab	19.39±0.40 bc
AG MF	3.39±0.05 c	48.03±1.35 cd	4.05±0.84 a	19.99±1.68 ab
AG F	3.17±0.01 d	45.56±1.53 d	4.56±0.35 a	21.34±0.46 a
TR white	5.55±0.12 a	65.33±0.69 a	1.30±0.08c	9.29±0.34 d
TR C	2.70±0.12 b	48.49±1.58 a	4.48±0.24 b	18.53±0.78 c
TR CM	2.68±0.07 b	45.90±1.19 a	4.79±0.16 b	19.25±0.20 bc
TR M	2.67±0.05 b	45.39±1.25 a	4.67±0.38 b	19.76±0.54 abc
TR MF	2.63±0.02 b	45.46±1.21 a	5.30±0.51 ab	20.73±0.91 ab
TR F	2.54±0.00 b	45.22±1.23 a	5.77±0.31 a	21.27±0.52 a

Note: Samples with different letters in the same column differ significantly ($p < 0.05$).C: coarse, CM: coarse C: coarse, CM: coarse to medium, M: medium, MF: medium to fine, F: fine.

Table 2.3. Texture profiles of breads.

Sample	Hardness (g)	Springiness (%)	Gumminess (g)	Chewiness (g)
Zenda white	273.52±16.86 e	164.95±13.33 a	217.43±11.91 e	358.37±30.90 d
Zenda C	654.81±34.17 d	152.72±13.47 a	507.26±25.3 d	773.86±71.44 ab
Zenda CM	812.58±86.17 c	120.88±17.61 b	585.26±54.83 c	702.2±82.83 b
Zenda M	799.76±51.01 c	101.67±2.87 bc	571.28±32.24 c	581.24±43.32 c
Zenda MF	1025.42±32.39 b	99.52±1.93 c	696.01±23.25 b	692.73±28.97 b
Zenda F	1256.77±36.43 a	96.68±1.55 c	838.00±25.61 a	810.12±26.27 a
Tatanka white	153.44±13.09 d	179.88±1.62 a	126.01±9.83 d	226.68±17.97 c
Tatanka C	780.63±51.71 c	105.11±12.39 b	550.78±32.03 c	577.24±58.85 b
Tatanka CM	911.72±35.34 b	97.39±1.66 b	632.02±27.64 b	615.79±34.63 b
Tatanka M	753.90±27.73 c	105.27±11.05 b	527.50±14.86 c	554.36±49.32 b
Tatanka MF	918.47±49.61 b	96.89±1.59 b	610.45±32.78 b	590.99±24.10 b
Tatanka F	1216.17±47.16 a	96.17±1.87 b	792.71±38.21 a	762.61±44.26 a
AG white	134.49±3.04 e	181.57±3.40 a	113.50±2.70 e	206.01±3.41 c
AG C	465.48±21.16 c	139.50±27.04 b	350.46±14.54 c	489.30±99.56 ab
AG CM	342.48±11.81 d	164.24±9.26 ab	276.38±14.45 d	452.9±16.29 b
AG M	413.73±24.98 cd	158.90±5.40 ab	318.86±17.56 cd	506.17±23.52 ab
AG MF	544.65±79.23 b	141.02±22.92 b	406.97±53.54 b	563.23±50.73 a
AG F	764.70±36.54 a	101.46±2.37 c	528.27±23.87 a	536.03±27.57 ab
TR white	160.84±5.46 e	178.29±7.22 a	136.71±4.55 d	243.63±10.76 d
TR C	1704.61±41.41 b	95.05±0.98 b	1137.15±24.46 ab	1080.84±27.26 ab
TR CM	1441.80±77.47 c	97.45±2.21 b	1012.56±53.02 c	986.42±50.86 bc
TR M	1331.66±71.65 d	96.83±1.61 b	934.74±85.04 c	904.47±76.15 c
TR MF	1517.00±69.46 c	94.93±1.03 b	1036.73±74.06 bc	984.39±74.23 bc
TR F	1966.35±47.52 a	93.2±1.83 b	1237.11±36.76 a	1152.99±38.79 a

Note: Results are presented as means ± standard deviations (n = 4). Samples with different letters in the same column differ significantly ($p < 0.05$) (AG, Ag gallant; TR, turkey red; C: coarse, CM: coarse C: coarse, CM: coarse to medium, M: medium, MF: medium to fine, F: fine).

Table 2.4. Morphological characteristics of bread slice produced from whole wheat flour and white flour.

Sample	Number of cells/slice area (mm^2)	Area of cells / %	Wall thickness / mm	Cell diameter / mm
Zenda white	0.75±0.02 a	49.23±0.22 c	0.42±0.01 b	1.70±0.06 c
Zenda C	0.71±0.01 ab	50.78±0.26 ab	0.43±0.00 ab	1.85±0.02 abc
Zenda CM	0.68±0.01 b	51.15±0.17 a	0.44±0.00 a	1.97±0.11 a
Zenda M	0.70±0.01 b	50.53±0.33 b	0.44±0.01 a	1.87±0.05 ab
Zenda MF	0.72±0.01 ab	50.65±0.15 ab	0.43±0.00 a	1.88±0.04 ab
Zenda F	0.74±0.03 a	49.65±0.23 c	0.43±0.01 ab	1.79±0.05 bc
Tatanka white	0.67±0.01 c	50.23±0.33 ab	0.43±0.00 a	1.82±0.09 a
Tatanka C	0.77±0.02 a	50.23±0.20 ab	0.41±0.00 b	1.68±0.03 bc
Tatanka CM	0.74±0.01 b	50.65±0.11 a	0.42±0.00 ab	1.76±0.03 abc
Tatanka M	0.73±0.00 b	50.60±0.19 a	0.43±0.00 a	1.80±0.05 ab
Tatanka MF	0.77±0.00 a	50.30±0.10 ab	0.42±0.00 ab	1.74±0.01 abc
Tatanka F	0.78±0.02 a	50.00±0.30 b	0.41±0.00 b	1.64±0.06 c
AG white	0.71±0.01 a	50.08±0.32 d	0.42±0.01 ab	1.83±0.05 b
AG C	0.71±0.01 a	51.33±0.11 b	0.43±0.00 ab	1.85±0.04 b
AG CM	0.66±0.01 b	52.13±0.22 a	0.44±0.00 a	2.02±0.06 a
AG M	0.66±0.02 b	51.98±0.26 a	0.44±0.00 a	1.99±0.08 a
AG MF	0.67±0.02 b	52.03±0.04 a	0.44±0.01 ab	2.03±0.05 a
AG F	0.72±0.01 a	50.68±0.11 c	0.42±0.00 b	1.75±0.03 b
TR white	0.68±0.05 b	50.40±0.54 a	0.43±0.01 a	1.94±0.19 a
TR C	0.89±0.03 a	48.85±0.38 b	0.39±0.00 b	1.45±0.04 b
TR CM	0.83±0.02 a	49.60±0.34 ab	0.41±0.00 b	1.54±0.03 b
TR M	0.83±0.03 a	49.55±0.40 ab	0.41±0.01 b	1.55±0.05 b
TR MF	0.83±0.03 a	49.58±0.54 ab	0.41±0.01 b	1.59±0.12 b
TR F	0.89±0.02 a	48.80±0.21 b	0.39±0.00 b	1.43±0.01 b

Note: Results are presented as means ± standard deviations ($n = 4$). Samples with different letters in the same column differ significantly ($p < 0.05$). (AG, Ag gallant; TR, turkey red; C: coarse, CM: coarse C: coarse, CM: coarse to medium, M: medium, MF: medium to fine, F: fin

Chapter 3 - Comparing bread-making properties of white and whole wheat flours from 64 different genotypes: a correlation analysis[1]

Abstract

Quality attributes of wheat and its flour strongly influence the processability, end-product performance, and consumer acceptability of wheat-based products. The quality of whole wheat flour (WWF) has been estimated using white flour (WF) methodologies, which raised concerns due to the uncertain correlations between WF and WWF. Strong correlations were identified between the WF and WWF for protein content and flour water absorption (r = 0.977 and 0.713, respectively), pasting properties of peak and final viscosity (r = 0.851 and 0.698, respectively), and the optimum mixing time determined by Mixograph (r = 0.825). In contrast, the bread volumes and water retention capacity were weakly correlated to WWF versus WF (r = 0.480 and 0.277, separately). There was a negative relationship between the peak value of WF and hardness of the white bread (r= - 0.582), while it was not present for the WWF bread. This comparatively evaluation of the dough rheological properties and bread-making performance of WF and WWF from 64 wheat genotypes provide valuable information for the baking industry to predict the bread-making performance of whole wheat flour.

Keywords: Whole wheat flour; dough rheology; correlation analysis; bread; wheat quality.

[1]Li, Cheng, Gengjun Chen, Michael Tilley, Richard Chen, and Yonghui Li. " Comparing bread-making properties of white and whole wheat flours from 64 different genotypes: A correlation analysis." Under Review.

3.1. Introduction

Whole wheat grains have gained popularity among consumers as they seek healthier diet options. Unlike conventional white flour (WF), which mainly serves as a source of carbohydrates, whole wheat flour (WWF) contains high levels of dietary fibers, bioactive phenolic compounds, antioxidants, vitamins, and minerals due to the inclusion of bran and germ (Spaggiari et al., 2020). Additionally, the presence of bran in WWF slows down the rate of glucose release during digestion, making whole wheat bread a medium glycemic index food that supports a healthy diet (Suyun Lin et al., 2022a). However, some negative or unique effects are observed for whole wheat bread compared to white bread, such as lower loaf volume and distinctive crumb texture (C. Li, Tilley, Chen, Siliveru, & Li, 2023). These effects are likely a result of bran and germ, which can affect the performance and quality of whole wheat bread.

Generally, the WF bread-making quality can be related to multiple flour quality parameters, such as protein content, wet/dry gluten content, damaged starch, dough rheology, and gluten composition. Studies have found higher correlation coefficients between gliadin ($r = 0.80$), glutenin ($r = 0.76$), and glutenin macropolymer ($r = 0.80$) and white bread volume in common wheat, compared to the correlation with crude protein content ($r = 0.71$) (Thanhaeuser, Wieser, & Koehler, 2014). Additionally, it has been found that glutenin content primarily affects dough development time and white bread volume (Guzmán et al., 2022). On the other hand, damaged starch (range from 6.1 - 17.7%) has been found to have a significantly negative correlation with bread volume ($r = -0.95$) (Barrera, Pérez, Ribotta, & León, 2007).

While these findings have been effective in estimating the bread quality, difficulties about their applicability to WWF and bread have arisen due to the complex interactions induced by the components in bran and germ. Previous studies have shown inconsistencies in the correlation among bread volume, water retention capacity, and mixing properties when comparing WWF and WF using four different wheat samples, indicating that the baking potential of WWF could not be accurately predicted using data from WF (Bruckner, Habernicht, Carlson, Wichman, & Talbert, 1998; Seyer & Gélinas, 2009). These findings suggest that additional research is needed to better understand the relationships between the WF and WWF quality and develop more applicable methods for predicting the bread-making quality of WWF.

This study was aimed to provide a systematic quality analysis of WF and WWF from 64 different wheat lines and to determine if any correlations can be established between WF and WWF quality parameters. Both the WF and WWF were thoroughly characterized in terms of composition, water retention capacity, dough mixing and pasting properties, bread volume and texture. Additionally, we also explored the correlation between the WF system and the WWF system. The result of this study will be beneficial for breeders and producers to predict the bread-making performance of WWF.

3.2. Materials and methods

3.2.1. Wheat samples and whole wheat flour preparation

Sixty-four hard red winter wheat samples were collected from the Kansas region, representing a wide range of bread baking potential. The white flour (WF) and bran parts (shorts were blended into the respective bran sample) were prepared using a previously established method (C. Li et al., 2023). The bran was further pulverized using a Wiley mill (Thomas Scientific, Philadelphia, PA, USA) equipped with a 2 mm opening screen. A portion of the white flour was blended with the corresponding bran according to the milling yield (Supplementary Table S1) to obtain reconstituted WWF. The milling yield of the WF was around 67%.

3.2.2. Chemical analysis

The moisture contents of white (WF) and corresponding whole wheat flour (WWF) were determined according to AACC method 44-15.02 (AACC, 2010). The white flour protein content was determined with a near-infrared (NIR) analyzer (AACC method 39-10A) (AACC, 2010). The complex composition of reconstituted whole wheat flour necessitated the use of nitrogen combustion analyzer (LECO Corp., USA) to measure the protein content, and it was calculated with a conversion factor of 5.7, per the AACC method 46-30 (AACC, 2010).

3.2.3. Water retention capacity of flours

The water retention capacity of WF and WWF was determined according to AACC method 56-11(AACC, 2010). The water retention capacity was calculated as g water retained per g sample and expressed as %, as shown by the equation: Water retention capacity (%) = (W_1/W_2 – 1) x (86/ (100- flour moisture (%)) x 100, where W_1 was the weight of flour and water after centrifugation, and W_2 was the flour sample weight.

3.2.4. Mixograph analysis

The mixing characteristics of WF and WWF were evaluated in duplicate using a Mixograph (National Manufacturing, USA) in accordance with AACC method 54-40 (AACC, 2010). Flour (10 g, 14% moisture content base) and water were mixed for 10 min. Water absorption (%), dough development time (MPT), peak value (PVE), curve tail integral (CTI), breakdown, and slope were determined.

3.2.5. Pasting properties

The pasting characteristics of the WF and WWF were analyzed using a Rapid Visco Analyzer (RVA) (Perten Instruments, Australia). The RVA curve was used to capture the following properties: peak viscosity (PV), trough/hold viscosity (trough), breakdown viscosity (BKD), final viscosity (FV), setback viscosity (setback), pasting temperature (TEMP) and peak time. Each sample was analyzed in triplicate.

3.2.6. Bread making

WF and WWF breads were baked according to the AACC Approved Method 10–10.03 (international, 2010). The formulation was adjusted according to method (C. Li et al., 2023), utilizing 100g WWF or WF. After separating the dough by weight, two loaves of bread were baked for each treatment. After 2 hours of cooling at room temperature, The loaf weight was recorded and the volume was measured according to AACC method 10-05.01 (, 2010).

3.2.7. Crumb characteristics

A C-Cell Bread Imaging System (Calibre Control International Ltd., UK) was used to analyze the center slices from each loaf. The number of cells per slice area (cells/area), area of cells, cell wall thickness, and cell diameter were collected.

3.2.8. Texture profile analysis (TPA)

The texture properties of the bread slices were analyzed according to the method (C. Li et al., 2023) using a TA-XT2 Texture Analyzer (Stable Micro System, UK) with a 25-mm diameter cylinder probe. Four slices (15 mm thick) from the center of each loaf were analyzed.

3.2.9. Statistical analysis

The results were analyzed through analysis of variance (ANOVA). Means were separated using Tukey's standardized range test (SAS Studio, SAS Institute Inc., USA) with significance established at $P \leq 0.05$ using a one-way ANOVA followed procedure at 5% significance level. The least significant difference (LSD) test was conducted to determine significant differences in the mean values ($p < 0.05$). Pearson correlation analysis was conducted to establish the correlation between WF and WWF on rheology, dough, and bread properties.

3.3. Results and discussion

3.3.1. Moisture and protein content of whole wheat flour (WWF) and white flour (WF) samples

The average moisture and protein content of the sixty-four WF and corresponding WWF are presented in **Table 1** and Supplementary Table S2. The protein content of WWF was 11.05% on average, which was generally higher than that in the corresponding WF (8.99%). The average moisture content in WF and WWF were 13.78 and 12.04%, separately. The moisture content for both WF and WWF is within the limit established by FDA's Code of Federal Regulations Title 21(FDA, 2014), which is not more than 15%. The lower moisture content in WWF compared to WF can be attributed to the different moisture distribution in the endosperm and bran/germ after kernel tempering, as well as the additional milling process of the bran.

The regression between the protein content in WF and WWF could be described by the equation: protein content (WF) = 0.9548 × protein content (WWF) - 1.5625 ($R^2 = 0.9418$) **(Figure 1)**, suggesting a positive relationship between the two. The increased protein content in WWF compared to WF is due to the presence of bran, germ and endosperm, with bran contributing 20% of the total grain protein and germ accounting for 8% (MacMASTERS, Hinton, & Bradbury, 1971). Similarly, a strong protein correlation of 0.93-0.97 was discovered between the WF and WWF conducted with 32 winter wheat genotypes grown at Huntley, Bozeman and Havre in 1997 and 1998 (Bruckner et al., 1998). It is well established that protein generally plays a dominant role in determining the properties of dough and bread-making.

3.3.2. Water retention capacity (WRC)

The water retention capacity test evaluates the water-binding ability of flour components and provides insights into the flour quality, functionality, and end-use performance. The result in in **Table 1** shows that the WRC values of the WWF were higher than those of the corresponding WF, with WRC of WWF averaging 62.34% and WF averaging 56.07%. Pearson correlation between the WRC values of WF and WWF was positive but not statistically significant ($r = 0.277$). It was reported that four hard red spring wheat samples had a WF of 73 to 85% and a WWF of 98 to 111% on WRC (Seyer & Gélinas, 2009). The difference was due to the test method used and the variation of the samples. The difference in WRC of WWF and WF is due to the flour composition difference, where components like cellulose, arabinoxylan, damaged starch, and β-glucans in WWF play a greater role in water binding (De Bondt, Liberloo, Roye, Goos, & Courtin, 2020).

3.3.3. Mixograph properties

The dough mixing properties of WWF and WF were evaluated and compared using a Mixograph (**Supplementary Table S3&4).** In general, the water absorption of dough made with

WWF was around 3% higher than that of WF. There was a positive correlation between the water absorption of WF and WWF (r = 0.713, p <0.0001) (**Table 2**). The water absorption was shown to be positively and significantly linked with protein content in WF and WWF (r = 0.972, r = 0.740, respectively). A previous study showed that water absorption increased in WWF with an increase in protein content, fiber incorporation, and damaged starch addition (Goldstein, Ashrafi, & Seetharaman, 2010).

The optimal formation of the gluten network and the maximal dough resistance, as determined by protein and starch, is referred to as the ‘dough development time (MPT)’ in the Mixograph test. After achieving the MPT additional mixing results in a decrease and narrowing (break down) of the trace as the dough gradually loses its elasticity and becomes sticky. The average MPT for WF was 6.8 min while the average MPT for WWF was 6.49 min (**Supplementary Tables S3&4**). The relationship of MPT between WF and WWF are presented in **Figure 1,** and these were strongly correlated (r = 0.825, p < 0.0001) (**Table 2).** The MPT of WF was negatively correlated to the protein content and water absorption (r = -0.431 and -0.455, respectively) (**Table 2**). Previous studies also showed that the MPT decreased with the addition of different level of wheat bran or WWF into the WF (Bader Ul Ain et al., 2020; Bae, Lee, Hou, & Lee, 2014).

The area below the midline curve from the beginning to the end of the mixing process (10 min) is known as CTI, and it describes the energy required during the dough mixing process. For WF, CTI showed positive correlation with PVE (r = 0.957, p ≤ 0.001), and MPT had a negative correlation with breakdown (r = -0.714, p ≤ 0.001) (**Table 2**). There was a similar correlation on WWF with r = 0.839 and r = -0.801, respectively. Protein content correlated strongly with PVE and CTI in WF (r = 0.768 and r = 0.703, respectively) (Table 2). Protein content, on the other hand, had a substantial association with PVE but a little correlation with CTI (r = 0.471 and r = 0.252) for WWF. Previous studies reported a strong correlation (r > 0.5) between bran protein and mixing properties including PVE, peak width, and total area of reconstituted WWF (WF to bran and shorts ratio: 71:29) (Navrotskyi, Guo, Baenziger, Xu, & Rose, 2019). The strong correlation between some major Mixograph parameters of WF and WWF suggests that WF can be used to predict the dough mixing properties of WWF.

3.3.4. Flour pasting properties measured by RVA

The RVA viscometer has been commonly used to study the pasting properties of starch, with the results being useful for predicting the quality of bread-making. During heating, hydration occurs where water enters the interior of the starch and binds with other components like protein. The pasting properties of the WF and WWF are shown in **Supplementary Tables S5 & S6**. The RVA curve of selected WF and WWF are presented in **Figure 2**, with significant differences observed between the WF and WWF in terms of peak, breakdown, and setback viscosities.

Overall, WF exhibited much higher peak, trough, breakdown, final, and setback viscosity, but lower pasting temperature compared to WWF. The reduction in pasting parameter values of the WWF can be viewed as a benefit for simpler cooking and less retrogradation. Among all wheat flour samples, sample No. 62, 58, 61 and 64 had the lower peak viscosity in both WF and WWF, while No. 1 had the highest peak viscosity. Similar results were also reported when comparing wheat flour and wheat blend (85% refined flour +15% whole grain) (Ragaee & Abdel-Aal, 2006), and comparing WF, 50% WWF, and 100% WWF (Bae et al., 2014).

WF has an average breakdown viscosity of 942.42 cP, while WWF has an average breakdown viscosity of 626.13 cP. (**Table 1**). The reason for the difference was due to the WF with higher starch content being more fragile than WWF, as evidenced by its larger breakdown viscosity. The flour slurries were subjected at a constant elevated temperature and mechanical shear stress, which caused the swollen granules to rupture and the amylose to leach out. Furthermore, WWF had a lower setback viscosity compared to WF during cooling, which is due to the reduced retrogradation and reordering of the starch molecules (Copeland, Blazek, Salman, & Tang, 2009). The lower final viscosity values of WWF than WF also indicate a lower resistance to heating and shearing, which led to the formation of a less viscous paste or gel after cooling. It is impossible to disregard the dilution of starch caused by the inclusion of bran in whole wheat flour, bran's interference hindered the maximal starch pasting viscosity from being achieved (Lai, Hoseney, & Davis, 1989).

The average pasting temperature of WF (69.3 °C) was found to be relatively consistent across samples, whereas WWF required a higher temperature (82.76 °C) and wider range to reach the peak viscosity (**Table 1**). The increased pasting temperature of WWF indicates a decreased ability of the starch granules to absorb water and swell (Wani et al., 2014). The higher starch content and larger surface area of WF lead to higher frictional force that the molecule must overcome to flow, resulting in an increase in viscosity compared to WWF. In addition to the differences in starch content, the characteristics and pasting properties of WWF are further affected by its protein, bran, and their interaction with starch.

Furthermore, all selected RVA parameters of WF and WWF exhibited highly significant positive correlations ($r > 0.588$, $p < 0.0001$), except for TEMP ($r = 0.075$) as determined by Pearson's correlation coefficient (**Table 2).** In particular, the peak and breakdown viscosities showed a strong correlation between WF and WWF ($r = 0.851$, and $r = 0.883$, respectively). This correlation reflects the level of starch gelatinization, protein denaturation, flour composition, and their interactions.

3.3.5. Bread specific loaf volume

Bread specific volume is known to be influenced by factors such as protein content and quality, as well as other grain and flour characteristics. As seen in **Table 1** and Supplementary Table S7, bread made with WF, and WWF show differences in baking performance. Bread made with WWF had a lower average specific volume of 2.09 cm^3/g compared to those made with WF, which had an average value of 4.10 cm^3/g. This result is predicable since fiber in WWF that interacts with the gluten network and can decrease loaf volume (Seyer & Gélinas, 2009). The reduction in loaf volume with the addition of bran is believed to be due to the following factors. Firstly, the physical disruption of the dough foam structure by wheat bran minimizes gas retention during fermentation, leading to a decrease in bread volume (X. Xu, Xu, Wang, & Zhou, 2018). Secondly, wheat bran changed the water distribution in dough by competing with gluten for available water and causing the formation of additional β-sheets in the gluten structure, which can adversely impact loaf volume, as proteins play a role in maintaining proofed dough volume through the ability to maintain a certain critical threshold of β-turn protein structures (Bock, 2019).

Pearson's correlation analysis revealed a statistically significant and weak correlation between the specific volume of bread made with WF and WWF ($r = 0.480$, $p < 0.001$) (**Table 3**). The protein content was moderately correlated with the specific volume of both WF ($r = 0.66$) and WWF bread ($r = 0.611$). Water absorption from the Mixograph was found to be the parameter most significantly correlated to specific volume, with correlation coefficients of 0.632 in WF and 0.570 in WWF.

3.3.6. Bread textural properties

The texture of bread is a crucial attribute for consumers when evaluating the quality and appeal of the products. In this study, the textural properties of whole wheat bread and white bread were evaluated through texture profile analysis (TPA). Results showed that compared to white bread, whole wheat bread had higher values of hardness, gumminess, and chewiness (**Supplementary Tables S8 & S9**). The average hardness value of white bread was 685.34 g, while whole wheat bread had an average hardness value of 2227 g (**Table 1**). This difference of white and whole wheat bread in texture properties may be due to the lower specific volume and more compact crumb structure of whole wheat bread (Suyun Lin et al., 2022a).

Our findings showed negative and strong correlations between the hardness value and specific volume for both white and whole wheat bread (r = -0.683 and -0.742, respectively) (**Table 3**). Similarly, gumminess and chewiness values of whole wheat bread were also strongly correlated with its specific volume (r = - 0.76, and - 0.732, separately). In addition, there was a moderate and negative relationship between white bread hardness and water absorption, PVE, and CTI of WF in the Mixograph (r = -0.490, -0.582, -0.554, respectively) (**Table 4**). The physical disruption of the gluten network structure by wheat bran alters gas retention, leading to a decrease in bread texture, is an important factor (X. Xu et al., 2018).

3.3.7 C-cell properties of bread crumb

The cell-to-total area ratio (i.e., cell area%) in a bread slice provides insight into the number of gas bubbles trapped during mixing and proofing. The C-cell properties of bread made with WWF and WF are shown in **Supplementary Tables S10 and S11**. During bread making, bran in the WWF can diminish gluten strength and gas retention capacity, causing disruptions and inhomogeneities in the porous structure of crumb, resulting in a different cell structure (Dobraszczyk & Salmanowicz, 2008). In addition, the cell area of WWF bread displayed a negative correlation with its hardness, gumminess, and chewiness (r = -0.497, -0.521, -0.506, respectively) (**Table 4**). A previous study also reported a similar correlation between cell area and hardness properties on day 1 with a value of -0.531 (Junejo et al., 2021). Furthermore, Wang et al. showed that dough extension and resistance were significantly correlated to cell properties (cell area, cell diameter, and cell wall thickness) of steam bread using reconstituted WWF (N. Wang, Hou, & Dubat, 2017).

3.4. Conclusion

Analysis of 64 white flours and corresponding whole wheat flours revealed varying correlations for quality performance traits. A strong correlation was observed for protein content, water absorption, and mixing peak time between white and whole wheat flours. White flour showed much higher peak, trough, breakdown, final, and setback viscosity values, while a lower pasting temperature compared to whole wheat flour. Furthermore, a strong correlation was observed for the peak and breakdown viscosities between white and whole wheat flour. There was a weak correlation for specific volume between bread made from white and whole wheat flour. Among all parameters examined, protein content and water absorption were strongly correlated with the specific volume for both white and whole wheat flours. Compared to white bread, whole wheat bread had higher values of hardness, gumminess, and chewiness. This comparative evaluation of the dough rheological properties and bread-making performance of WF and WWF from 64 wheat genotypes offers valuable insights for the baking industry, enabling the prediction of the bread-making performance of whole wheat flour.

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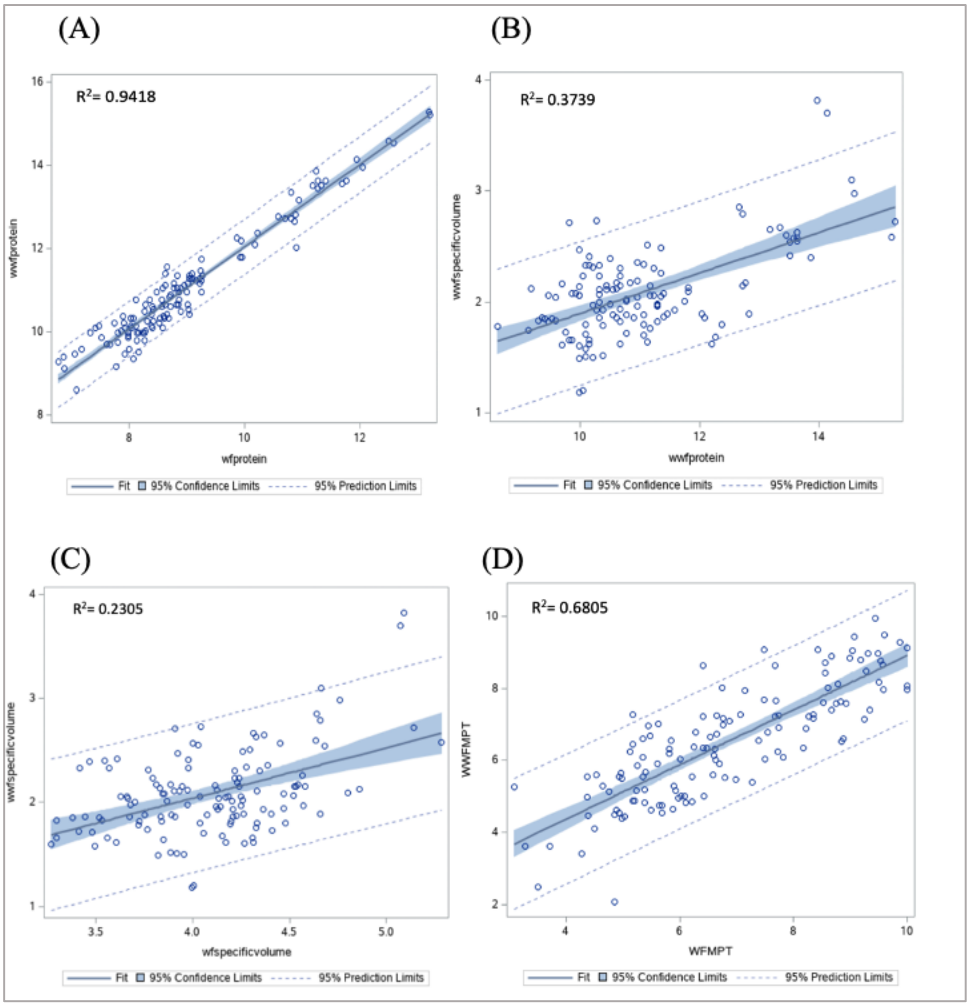

Figure 3.1. The relationship between: (A) white flour (WF) protein and whole wheat flour (WWF) protein, (B) WWF protein and WWF bread specific volume, (C) WF bread specific volume and WWF bread specific volume, (D) dough development time (MPT) of WF and WWF.

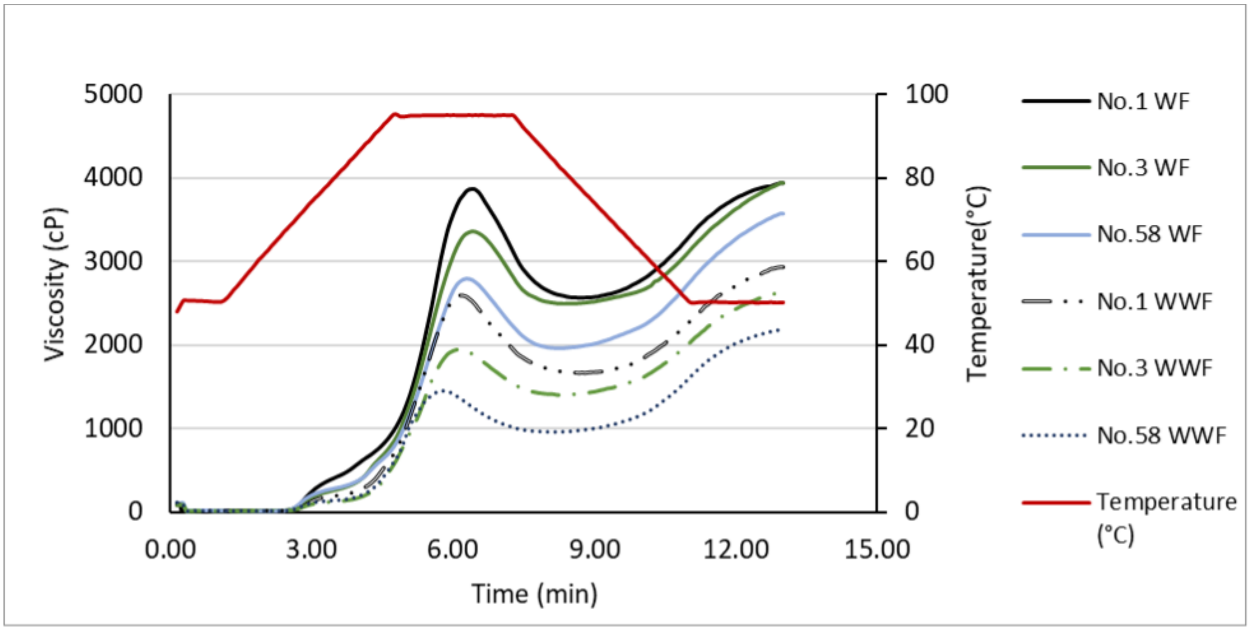

Figure 3.2. RVA curve of selected white flour (WF) and whole wheat flour (WWF).

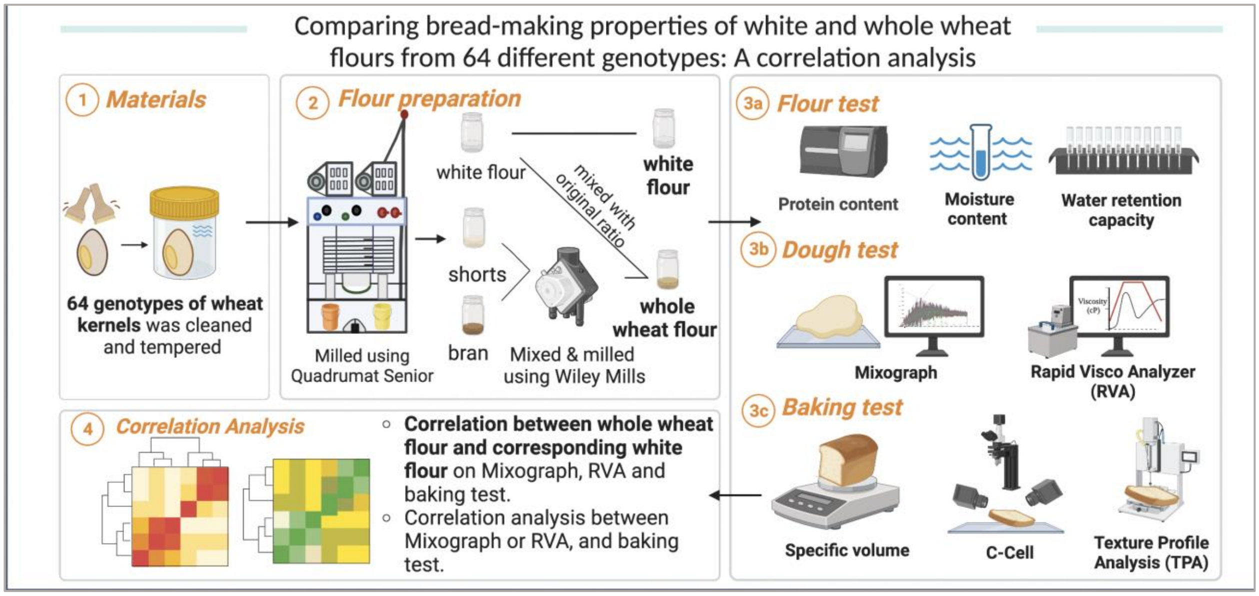

Figure 3.3. Graphic abstract.

Table 3.1. Physicochemical properties of white flour (WF) and whole wheat flour (WWF).

	Variable	Mean	Std Dev	Minimum	Maximum	LSD	MSE
Protein	WF protein (%)	8.99	1.35	6.77	13.22	0.256	0.016
	WWF protein (%)	11.05	1.37	8.61	15.28	0.237	0.782
Moisture	WF moisture (%)	13.78	0.28	13.27	14.45	0.238	0.014
	WWF moisture (%)	12.04	0.63	10.35	14.00	0.266	0.018
WRC	WF WRC (%)	56.07	4.28	50.09	68.43	1.314	0.432
	WWF WRC (%)	62.34	1.70	56.96	66.42	1.390	0.469
Mixograph	WF absorption (%)	57.35	2.05	53.80	63.90	0.997	0.249
	WWF absorption (%)	60.24	2.24	56.00	67.00	1.370	0.470
	WF MPT (min)	6.80	1.72	3.09	10.00	1.513	0.573
	WWF MPT (min)	6.49	1.58	2.08	9.93	0.640	0.103
	WF PVE (%)	32.28	5.06	21.87	51.96	7.213	13.038
	WWF PVE (%)	28.05	3.93	20.33	40.80	1.434	0.516
	WF CTI (%)	249.18	40.95	165.61	411.30	57.750	835.67
	WWF CTI (%)	211.34	32.40	137.82	303.16	13.340	44.581
	WF Breakdown (%)	6.35	4.40	-0.07	17.44	7.018	12.341
	WWF Breakdown (%)	12.27	10.14	0.06	32.07	3.974	3.958
	WF slope (%)	3.54	3.38	-0.61	19.12	5.259	6.930
	WWF slope (%)	4.31	2.59	0.15	18.25	3.526	3.115
RVA	WF PV (cP)	3342.0	281.97	2731.00	3886.00	66.190	1103.1
	WWF PV (cP)	1985.0	244.11	1471.00	2594.00	101.1	2561.8
	WF trough (cP)	2400.0	241.99	1881.00	3035.00	131.80	4373.6
	WWF trough (cP)	1359.0	139.87	965.00	1662.00	91.8	2144.2
	WF BKD (cP)	942.42	231.91	577.00	1548.00	135.90	4652.6
	WWF BKD (cP)	626.13	159.32	388.00	1005.00	65.8	1085.8
	WF FV (cP)	3943.0	267.22	3432.00	4583.00	94.60	2255.5
	WWF FV (cP)	2730.0	191.39	2199.00	3115.00	105.1	2769.6
	WF Setback (cP)	1543.0	109.36	1288.00	1868.00	76.020	1455.3

	WWF Setback (cP)	1372.0	103.59	1177.00	1633.00	63.7	1016.0
	WF PKT (min)	6.37	0.15	6.00	6.73	0.154	0
	WWF PKT (min)	6.00	0.13	5.73	6.27	0.094	0.002
	WF TEMP (°C)	69.30	0.72	67.60	72.55	0.991	0.2
	WWF TEMP (°C)	82.76	7.73	70.10	89.65	0.893	0.200
Bread	WF specific volume (cm^3/g)	4.10	0.41	3.27	5.29	0.139	0.005
	WWF specific volume (cm^3/g)	2.09	0.41	1.18	3.82	0.101	0.003
	WF cells /area (mm^{-2})	0.90	0.038	0.800	0.970	0.107	0.003
	WWF cells/area (mm^{-2})	0.86	0.07	0.71	1.01	0.064	0.002
	WF cells area (%)	49.63	0.77	48.20	51.97	0.774	0.308
	WWF cells area (%)	49.17	0.70	47.31	50.78	0.638	0.209
	WF wall thickness (mm)	0.40	0.01	0.38	0.46	0.014	0.000
	WWF wall thickness (mm)	0.41	0.05	0.38	0.93	0.014	0
	WF cell diameter (mm)	1.51	0.09	1.34	1.80	0.096	0.005
	WWF cell diameter (mm)	1.55	0.11	1.31	1.84	0.099	0.005
	WF hardness (g)	685.34	146.93	374.40	1247.00	67.31	3496.50
	WWF hardness (g)	2227	1033	688	6647	193	28822
	WF springiness (g)	105.54	13.66	93.60	155.43	11.18	96.44
	WWF springiness (g)	87.30	4.92	73.85	99.90	2.886	6.428
	WF gumminess	408.82	71.93	257.34	689.68	38.48	1142.71
	WWF gumminess	1138	403.64	529.81	2909.00	87.6	5927.071
	WF chewiness	431.62	99.79	273.56	824.58	56.78	2488.11
	WWF chewiness	985.92	330.29	499.03	2394.00	86.3	5744.948

*WF, white flour; WWF, whole wheat flour; Std Dev, standard deviation; LSD, least significant difference; MSE, mean square error. WRC, water retention capacity (%); MPT, dough development time (Min); PVE, peak value (%); CTI, curve tail integral (%Tq*Min); PV, peak viscosity (cP); Trough, Trough viscosity (cP); BKD, Breakdown viscosity; FV, final viscosity (cP); Setback, setback viscosity (cP); PKT, peak time (Min); TEMP, Pasting temperature (°C); Cells/area, total number of cells per area.

Table 3.2. Correlation coefficients between white flour (WF) and whole wheat flour (WWF) on Mixograph and RVA pasting characteristics).

WF						WWF								
Trough	BKD	FV	Setback	PKT	TEMP	PV	trough	BKD	FV	Setback	PKT	TEMP		
0.618*	0.571*	0.434*	-0.306	0.132	0.307	0.851*	0.727*	0.665*	0.523*	-0.016	0.278	-0.122		PV
	-0.293	0.912*	0.017	0.770*	0.272	0.354*	0.699*	-0.072	0.679*	0.31	0.646*	0.447*		Trough
		-0.424*	-0.389*	-0.643*	0.09	0.665*	0.155	0.883*	-0.072	-0.343*	-0.336	-0.614*	WF	BKD
			0.424*	0.656*	0.053	0.138	0.569*	-0.289	0.698*	0.521*	0.537*	0.443*		FV
		WF protein		-0.101	-0.474*	-0.446*	-0.157	-0.546*	0.204	0.588*	-0.117	0.095		Setback
	WWF protein	0.977*			0.306	-0.04	0.338*	-0.359*	0.374*	0.235	0.677*	0.637*		PKT
	Absorption	0.972*				0.194	0.23	0.096	0.042	-0.233	0.231	0.075		TEMP
	MPT	-0.431*	-0.455*				0.787*	0.841*	0.572*	-0.007	0.246	-0.247		PV
	PV	0.768*	0.672*	-0.422*				0.329	0.849*	0.219	0.621*	0.122		Trough
WF	CTI	0.703*	0.611*	-0.485*	0.957*				0.131	-0.202	-0.168	-0.486*	W WF	BKD
	Breakdown	0.277	0.318	-0.714*	0.189	0.204				0.701*	0.458*	0.228		FV
	slope	0.539*	0.524*	-0.354*	0.389*	0.298	0.459*				0.008	0.257		Setback
	Absorption	0.740*	0.713*	-0.433*	0.510*	0.498*	0.239	0.371				0.440*		PKT
	MPT	-0.305	-0.323	0.825*	-0.352*	-0.371*	-0.646*	-0.232	-0.177					
WWF	PV	0.471*	0.721*	-0.215	0.591*	0.506*	0.163	0.331	0.237	-0.281				
	CTI	0.252	0.419*	0.123	0.341	0.281	-0.084	0.113	-0.044	0.042	0.839*			
	Breakdown	0.333	0.392*	-0.729*	0.432	0.459*	0.551*	0.232	0.301	-0.801*	0.261	-0.181		
	slope	0.402*	0.509*	-0.434*	0.459	0.397*	0.31	0.500*	0.275	-0.497*	0.495*	0.247		
			WF						WWF					
		WWF protein	Absorption	MPT	PV	CTI	Breakdown	slope	Absorption	MPT	PV	CTI		

MPT, Midline peak time (Min); PVE, peak value (%); CTI, curve tail integral (%Tq*Min). PV, peak viscosity (cP); Trough, Trough viscosity (cP); BKD, Breakdown viscosity; FV, final viscosity (cP); Setback, setback viscosity (cP); PKT, peak time (Min); TEMP, Pasting temperature (°C). *Significant at p ≤ 0.0001.

Table 3.3. Correlation coefficients between specific volume of bread made with white flour (WF) and whole wheat flour (WWF) and corresponding protein contents and selected rheological properties.

	WF specific volume
WF protein	0.660*
WF water absorption	0.632*
WF PVE	0.510*
WF CTI	0.437*
WF hardness	-0.683*
WF springiness	0.089
WF gumminess	-0.523*
WF chewiness	-0.364
WWF specific volume	0.480*
WWF protein	0.611*
WWF water absorption	0.570*
WWF PVE	0.350
WWF CTI	0.166
WWF hardness	-0.742*
WWF springiness	0.560*
WWF gumminess	-0.760*
WWF chewiness	-0.732*
	WWF specific volume

PVE, peak value (%); CTI, curve tail integral (%Tq*Min). *Significant at $p \leq 0.0001$.

Table 3.4. Correlation coefficients between textural properties of bread made with white flour (WF) and whole wheat flour (WWF) and corresponding rheological properties and crumb cell properties.

	Water absorption	MPT	PVE	CTI	PV	Setback	Cell area (%)
WF hardness	-0.490*	0.047	-0.582 *	-0.554 *	-0.287*	0.417*	-0.473*
WF springiness	0.177	-0.071	-0.086	-0.084	-0.018	-0.016	0.348*
WF gumminess	-0.298	0.016	-0.490 *	-0.463 *	-0.284	0.405*	-0.374*
WF chewiness	-0.162	0.005	-0.400 *	-0.383 *	-0.237	0.302	-0.036
WWF hardness	-0.399*	-0.065	-0.243	-0.138	-0.340*	-0.021	-0.497*
WWF springiness	0.385*	-0.048	0.044	-0.082	0.106	-0.075	0.104
WWF gumminess	-0.384*	-0.055	-0.235	-0.129	-0.358*	0.019	-0.521*
WWF chewiness	-0.357*	-0.061	-0.250	-0.151	-0.363*	0.021	-0.506*

MPT, dough development time (Min); PVE, peak value (%); CTI, curve tail integral (%Tq*Min); PV, peak viscosity (cP); Setback, setback viscosity (cP). *Significant at $p \leq 0.0001$.

Chapter 4 - Unraveling the influence of wheat bran chemical composition, lipolytic enzyme activities, and phenolic components on the bread-making properties of reconstituted whole wheat flours[1]

Abstract

With the complex nature of wheat bran, understanding of the impact of bran chemistry and composition on the bread qualities are lacking. The present study aimed to explore the relationship between bran composition and functionality and its effect on the bread-making properties of reconstituted whole wheat flours (WWF). Ten different wheat genotypes' bran were mixed with a control white flour to form ten different reconstituted WWF (bran/flour=15/85). Results revealed that the lipid content of bran was positively correlated with mixing peak time (MPT) and water absorption, but negatively associated to water retention capacities, antioxidant capacity, as well as some Mixograph properties. A significantly negative correlation was observed between the MPT and ABTS scavenging capacity (r = -0.729), and between bran lipoxygenase activity and bread volume (r = -0.804). Our findings provide valuable insights for the baking industry to assess the quality of whole wheat products.

Keywords: Wheat bran; whole wheat bread; Mixograph; bran enzyme; lipoxygenase

[1] Li, Cheng, Wei Wu, Michael Tilley, Richard Chen, and Yonghui Li. " Unraveling the Influence of Wheat Bran Chemical Composition, Lipolytic Enzyme Activities, and Phenolic Components on the Bread-making Properties of Reconstituted Whole Wheat Flours." Under Review.

4.1. Introduction

Wheat bran constitutes up to 20% of total wheat grain, is rich in soluble and insoluble dietary fibers and bioactive compounds, making it a popular ingredient in bread formulations for those seeking health benefits. Recent studies have shown that wheat bran contains high levels of phytic acid and dietary fiber that can facilitate removal of toxins from the digestive system and reduce the incidence of colorectal cancer (Alkandari et al., 2021; Jimenez-Pulido et al., 2022). Additionally, wheat bran has been shown to play a significant function in regulating the cellular oxidative state, preventing inflammation, and may offer protection against obesity, diabetes, hypertension, and gastrointestinal diseases (Fardet, 2010).

While wheat bran offers numerous health benefits, its incorporation into bread-making results in bread of inferior quality, including low volume, bitter flavor, and poor crumb structure. These negative effects are caused by the composition and physicochemical characteristics of wheat bran, such as fibers, phenolic compounds, enzymes, and their interactions with flour constitutes affect the dough gluten proteins network (Pietiäinen, Moldin, Ström, Malmberg, & Langton, 2022). The physiochemical characteristics of wheat bran significantly impact flour, dough, and baking performance. Specifically, the active components of wheat bran could adversely impact the solubility and retrogradation of starch. Protein and polyphenols of wheat bran have a favorable impact on the dough viscoelastic properties when combining various brans with single refined flour (Navrotskyi et al., 2019). The water retention capacity (WRC) of bran is an important indicator of its quality, with less WRC implying better performance of the bread product. In addition, major bran components such as lipids, water extractables, hydrolysable phenolics, and fiber, also play important roles in breadmaking (Khalid, Ohm, & Simsek, 2017). The bran fibers tend to reduce bread volume and negatively impact texture, as they compete with gluten for water and weaken the dough (Zhou et al., 2021). In conjunction with the deterioration of dough consistency and baking performance of whole-wheat bread, the proteins' higher solubility in the crumb with bran was reflective of the bran components' interaction and degradative effects on protein’s functionality.(Khalid et al., 2017).

The antioxidant capabilities of wheat bran is primarily driven by the presence of 8 phenolic acids, including ferulic acid, chlorogenic acid, *p*-coumaric acid, caffeic acid, vanillic acid, gallic acid, quercetin, syringic acid, etc. (L. Zhang et al., 2023). During bread making, the phenolic compounds alter gluten network formation by interfering with disulfide-sulfhydryl interchange reactions between gluten proteins, (Girard & Awika, 2020) which may reduce the aggregation of high-molecular-weight gluten while increasing the extractable proteins (Han & Koh, 2011) reducing bread quality. Previous studies have demonstrated that double-bond containing compounds such as ferulic acid and fumaric acid reduce dough mixing stability but enhance yeast activity (Su et al., 2019). Moreover, the presence of enzymes, particularly lipase, lipoxygenase (LOX), and peroxidase, in wheat bran affects the quality of whole wheat flour and finished baked goods (Poudel & Rose, 2018). This includes the LOX produces active peroxides that oxidation of the free thiol groups in glutenin, enhancing the development of gluten macropolymers (Pourmohammadi & Abedi, 2021). However, the aforementioned enzymes can pose storage challenges and affect the flavor of products, as they catalyze the hydro-peroxidation of polyunsaturated fatty acids, causing off-flavors (Poudel & Rose, 2018).

The utilization of wheat bran as a prominent nutritional ingredient and in whole wheat flour holds great potential. However, the influence of wheat bran functionality and composition on rheological characteristics of the dough and bread performance remains unclear and requires further research. Therefore, the goals of the present research were to determine the physicochemical properties, including phenolics, antioxidants, and oxidation-related enzymes characteristics such as lipase, lipoxygenase, and peroxidase using various wheat brans; and to investigate the relationship between phenolic compositions, lipolytic activities in wheat bran and their effect on the qualities of reconstituted hard whole wheat flour dough and bread-making.

4.2. Materials and methods

4.2.1. Materials and chemicals

Ten hard red winter wheat lines (named A-J) were provided from Kansas (U.S.) area. Wheat kernels were tempered to 15% moisture and milled using a Quadrumat Senior laboratory mill (CW Brabender Instruments, NJ, U.S.) to produce bran (containing shorts) and flour, following the AACC Method 26-50.01 (AACC,2010). The yield of the straight-grade flour was between 65-70%. The obtained bran was milled additionally using a Perten laboratory mill 3303 (Perkin-Elmer, MA, U.S.) for particle size reduction. Reconstituted whole wheat flour (WWF) was obtained by mixing each of the re-ground bran with a control commercial white flour (protein content 11.14%) at a ratio of 15 to 85. The process of WWF preparation is outlined in **Figure S1** (Supplementary Document). General chemicals, UPLC grade solutions and analytical standards were purchased from Fisher Scientific (Hampton, NH, U.S.) or Sigma Aldrich Chemistry (MO, U.S.).

4.2.2. Chemical composition

Protein content of the re-ground wheat bran was measured using a nitrogen determinator (LECO Corp., MI, U.S.) with a factor of 5.7, as described by the AACC Method 46-30 (AACC, 2010). The moisture content was quantified using an air-oven, as per the AACC Methods 44–15.02 (AACC, 2010). The crude lipid content was measured through ethyl ether extraction of the total lipids. The total, soluble, insoluble fiber of reground wheat bran were determined using AACC method 32-07.01 (AACC, 2010).

4.2.3. Bran hydration properties

The water retention capacity of the reground bran was evaluated according to the AACC method 56-11.02 (AACC, 2010). Briefly, 2.0g of wheat bran (W1) was soaked in 10mL of deionized water for 20 min while being stirred at intervals of 5, 10, 15, and 20 min, and then centrifuged, a 10-min draining step was performed. The water retention capacity was calculated and expressed as a percentage based on 14% moisture content.

4.2.4. Extraction of bran enzymes

The extraction of bran enzymes was conducted as described in the literature (Gökmen, Serpen, Atli, & Köksel, 2007). Briefly, 1 g of bran sample was mixed with 20 mL of 0.1 M sodium phosphate buffer in a tube at room temperature. The mixture was then centrifuged to obtain crude extract of bran enzymes, which was used for further analysis.

4.2.5 Measurement of lipase, peroxidase (POD) and lipoxygenase (LOX) activities in the wheat bran extract

Lipase activity was measured as described (Piechowiak, Józefczyk, & Balawejder, 2018) using canola oil instead of vegetable oil. The lipase activity was expressed in units per gram (U/g), where U was defined as the number of fatty acids produced after the 1-minute fat hydrolysis per gram of the bran sample. The LOX activity was measured as reported previously (Navrotskyi et al., 2019). The POD activity was measured as described (De Almeida, Pareyt, Gerits, & Delcour,

2014) in a mixture of the bran extract (50 μL), ABTS (9.1mM, 2.9 mL), and 0.3% (w/w) hydrogen peroxide (0.1mL) were added to a tube. The absorbance was measured at 405 nm. The enzyme activity was expressed as U/g, where U of LOX and POD was defined as the rate of rise in absorbance at 234nm and 405 nm, separately. All enzymatic activities were carried out from two extractions with replicated assays per extract.

4.2.6. Extraction of soluble wheat bran phenolics

The soluble phenolic compounds in wheat bran were extracted according to methods as described (Okarter, Liu, Sorrells, & Liu, 2010) with a few modifications. Briefly, wheat bran was extracted twice using 80% acetone with a ratio of 1:5. Subsequently, the solvent evaporated to 2 mL. The resulting solution was refilled to 5 mL with HPLC-grade methanol, followed by filtration through a 0.45 μm filter, and stored at a temperature of −20 °C. The solution was used to measure total phenolic content and antioxidant activities within 3 days.

4.2.7. Total phenolic content assay of wheat bran extract

The total phenolic content (TPC) of the soluble extract was assessed (Singleton & Rossi, 1965). wheat bran extract (100 μL) was combined with deionized water (7.9 mL) before being added to 500 μL of Folin-Ciocalteu reagent. After 5 min of 20% sodium carbonate solution (1.5 mL) was added and the absorbance at 765 nm was recorded after two hours. The TPC (μg GAE/ mg) was indicated as microgram gallic acid equivalence (GAE) per milligram of the wheat bran sample.

4.2.8. Antioxidant ABTS radical scavenging assay

The radical cation ABTS $^{\bullet+}$ scavenging activity of the wheat bran extract was determined following a previous procedure (Re et al., 1999) with some modification. Under dark conditions, 3.9 mL of 0.13 mM 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) solution was reacted with 50 µL bran extract for 10 minutes. The absorbance was then recorded at 734 nm.

4.2.9. Extraction of bound phenolics

The bound phenolics in wheat bran samples were extracted according to a previous method (Tian, Chen, Tilley, & Li, 2021) with some modification. Briefly, to extract insoluble-bound phenolics, the residue from the previous extraction of soluble phenolics was digested with 2 M sodium hydroxide at room temperature for 3 hours with shaking under nitrogen. The mixture was then acidified to pH 2 with 6 M hydrochloric acid and extracted with hexane to remove lipids. The organic phases were separated, dried, and reconstituted to a final volume of 2 mL UPLC grade methanol.

4.2.10. UPLC phenolic acid composition analysis

The insoluble phenolic acid compositions in the wheat bran extract were analyzed using a UPLC-DAD-ESI-Q-TOF-MS/MS system equipped with a CORTECS UPLC C18 column (Waters Corporation, MA, USA). The mobile phase consisted of water containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). The programmed run using a constant flow rate of 0.4 mL/min with the solvent A: 91% in first 72 s, 91 to 84% A in 120 s, 84 to 81% in 102 s, 81 to 79% in 86 s, 79% in 60 s. The peaks of the components were identified at 280 nm. The individual phenolic compounds were quantified by comparing the detected area of the peaks to calibration curves determined using simple phenolic acids.

4.2.11. Mixograph analysis

The mixing properties of the flours were evaluated in duplicate utilizing a Mixograph (National Manufacturing, NE, U.S.) with 10 g bowl following AACC method 54-40 (AACC, 2010). The water absorption (%), dough development time (MPT, time to peak), peak value (PV), curve tail integral (CTI), breakdown, and slope were collected.

4.2.12. Bread baking and loaf volume measurement

Breads with the reconstituted whole wheat flour were produced in duplicate following the AACC Approved Method 10–10.03 (AACC, 2010). The bread formulation and procedure were modified according to our previous study (C. Li et al., 2023). After 2 hours of cooling, volume of bread was analyzed and recorded according to AACC Approved Method 10-05.01 (AACC, 2010).

4.2.13. Statistical analysis

Analyses were conducted at least duplicated by SAS Studio (SAS Institute Inc., Cary, NC, U.S.). The significant differences were presented among the values using one-way analysis of variance followed by Duncan's significant difference test at 95% of confidence level.

4.3. Results and discussion

4.3.1. Proximate composition and hydration properties of wheat bran

The proximate composition of the wheat bran is presented in **Table 1.** The protein content of the samples ranged from 9.95% (sample J) to 15.25 % (sample A), while the crude lipid content ranged from 4.90% (sample J) to 6.97% (sample D). Sample J had the lowest values of both protein and lipid contents among all the samples. A previous report showed that the wheat bran not only has a relatively high (approximately 15.5%) protein content, but also has a more balanced amino acid profile compared to endosperm protein (Alzuwaid, Sissons, Laddomada, & Fellows, 2020) The incorporation of bran into wheat flour tends to result in higher content of proteins, lipids, and minerals (C. Li et al., 2023).

The dietary fiber in wheat bran is a key characteristic that distinguished it from refined wheat flour and provides potential health-promoting benefits. The dietary fibers are resistant to digestion but can be fermented by gut microbiota. Dietary fiber is classified into soluble (SDF) and insoluble dietary fiber (IDF) by its solubility in water. The SDF, IDF values of the bran are presented (**Table 1)**. Sample F had the highest IDF content (40.71%), and sample B had the lowest IDF (28.73%). There was no statistically significant difference among the ten selected wheat samples in IDF and SDF. A previous study showed that wheat bran contains various dietary fibers, such as cellulose, arabinoxylan, beta-glucan, resistant starch and others (Bach Knudsen, Nørskov, Bolvig, Hedemann, & Laerke, 2017). Additionally, arabinoxylan which belongs to SDF is the predominant component of dietary fiber, accounting for nearly 50% of the total dietary fiber (J. Liu, Yu, & Wu, 2020).

The hydration properties of the brans are shown in **Table. 1**. The water retention capacities of the brans varied from 196.2 to 247.5%. Notably, Sample J, which had the lowest protein and lipid content, had the highest water retention capacity. This may be attributed to its relatively higher amount of total carbohydrates, which may contribute to the higher water retention capacity, as carbohydrates are known to absorb more water. The variations in the bran water retention capacity can be attributed to the distinctive characteristics of the samples. Bran has pores of varying sizes from nanometers to micrometers, thus the absorbing and trapping of water in these capillaries is crucial to water retention. Microscopy assessment revealed that the grinding process of bran caused the tissues to disintegrate and sperate, leading to the loss of micropores and altering the water retention capacity (Jacobs, Hemdane, Dornez, Delcour, & Courtin, 2015). Previous research determined that the water retention capacity for coarse (783 μm) and fine (463 μm) particle sized bran ranged from 168 to 268.4% and 198.5 to 297.7%, respectively (Navrotskyi et al., 2019) Wheat bran tended to have much higher water retention capacity compared to refined flour, which can be partially attributed to its higher arabinoxylan and fiber content (Kaur et al., 2019).

4.3.2. Enzymatic activities of wheat bran extract

The enzymatic activities of lipase, lipoxygenase (LOX) and peroxidase (POD) in wheat bran are shown in Table 2. The lipase activity of the samples ranged from 2.08 (sample C) to 2.38 U/g (sample H) with no significant difference between the samples. Previously, the lipase activity in wheat bran has been found to range from 0.6 to 2.6 U/g, which was substantially connected to the buildup of the free fatty acids and could be used to assess the shelf-life (Rose & Pike, 2006). The highest POD values were observed in samples C, F, G, and I (576.8-622.8 U/g), while sample E (310.2 U/g) had the lowest POD value. Previous research found that wheat bran can have up to 509.22 U/g of POD, and the activity can be significantly reduced through conventional heating

(Habuš et al., 2021). Regarding LOX activity, sample I and J had the highest values (98.5 and 98.2 U/g), while sample F had the lowest value (72.7 U/g). A previous study found that the lipoxygenase activity ranged from 11.1 to 143.1 U/g by analyzing 79 wheat bran samples (Navrotskyi et al., 2019). These findings demonstrate obvious variations in the bran LOX and POD activities from different wheat genotypes.

Wheat lipase, primarily found in the bran component, can lead to hydrolytic rancidity in flour. The volatile compounds resulting from lipid hydrolysis and oxidation can cause the flour to have a musty and earthy odor, leading to decreased sensory quality. Furthermore, the polyunsaturated fatty acids are catalyzed by lipoxygenase, resulting in oxidation products that deteriorate the end-use products (Galliard & Gallagher, 1988). As a result of its interaction with polyunsaturated fatty acids, LOX also imparts an off-flavor and off-odor to the flour (Shahidi & Hossain, 2022).

Similar to lipase, LOX and POD are also substantially concentrated in the bran (Rani et al., 2001). These enzymes may contribute to the decline of initial antioxidants during storage or in wheat dough or to the degradation of carotenoids throughout the dough and breadmaking process (Žilić et al., 2010). Due to the negative impact of these enzymatic activities, appropriate strategies for inactivating the enzyme activities could be considered, such as steam treatment (Guo, Wu, & Zhu, 2020) or microwave treatment (M. Li, Sun, & Zhu, 2017).

4.3.3. Total phenolic content (TPC) and ABTS $^{\bullet+}$ free radical scavenging activity of bran soluble extracts

The total phenolic content (TPC) of the bran samples, represented as gallic acid equivalents, was analyzed and compared (Table 2). The TPC among bran extracts ranged from

2.481 (Sample A) to 3.048 mg/g (Sample H) of bran. Variations in the capacity to quench ABTS radicals were observed among the bran samples (Table 2). Sample G had the strongest antioxidant activity based to the lowest %ABTS remaining value, followed by sample A, E, and J. There was no correlation between the TPC and ABTS scavenging activity from this study. Overall, this was consistent with earlier studies (Ghamry, Zhao, & Li, 2023; Liangli Yu et al., 2002; Liangli Yu, Perret, Harris, Wilson, & Haley, 2003). While phenolics are considered the primary contributor to the antioxidant properties in wheat bran, other factors such as enzymes, peptides, free amino acids, aromatic amines, and sulfur containing compounds, may also play a significant role. More thorough investigation is needed to explore the underlying physiological mechanisms of the bran antioxidants.

4.3.4. Wheat bran profile for insoluble phenolic acids

Figure 1 shows the typical chromatograms of phenolic acid standards (A), phenolic components in insoluble fraction of wheat bran (B), and MS spectra of the insoluble fraction in wheat bran with peak assignments at 1.874 min (C) and 2.490 min (D). Insoluble fraction of wheat bran contained predominantly *p*-coumaric acid and *trans*-ferulic acid (**Figure 2**). In the insoluble fractions, *Trans*-ferulic acid concentration ranged from 1437.61 to 1902.99 µg/g (**Figure 2**). *p*-Coumaric acid was likewise relatively rich in the insoluble fraction of wheat bran, with a concentration range of 36.64 to 81.83 µg/g. These findings were expected as previous research also showed that the predominant phenolic components in cereals are found in the insoluble fraction, and *trans*-ferulic acid is the most prevalent phenolic acid in wheat, makes up more than 90% of all phenolic acids (Tian et al., 2021). Bound polyphenol compounds in wheat bran were linked through ester or ether bonds to the pericarp and aleurone layer cell walls. The lignocellulosic fiber of bran can be effectively broken down through thermal process, which increases the bound phenolic compounds to be extracted (Y. Li, Li, Wang, & Li, 2022).

4.3.5. Mixing properties of reconstituted whole wheat flour

Assessing the mixing properties is crucial for determining flour quality. Thus, it is necessary to study the reconstituted whole wheat flours with different wheat bran. The Mixograph characteristics of the refined flour and reconstituted whole wheat flour are presented (**Table 3**) and Mixograms of reconstituted whole wheat flour are shown in Figure S2. Sample H had the highest water absorption (64.5%), while sample I and J had the lowest (61.5%). We did not observe an obvious correlation between the water absorption and the water retention capacity or dietary fiber (**Table 4**). A previous study showed that fibers in reconstituted whole wheat flour can interfere with hydration of protein and starch, weakening the gluten structure in dough (Zhou et al., 2021). However, a negative correlation (r = -0.729) existed between the mixing peak time (MPT) and ABTS scavenging ability (**Table 4**). Antioxidants can influence the aggregation of gluten proteins by impacting disulfide-sulfhydryl exchange reactions (Huang et al., 2018). Phenolic acids make up the majority of the antioxidant compounds, and adding various phenolic acids has been shown to shorten mixing times and increase dough tolerance to shearing (Han & Koh, 2011). We observed that the optimum mixing time for whole wheat flour was similar to or longer than that of the white flour. Additionally, the curve tail integral and slope values between white and whole wheat flour showed no significant difference. Sample J had the lowest protein content among the samples and the shortest optimum mixing time and relatively low peak value. It appears that brans deteriorate flour mixing capacities, as previously reported. Polyphenols and protein contents exhibited favorable and significant association with the characteristics of the dough (Navrotskyi et al., 2019).

4.3.6. Bread volume and correlation analysis between bran characteristics and dough or baking quality

The volume of bread made with the reconstituted whole wheat flour with 15% difference brans is presented in **Table 3**. Compared to the loaf volume of control bread (665 cm^3), sample A, F, G had relatively larger volumes (640.0-652.5 cm^3), while sample I and J had the lowest volumes (590.0-602.5 cm^3), which appeared also have the lowest bran protein content 9.95-10.0% compared to others. However, the Pearson correlation coeeficient between bran protein and loaf volume was only 0.278, implying very weak or nearly no corelation (**Table 4).** Similar results were reported previously, showing that using whole wheat flour with a higher protein concentration did not always result in a larger bread loaf volume (Li, Tilley, Chen, Siliveru, & Li, 2023).

The Pearson correlation between various properties of wheat bran, dough, and bread volume is presented in **Table 4**. The lipid concentration of the wheat bran was negatively correlated with the water retention capacity, antioxidant capacity, mixing peak value, curve tail integral, breakdown, and slope, but has a positively correlation with the dough peak mixing time and water absorption. On the other hand, the major characteristic that was related to bread volume was lipoxygenase activity, with a significant negative correlation (r = -0.804), as shown in **Table 4**. The bread volume was also weakly correlated with water absorption (r = 0.483). A prior study found a weak association between LOX and baking parameters (Every, Simmons, & Ross, 2006). However, the elevated levels of ash, glutathione, and protein thiols in those fractions with increased LOX were found to have a negative impact, and these strong capacity for reducing ingredients may lead to poor-quality bread (Jacobs, Bogaerts, Hemdane, Delcour, & Courtin, 2016). Despite this, adding recombinant LOX (20 U/g) to the dough was shown to increase mixing stability time and improve loaf volume, while adding 10 U/g or 30 U/g LOX resulted in small loaf volume (C. Zhang et al., 2013). Other researchers, however, reported no significant relationship between LOX and bread volume (Navrotskyi et al., 2019). It is possible that several other factors, including wheat variety, protein and bran composition, flour characteristics, testing method, and baking procedures, play an integral role in how LOX affects bread quality. Further research is suggested to better comprehend the underlying mechanisms as well as the relationships between LOX and other flour components during baking.

4.4. Conclusion

In this study, the influence of bran composition on dough and baking characteristics of whole wheat flour was investigated by reconstituting different bran samples with single refined flour. In bound phenolic compounds in wheat bran, the greatest proportions of phenolic acids were represented by trans-ferulic acid and the second was *p*-coumaric acid. The lipid content of the wheat bran was negatively correlated with the water retention capacity, antioxidant capacity, mixing peak value, curve tail integral, breakdown, and slope, but positively correlated with the dough development time and water absorption. An inverse relationship (r = -0.729) between the mixing peak time and ABTS scavenging ability was also identified. The bread volume was significantly negatively correlated with lipoxygenase activity (r = -0.804). Although our findings suggest that lower concentration of lipoxygenase activity results in larger bread volume, this relationship is based on correlation and does not establish causation. Further studies are necessary to comprehend the mechanisms of lipoxygenase activity impact the breadmaking. These findings contribute to the whole wheat flour quality assessment and effective industrial application.

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Table 4.1. Protein, crude lipid, fiber, and water retention capacity (%) of wheat brans from different genotypes (A-J).

Sample	Protein (%)	Crude lipid (%)	IDF (%)	SDF (%)	WRC (%)
A	15.25±0.04 a	5.48 ± 0.03 cd	33.92 ± 0.03 a	1.48 ±0.16 a	219.42 ± 18.50 ab
B	14.56±0.04 a	6.35 ± 0.16 abc	28.73 ± 0.42 a	1.00 ± 0.32 a	196.21 ± 2.58 b
C	13.68±0.24 b	6.05 ± 0.26 bc	35.64 ± 0.76 a	1.17 ± 0.10 a	217.73 ± 3.15 ab
D	12.23±0.04 c	6.97± 0.07 a	32.35 ± 0.43 a	1.80 ± 0.41 a	200.54 ± 2.20b
E	11.80±0.00 c	5.13 ± 0.16 d	30.13 ± 2.68 a	1.63 ± 0.83 a	216.13 ± 12.09 ab
F	10.86±0.28 d	5.13 ± 0.23 d	40.71 ± 1.56 a	1.58 ± 0.10 a	216.62 ± 4.56 ab
G	10.29±0.12 de	6.05 ± 0.14 bc	35.54 ±0.65 a	1.34 ± 0.03 a	221.28 ± 2.53 ab
H	10.12±0.04 e	6.53 ± 0.14 ab	35.61 ± 1.73 a	1.49 ± 0.31 a	218.00 ± 2.27 ab
I	10.00±0.04 e	6.24 ± 0.14 abc	32.21 ± 5.39 a	1.13 ± 0.13 a	198.18 ± 0.75 b
J	9.95±0.04 e	4.90 ± 0.13 d	35.45 ± 4.68 a	1.48 ± 0.57 a	247.54 ± 4.55 a

IDF, insoluble fiber; SDF, soluble fiber; WRC, water retention capacity. Samples with different letters differ significantly ($p < 0.05$).

Table 4.2. Lipase, peroxidase (POD), lipoxygenase (LOX) activities, total phenolic contents (TPC), and ABTS antioxidant activities of wheat bran from different genotypes (A-J).

Sample	Lipase activity (U/g)	POD (U/g)	LOX (U/g)	TPC (ug GAE/mg bran)	ABTS remaining (%)
A	2.25 ± 0.10 a	474.0 ± 11.6 b	74.6 ± 0.0 c	2.481 ± 0.017 d	44.78 ± 0.49 d
B	2.13 ± 0.02 a	432.6 ± 13.4 bc	88.6 ± 1.4 b	2.596 ± 0.024 bcd	56.61 ± 0.80 a
C	2.08 ± 0.03 a	622.8 ± 8.8 a	87.2 ± 2.8 b	2.713 ± 0.008 bc	53.21 ± 0.54 ab
D	2.18 ± 0.02 a	446.0 ± 8.8 b	76.4 ± 0.6 c	2.535 ± 0.031 cd	49.46 ± 1.07 bc
E	2.20 ± 0.10 a	310.2 ± 5.8 d	80.4 ± 1.5 bc	2.614 ± 0.036 bcd	44.87 ± 0.40 d
F	2.15 ± 0.10 a	587.6 ± 10.8 a	72.7 ± 0.0 c	2.781 ± 0.063 b	48.21 ± 0.89 cd
G	2.25 ± 0.10 a	576.8 ± 0.4 a	75.4 ± 0.9 c	2.533 ± 0.019 cd	43.79 ± 0.31 d
H	2.38 ± 0.08 a	430.4 ± 2.0 bc	76.0 ± 0.1 c	3.048 ±0.012 a	55.31 ± 0.22 a
I	2.18 ± 0.07 a	587.8 ± 1.8 a	98.5 ± 3.8 a	2.503 ± 0.032 cd	52.95 ± 1.16 ab
J	2.33 ± 0.08 a	394.2 ± 8.2 c	98.2 ± 1.2 a	2.518 ±0.078 cd	44.06 ± 0.94 d

Samples with different letters differ significantly ($p < 0.05$).

Table 4.3. Mixograph properties and bread volume of white flour (WF) and reconstituted whole wheat flour (15% bran added, A-J).

Sample	Water absorption (%)	Peak time (min)	Peak value (%)	Curve tail Integral (%Tq*Min)	Slope (%/min)	Bread loaf volume (cm^3)
WF	57.9 ± 0.0 c	5.2 ± 0.2 d	32.8 ± 1.0 ab	274.9 ± 13.3 a	3.2 ± 0.8 a	665.0 ± 5.0 a
A	63.5 ± 0.5 ab	6.5 ± 0.3 abc	33.5 ± 0.5 ab	257.7 ± 9.3 a	4.4 ± 1.5 a	647.5 ± 2.5 abc
B	63.5 ± 0.5 ab	7.4 ± 0.2 a	32.4 ± 0.5 b	248.4 ± 7.4 a	1.9 ± 0.9 a	617.5 ± 2.5 def
C	63.5 ± 0.5 ab	6.5 ± 0.0 abc	33.6 ± 0.1 ab	261.4 ± 1.3 a	2.8 ± 1.0 a	617.5 ± 2.5 def
D	63.5 ± 0.5 ab	7.3 ± 0.0 a	32.9 ± 0.7 ab	248.2 ± 10.5 a	2.1 ± 0.3 a	627.5 ± 2.5 cde
E	63.5 ± 0.5 ab	6.3 ± 0.1 bc	35.0 ± 1.3 ab	271.0 ± 13.0 a	4.0 ± 0.2 a	610.0 ± 0.0 efg
F	62.5 ± 0.5 ab	6.2 ± 0.0 bc	36.6 ± 0.2 a	289.0 ± 5.2 a	5.0 ± 1.4 a	640.0 ± 0.0 bcd
G	63.5 ± 0.5 ab	6.5 ± 0.2 abc	35.4 ± 0.8 ab	278.6 ± 8.7 a	4.2 ± 0.2 a	652.5 ± 12.5 ab
H	64.5 ± 0.5 ab	7.3 ± 0.2 a	33.4 ± 0.7 ab	249.7 ± 11.1 a	1.1 ± 0.4 a	622.5 ± 2.5 def
I	61.5 ± 0.5 b	7.0 ± 0.1 ab	32.5 ± 0.7 b	257.6 ± 9.0 a	1.5 ± 0.4 a	602.5 ± 2.5 fg
J	61.5 ± 0.5 b	6.0 ± 0.2 cd	33.8 ± 0.2 ab	268.9 ± 8.1 a	3.6 ± 0.0 a	590.0 ± 0.0 g

Samples with different letters differ significantly ($p < 0.05$).

	Protein	Lipid																
WRC	-0.283	-0.608	**WRC**															
Lipase	-0.365	-0.085	0.554	**Lipase**														
POD	-0.056	0.174	-0.142	-0.301	**POD**													
LOX	-0.170	-0.084	0.059	-0.138	-0.008	**LOX**												
ABTS	-0.146	-0.614	0.497	0.177	-0.197	-0.199	**ABTS**											
IDF	-0.310	-0.235	0.430	0.240	0.406	-0.334	0.112	**IDF**										
SDF	-0.112	-0.062	-0.097	-0.214	-0.242	-0.249	0.353	-0.054	**SDF**									
TDF	-0.322	-0.243	0.419	0.216	0.380	-0.360	0.151	0.994	0.054	**TDF**								
Absorption	0.336	0.365	-0.080	0.074	-0.183	-0.633	-0.231	0.113	0.057	0.118	**Absorption**							
MPT	0.166	0.825	-0.541	0.016	-0.058	-0.058	-0.729	-0.259	-0.182	-0.279	0.447	**MPT**						
PV	-0.290	-0.522	0.218	-0.012	0.122	-0.434	0.514	0.262	0.260	0.290	-0.199	-0.660	**PV**					
CTI	-0.314	-0.567	0.196	-0.083	0.237	-0.141	0.518	0.152	0.161	0.169	-0.500	-0.757	0.930	**CTI**				
Breakdown	0.318	-0.518	0.080	-0.323	-0.112	0.148	0.174	-0.043	0.094	-0.033	-0.100	-0.613	0.292	0.350	**Breakdown**			
slope	0.078	-0.638	0.287	-0.261	0.017	-0.276	0.704	0.299	0.281	0.329	-0.128	-0.753	0.604	0.577	0.465	**slope**		
TPC	0.525	-0.031	-0.492	-0.348	-0.232	0.076	0.093	-0.381	0.251	-0.354	-0.100	-0.058	-0.125	-0.070	0.434	0.248	**TPC**	
Bread	0.278	0.164	-0.154	-0.113	0.353	-0.804	0.228	0.297	0.142	0.312	0.483	0.028	0.308	0.142	-0.186	0.382	0.058	**Bread**
0-0.3	0.3-0.5	0.5-0.7	0.7-1.0															

Table 4.4. Pearson correlation between different properties of wheat bran and bread volume.

Protein, protein content (%); Lipid, lipid content (%); WRC, water retention capacity (%); Lipase, lipase activity (U/g); POD, peroxidase activity (%), LOX, lipoxygenase activity (%), ABTS, ABTS scavenging ability (%); IDF, insoluble dietary fiber (%); SDF, soluble fiber (%), TDF, total dietary fiber (%); Absorption, Mixograph water absorption (%); MPT, Mixograph peak time (min); PV, Mixograph peak value (%); CTI, Mixograph curve tail integral (%Tq*Min); Breakdown, Mixograph breakdown (%); Slope, Mixograph slope (%/min); TPC, total phenolic content (%); Bread, bread volume (cm^3).

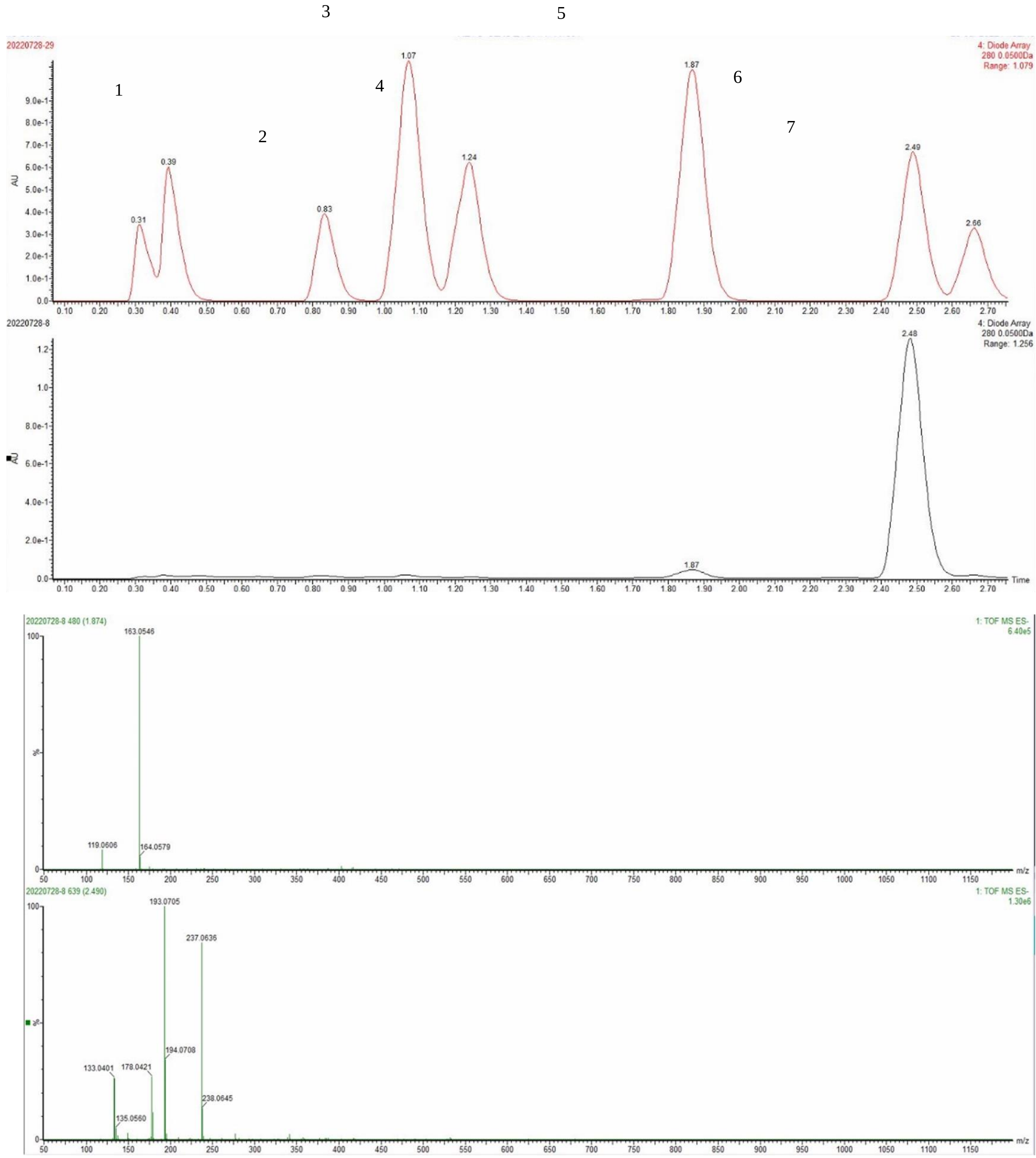

Figure 4.1. UPLC chromatogram of (A), standard phenolic components (B), chromatogram of phenolic components in typical insoluble fraction of wheat bran.
1-7: (1) gallic acid, (2) 4-hydroxybenzoic acid, (3) Caffeic + vanillic acid, (4) syringic acid, (5) *para*-coumaric acid, (6) *trans*-ferulic, (7) sinapic acid; MS spectra of the typical insoluble fraction in wheat bran with peak assignments at 1.874 min (C) and 2.490 min (D).

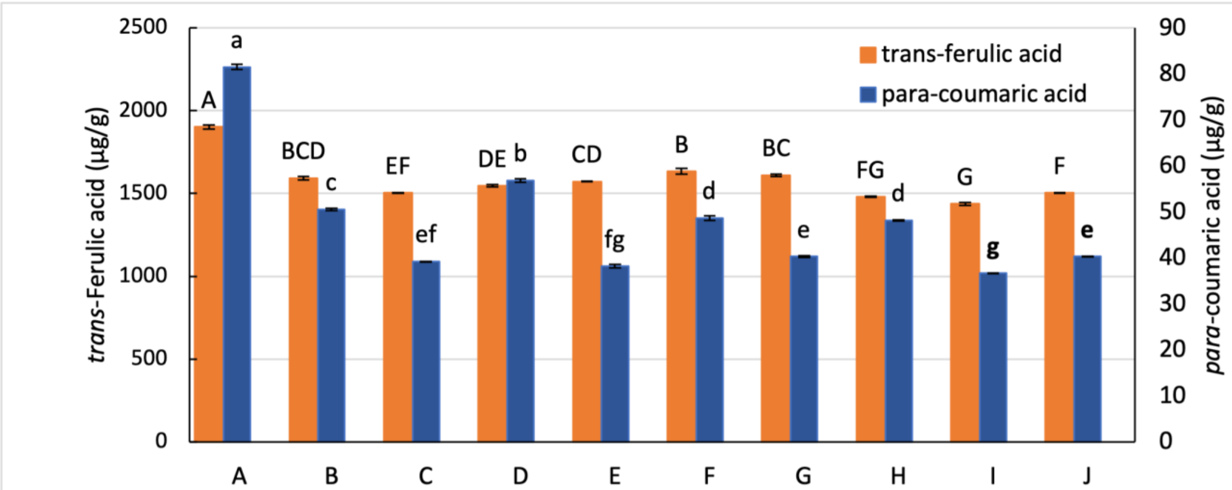

Figure 4.2. *para*-Coumaric and *trans*-ferulic acid contents in insoluble fraction of wheat bran. Samples with different letters differ significantly ($p < 0.05$).

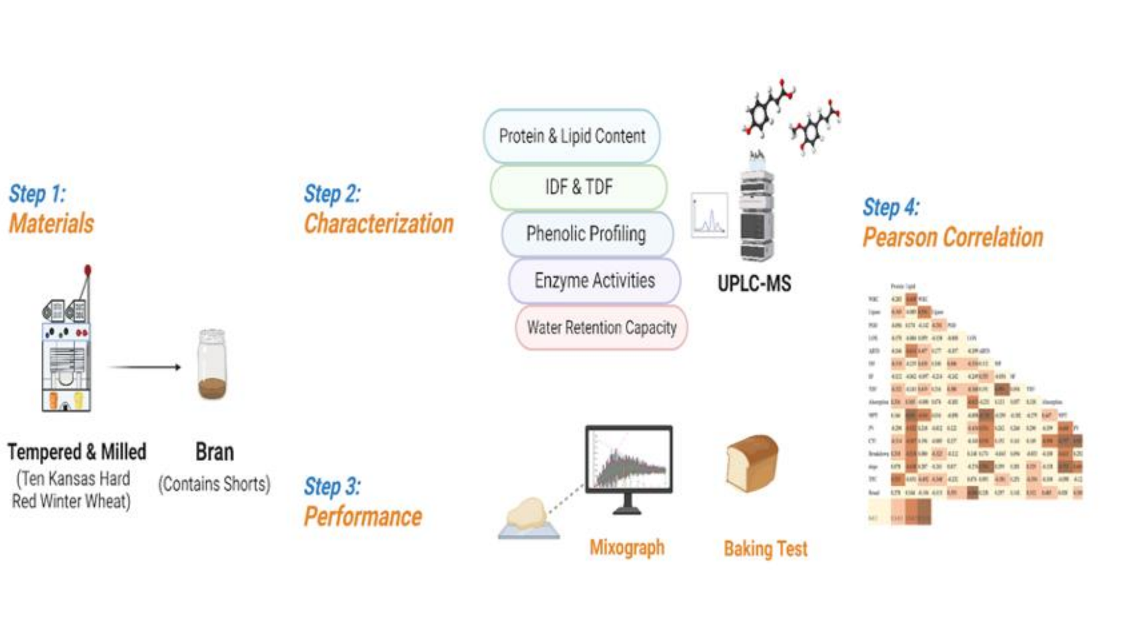

Figure 4.3. Graphic abstract.

Chapter 5 - Exploring the effects of wheat bran extract on gluten structure, dough rheological, and bread characteristics

Abstract

Wheat bran, characterized as a predominately dietary fiber and protein-based component, possesses diverse physicochemical properties including water hydration, rheology, and baking quality. In this study, wheat bran extract (WBE) was isolated, which exhibited a rich in carbohydrates (50.3%) and proteins (24.25%). Notably, the addition of 7.5% WBE significantly increased the bread volume to 4.84 cm^3/g, compared to the control bread volume of 4.18 cm^3/g. The addition of WBE also resulted in a remarkable improvement in dough properties. Firstly, the addition of WBE enhanced dough strength and led to higher peak values measured by Mixograph. Additionally, the addition of WBE resulted in increased peak, setback, breakdown and final viscosities measured by Rapid Visco Analyzer (RVA), and higher G′ and G″ modulus at a frequency of 1 Hz. Scanning electron microscopy analysis further revealed that the WBE could promote the aggregation of protein and starch within the dough. Regarding the ratio of gliadin and glutenin, it remained similar and increased with 5 and 7.5% WBE additions. Interestingly, WBE did not significantly affect starch gelatinization temperature or dough extension properties. These findings demonstrate that the inclusion of WBE in wheat flour presents a promising approach for producing high-quality bread that is enriched with dietary fiber and protein.

Keywords: Wheat bran; wheat bran extract; dietary fiber; gluten; dough rheology.

5.1. Introduction

Wheat bran, which constitutes the outer layers of the wheat grain, is an inexpensive byproduct generated during the wheat milling process. It possesses a layered structure that is made up of the outer pericarp (mainly composed of hemicellulose, cellulose, and little lignin), inner pericarp (rich in lignin), aleurone layer (abundant in hemicellulose and protein) and seat coat (Antoine et al., 2003a). Wheat bran hemicellulose, predominantly composed of arabinoxylans, exhibited diverse physicochemical properties such as water hydration and rheology (Pietiäinen et al., 2022). Due to its high dietary fiber content and bioactive components polyphenols and minerals, wheat bran has garnered attention for its promising numerous health benefits during consumptions (Coda et al., 2014). Consequently, these intriguing components possess significant potential for utilization in food applications, particularly in baking, intending to enhance the economic returns in cereal processing.

Bread, a widely consumed staple food, exhibits a remarkable global consumption rate of approximately 70 kg per person annually, with an annual production exceeding 9 billion kilograms (X. Wang, Lao, Bao, Guan, & Li, 2021). Traditionally, the bakery industry incorporates various additives into dough to enhance bread quality. However, growing safety concerns necessitate the exploration of alternatives to chemical additives. As a result, the addition of natural ingredients containing the compounds that are compatible to the chemical additives are highly promoted, and bran is also considerable recently. While the direct use of bran in bread were unfavorable on appearance, textural, sensorial, and rheological aspects of the dough and baked products. Therefore, it’s necessary to develop a proper method in order to withdraw the disadvantages of wheat bran maximumly.

Typically, dough rheology and baking product quality could be improved by altering the particle size, adding specific enzymes (e.g., a-amylase, xylanase, lipase, and protease), or wheat bran hydrolysate (Huiyan, Wang, Huang, Zheng, & Rayas-Duarte, 2010; C. Li et al., 2023; J. Li et al., 2012; Uttam, Padte, Raj, Govindaraju, & Kumar, 2023). Previously, different pretreatments following these traditional methods have been subjected to wheat bran to mitigate these drawbacks, while those methods are inconvenient, and some of them require more investigation (J. Li et al., 2012).

To develop a simpler method to reduce the cost, a systematic analysis between wheat bran components and corresponding functions is required in order to find out the useful parts of wheat bran in bakery quality improvement. In previous studies, the components of bran and their functions in bakery are listed along with their physical properties. According to previous studies, the components such as water-soluble fibers and water-soluble protein in wheat bran have huge impacts on the dough extensibility and baking quality (Uttam et al., 2023; P. Wang et al., 2019). Conversely, some researchers have reported that the inclusion of water insoluble arabinoxylan reduced dough elasticity and hindered protein formation in the range of 2.5 to 7.5%, potentially leading to unfavorable bread quality outcomes (Döring, Nuber, Stukenborg, Jekle, & Becker, 2015). The presence of insoluble arabinoxylan also impeded gluten formation and inhibited the thermal aggregation of gluten (Sun et al., 2022).

Consequently, we extracted these water-soluble components by simple water extraction, the quickest and most economical method, and evaluated their performance and investigate the impact of their concentration (0 - 7.5%) on composition, gluten structure, dough rheological, and quality of bread.

5.2. Materials and methods

5.2.1. Materials

Refined (white) wheat flour (protein content of 11.22% and moisture content of 7.9%), shortening, salt, sucrose, and yeast were purchased from the market. Hard and soft winter wheat bran was kindly provided from Hal Ross Flour Mill (Manhattan, Kansas). All the chemicals, and reagents used for the experiments were supplied from Fisher Scientific (Fairlawn, NJ, US) or Sigma-Aldrich (St. Louis, MO, US).

5.2.2. Extraction and characterization of the wheat bran extract (WBE)

5.2.2.1. Extraction of wheat bran extract (WBE)

To produce the wheat bran extract, wheat bran was mixed with distilled water in a ratio of 1:15 (v/w). The mixture was stirred at room temperature for two hours, followed by centrifugation at 8,000g for 15 minutes. The supernatants were collected and frozen at - 20°C and then lyophilized. The resulting lyophilized powder, labeled as the wheat bran extract (WBE), was stored at - 20°C for subsequent treatments or analysis.

5.2.2.2. Proximate composition analysis

Protein, moisture, and ash of wheat bran extract (WBE) were determined according to the approved AACC method (AACC, 2010). Dietary fiber was determined according to AOAC 991.43 (AOAC, 2007; Lee, Prosky, & Vries, 1992). The crude fat content was estimated by extracting total lipids with ethyl ether. The total carbohydrate content was obtained by deducting the amounts of protein, fat, ash, and moisture content from 100. The measurement of β-Glucan was conducted according to the AOAC Official Method 995.16 (AOAC, 2007).

5.2.2.3. GC monosaccharide composition analyses

To analyze the monosaccharide composition of wheat bran extract (WBE), gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) were employed based on the procedure outlined previously (Oxley, Currie, & Bacic, 2006). In brief, the lyophilized WBE was subjected to hydrolysis using 2.5 M trifluoroacetic acid (TFA) and heated at 100 °C for 4 hours. The hydrolysate was then dried using nitrogen gas, and the sample was acetylated with acetic anhydride and pyridine, followed by incubation at 100 °C for 2.5 hours. Next, the sugar alditols obtained from the hydrolysate were dissolved in dichloromethane (DCM) and analyzed using GC and GC–MS. The analysis was performed by scanning from 100 to 350 amu at 2.14 scans per second, with the GS-MS transfer line maintained at 260 °C. To establish the monosaccharide standards, including D-glucose, D-xylose, D-galactose, L-rhamnose, D-mannose, and D-arabinose, the sample derivatization procedure as described above was applied.

5.2.3. Mixograph characteristics

The dough mixing characteristics were evaluated in duplicate using a Mixograph (National Manufacturing, Lincoln, NE, USA) following the AACC approved method 54-40 (AACC, 2010). The Mixograms were processed using MixSmart software (v 3.40). The dough was prepared with white flour with four levels of lyophilized wheat bran extract (WBE) (0, 2.5, 5, and 7.5 percent). Flour samples (10g, db) and distilled water were mixed and water absorption (%), dough development time (MPT, time to peak), peak value (PV), curve tail integral (CTI), breakdown, and slope were collected.

5.2.4. Pasting characteristics

The pasting characteristics of the flours were evaluated using a Rapid Visco Analyzer (RVA) (Perten Instruments, Newport Scientific, Australia). In this analysis, 3 g of flour (14% moisture content basis) was added to a canister containing 25 ml of distilled water. The mixture was subjected to heating and cooling cycles for 13 minutes while being sheared at a constant shear rate of 160 rpm. The pasting temperature (TEMP), peak time, peak (PV), trough (trough), breakdown (BKD), setback (setback), and final (FV) viscosities were obtained. Each sample underwent a triplicate analysis.

5.2.5. Dough extensional and dynamic rheological properties

Dough extensional and dynamic rheological properties on doughs prepared from wheat flour with the addition of 0 - 7.5% wheat bran extract (WBE) were assessed using a TA-XTPlus texture analyzer (Stable Micro Systems, Godalming, UK) along with an SMS/Kieffer rig (Stable Micro Systems), and Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA, USA) according to the previous method (Chen et al., 2019). 10 g flour (14% moisture basis) was used, and the mixing time was based on the Mixograph results. Shear storage modulus (G′) and loss modulus (G″) were evaluated as strain 0.01 to 10%) at a frequency of 1 Hz, and as frequency (1Hz) at a strain of 0.1% at 25 °C. Each sample underwent at least triplicate analysis.

5.2.6. Scanning electron microscopy (SEM) analysis

The microstructure feature of wheat bran extract (WBE) and dough samples with four levels (0 – 7.5%) of WBE was viewed using an SEM (S-3500N, Hitachi Co. Japan) at two magnifications (100 ×, and 500 ×) with a sample working distance of 51 mm and an HT of 5 kV.

5.2.7. Thermal properties

The lyophilized dough samples were subjected to thermal analysis using differential scanning calorimetry (DSC) (Perkin Elmer, New Castle, DE, USA). Each sample (12mg) was scanned from 20 to 150 °C at a rate of 10 °C/min with a nitrogen gas flow rate of 50 mL/min. Starch retrogradation was determined by re-scanning the pan using the DSC after 7 days. Duplicate analyses were conducted for each sample. An empty DSC aluminum pan served as a reference. The temperature and enthalpy values were recorded using the Perkin Elmer Diamond DSC, which has been previously calibrated with pure Indium.

5.2.8. RP-HPLC analysis

The lyophilized dough sample (100 mg) was extracted twice for gliadin fraction using 1 ml of 60% ethanol for 12 minutes and extracted twice for glutenin fraction using 1.0 mL solution containing propan-1-ol, 1% dithioerythritol, and 0.05M Tris-HCl buffer (pH=7.4) for 45 minutes. Before analysis, the suspensions were centrifuged at 15,000×g for 10 min and the extracts were combined and then filtered. Gliadin and glutenin extracts were analyzed with an HP1050 Series HPLC (Agilent Technologies, Santa Clara, CA, US) according to the previous method (Chen et al., 2019). The gliadin and glutenin were identified at 210 nm and the quantitative analysis was based on their peak areas from the chromatograms.

5.2.9. FT-IR and protein secondary structures

The PerkinElmer Spectrum 400 FT-IR/FT-NIR Spectrometer (PerkinElmer, Inc., Waltham, MA, USA) equipped with an ATR cell was used to measure the infrared spectra of the lyophilized dough samples. Each sample was scanned in the range of 400–4000 cm^{-1} at an interval of 4 cm^{-1} with air as the background for a total of 64 scans. The spectra were converted to Gaussian shapes using OriginPro 2016 software to deconvolute the amide I region (1600–1700 cm^{-1}).

5.2.10. Bread characteristics

The bread preparation followed the AACC method 10–10.03 (AACC, 2010), and utilized the following formula: 100 g flour (14% moisture base), 3 g shortening, 0.2 g malt, 6 g sugar, 1.5 g salt, and 2.0 g dry yeast (Bellarine Red, PAK Group Pasadena, CA, USA). The mixing time and water addition were applied according to the Mixograph results. For each treatment, two loaves of bread were produced. After allowing the loaves to cool for 2 hours, the weight was measured, and the volume was determined using the AACC method 10-05.01 (AACC, 2010). Subsequently, the bread was sliced into 15 mm thick slices for further analysis.

The cell properties of two middle bread slices of bread were performed using a C-Cell Bread Imaging System (Calibre Control International Ltd., Appleton, Warrington, UK) following AACC method 10–18.01 (AACC, 2010). The texture properties of the bread were analyzed using a TA-XT2 Texture Analyzer (Stable Micro System, Godalming, Surrey, UK) with a 25-mm diameter cylinder probe. Slices from each treatment underwent a pre-test and test phase at a speed of 1.0 mm/s with a 50% strain, and a trigger force of 5 g was applied.

The color of the breadcrumb was assessed using a digital precise colorimeter (Cielab, Guangzhou, China). The values of L*(white), a*(red), and b* (yellow) were measured, and three replicates were conducted using the two-center slice from each treatment.

5.2.11. Statistical analysis

The data results were presented as mean ± standard deviation (SD). Statistical analysis was conducted using SAS Studio 3.7 (SAS Institute Inc., NC, US) employing one-way analysis of variance (ANOVA). Significance was determined at a p-value of less than 0.05 using Duncan's test.

5.3. Results and discussion

5.3.1. Chemical composition of wheat bran extract (WBE)

The wheat bran extract (WBE) was obtained by water extraction with a yield of 15.7% (w/w) and the proximate chemical compositions of WBE are shown in **Table 1**. The analysis reveals that WBE is rich in carbohydrates (50.3%) and protein (24.25%). Furthermore, WBE contains 9.5% total dietary fiber and 4.35% crude fat. These findings align with the previous literature results on the chemical composition of an aqueous extract of wheat bran (Luna-Valdez et al., 2017). They reported a similar concentration of 22.7% protein in the extract and highlighted the presence of Asp and Glu in considerable proportions regarding the amino acid profile (Luna-Valdez et al., 2017). The significant presence of acidic amino acids in albumins, which are more water-soluble, is expected due to the extraction method employed in this study.

A high ash content (13%) in the extract was observed, which can be attributed to the co-extraction of minerals that are present in substantial amounts in wheat bran. These findings have implications for the extraction process, as it enhances the nutritional values of the extract by providing additional minerals.

5.3.2. Sugar composition and β-glucans analysis

The sugar composition analysis of WBE revealed the presence of glucose, galactose, arabinose, xylose, and mannose in ratios of 7.09, 1.28, 0.57, 0.53, and 0.11, respectively (**Table 1**). Notably, the concentration of β-glucans in WBE was 0.52 %. Previous studies have associated the incorporation of 0-0.75% yeast β-D-glucan in dough with improved dough strength, adhesion, and bread quality (Suwannarong, Wongsagonsup, & Suphantharika, 2020). The Ara (arabinose) and Xyl (xylose) ratio serves as an indicator of the degree of substitution of the xylan backbone with Ara residues, and it depends on the anatomical regions of the wheat grain. Previous research reported that the value of the Ara/Xyl ratio in the water-extractable arabinoxylans was 0.6, which was similar to the ratio in the original wheat bran (Maes & Delcour, 2002). The discrepancy in A/X ratio suggests that different populations were isolated from distinct layers of the wheat bran. According to Barron, Surget, and Rouau (2007), the outer pericarp layer has a higher level of degree of arabinose substitution on the xylan backbone (average A/X ratio of 1.16) than the aleurone and intermediate layers (average A/X ratio of 0.44). The high ratio of Ara/Xyl ratio is 1.08 in WBE indicates the presence of arabinoxylans coming primarily from the outer pericarp of wheat bran.

5.3.3. Morphologies of wheat bran extract (WBE) and dough

The morphologies of wheat bran extract (WBE) and dough at different WBE levels were observed through SEM at a magnification of x100 and x500. The microstructure of WBE is depicted in **Fig.1A, and B**. Because most starch granules and adherent endosperm protein present in wheat bran were removed after extraction, WBE exhibits a laminated and sheet-like morphology with edge folds on the surface composed of relatively rough "flake-like" elements. Previous research has reported a similar sheet-like form of polysaccharides extracted from wheat bran, which aligns with the morphologies observed in WBE (Shang, Liu, Dong, Peng, & Zhu, 2021).

Figure 1C and 1D illustrate the microstructure of the control dough sample without the addition of WBE. Starch granules were connected and entrapped within the gluten matrix, forming a dense and ordered three-dimensional network. As the level of WBE addition in dough increased from 2.5 to 7.5% (**Fig. 1E - J**), the gluten matrix gradually became more continuous and aggregated, accompanied by more starch granules. This observation can be compared to previous studies that wheat bran disturbed the development of gluten network using SEM (Suyun Lin et al., 2022b). In contrast, the addition of WBE promoted the continuity of the gluten network in dough, and at higher concentration, it expanded the structure of gluten and facilitated the connection of starch granules to the gluten matrix.

5.3.4. Mixograph properties

The addition of wheat bran extract (WBE) had significant and positive effects on the mixing properties of the dough. **Table 2** presents the mixing parameters of doughs prepared with white flour and varying levels of WBE addition, as measured by the Mixograph. When WBE was added to white flour dough at the same 59% water absorption with control dough, the peak mixing time ranged from 5.0 to 5.6 min, which was similar to that of the control dough. The peak values increased significantly as WBE concentration increased from 0 to 7.5%, indicating that the addition of WBE resulted in a stronger dough. Curve tail integral, which represents the overall area covered by the Mixogram, was significantly higher in the doughs with WBE addition compared to the control dough. However, no significant differences were observed among the various levels of WBE addition. This finding provides evidence that WBE enhances the strength of the dough, requiring more energy to break down during mixing. During dough mixing, it is reasonable to assume the chemical reactivity of WBE interacted with gluten, leading to gluten aggregation. Similar to soluble fiber, WBE exhibited high reactivity, which depends on the functional groups participating in the formation of new bonds such as hydrogen bonds, with gluten and water (Q. Li, Liu, Wu, Wang, & Zhang, 2016). Similar results were previously reported that addition of 1 and 2% wheat endospermic cell wall to the wheat dough improved the peak values and dough stability (Bader Ul Ain et al., 2020).

5.3.5. Pasting properties

The pasting parameters of flour samples as presented in **Table 3 and Figure 2**, showed significantly changed ($p < 0.05$) with the addition of wheat bran extract (WBE). The addition of increasing concentration of WBE (0-7.5%) to white flour resulted in higher peak and final viscosities, ranging from 1239.0 to 1746.0 cP and 1508.0 to 1902.5 cP, respectively. Increases were also observed in breakdown and setback viscosities, ranging from 577.0 to 932.0 cP and 846.0 to 1088.5 cP, separately. Despite that, no significant differences were observed in peak time and pasting temperature among treatments. The increase in peak viscosities with higher concentrations of WBE addition is likely attributed to the greater degree of starch granules swelling during heating. It can be hypothesized that WBE promotes the contact between swollen starch granules, leading to higher peak viscosity. Similar results were reported in a previous study that used gum addition on chickpea-wheat flour (Jia, Ma, & Hu, 2020), suggesting that increased peak viscosity may be attributed to the decreased intermolecular forces and chain entanglements in the starch paste. This, in turn, resulted in the formation of more ordered double helical amylopectin side chain clusters, because of the interaction amongst starch functional group and WBE.

Upon reaching the peak, the viscosity of starch begins to decline due to disintegration of starch granules, the dissolution of starch molecular chains into the medium and the alignment of mixing direction (Klaochanpong, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2015). The breakdown viscosity reflects the stability of starch granules under heating and shearing conditions. The incorporation of wheat bran extract (WBE) into the flour significantly increased the breakdown viscosity compared to the control sample, indicating that the granules in the mixture with WBE exhibited higher resistance to thermal degradation than the control flour (Karim et al., 2008). The increase in the concentration of WBE substantially increases setback viscosity, which can be attributed to the higher leaching of starch component, facilitating the re-aggregation (H. Wang et al., 2020). In the cooling stage, a higher final viscosity was observed in starch with WBE, indicating a stimulatory effect of WBE on starch retrogradation. These observations emphasize the importance of selecting suitable WBE concentrations to control the retrogradation behavior of the sample. The final viscosity plays an important role in determining the product viscosity. An increase in paste viscosity was observed during the cooling process, which is due to the formation of a three-dimensional network and interaction of amylose with disintegrating granules (H. Wang et al., 2020). In our study, we observed a higher final viscosity in the sample with added WBE than that of the control flour. This could be attributed to the influence of WBE and the heating process, which caused significant inflation of the starch granules and full stretching of starch molecular chains, thereby accelerating the diffusion and promoted the formation of a solid structure. Furthermore, there is a strong correlation between the increase in peak value/setback and the increase in the specific volume of the white breads. These was agreement with previous reports

(Junejo et al., 2021; Karakelle, Kian-Pour, Toker, & Palabiyik, 2020). The results clearly demonstrate that WBE effectively improves the pasting properties of white flour during both heating and cooling stages.

5.3.6. Dough extension properties

Dough extension properties provide information about the viscoelastic behavior of dough, which ultimately affects the quality of final products. The Kieffer testing allows for the analysis of stress and strain. Dough strain hardening, characterized by a stress that surpasses the proportional increase in strain, is closely associated with the gluten matrix (Kindelspire, Glover, Caffé-Treml, & Krishnan, 2015). According to the results illustrated in **Table 4**, the rupture force of the control dough was measured at 15.27 g, while doughs with the addition of 2.5 to 7.5% wheat bran extract (WBE) exhibited rupture force ranging from 12.98 to 15.04 g. However, no significant differences in force values were observed ($p > 0.05$). The distance of doughs containing WBE increased from 21.35 to 30.97 mm whereas no significant difference was found. The variation in dough extension could be attributed to the interaction induced by the gluten polymer cross-links and aggregation with the influence of WBE.

5.3.7. Dynamic rheological measurements of doughs with wheat bran extract (WBE)

The oscillatory rheological properties of the dough were assessed using the small deformation test, specifically strain and frequency sweeps, as shown in **Figure 4**. Dough samples supplemented with WBE exhibited higher storage modulus (G′) and loss modulus (G″) than control dough. A higher G′ indicates a more elastic material, dough containing a higher WBE concentration, showed higher elastic properties. Previous studies also indicated that the viscoelastic properties of wheat doughs are consistent with gels formed of reversible cross-links, making them comparable to synthetic associative polymers (Peressini, Peighambardoust, Hamer, Sensidoni, & van der Goot, 2008). Depending on the frequency, dough with supplementary WBE exhibited lower elastic properties compared to the control dough, suggesting an antagonistic effect on the combination. Increases of G′ and G″ were observed and positively correlated with the WBE concentration. The changes in viscoelastic properties are likely to be due to the interaction between WBE and gluten and composition. One possibility is that the variation of G′ and G″ values with the addition of WBE is due to the behaviors of samples, which contain soluble fiber and relatively high protein concentration in WBE (24.25 %), and wheat flour (11.22%). Previous studies have shown that the addition of gluten-free flour, soy protein, and casein can alter both the G′ and G″ modulus (Matos, Sanz, & Rosell, 2014). Another possibility is the polysaccharide chains of the soluble dietary fiber, as the short-chain supplementation could promote starch granule swelling, thus increasing the G′ (Donatella Peressini & Sensidoni, 2009). The addition of WBE may impact the dough's elastic-solid behavior, affecting the softness and flexibility of the bread.

5.3.8. Thermal properties

The gelatinization parameters of the flour samples, including peak height, onset, end, peak temperatures, and Delta H, were calculated from the DSC thermograms and summarized in **Table 5**. Delta H represents the energy required for the melting of crystalline structure and can be used to analyze the degree of gelatinization of starch in cereal products (Genkina, Kozlov, Martirosyan, & Kiseleva, 2014) The gelatinization values of pastes containing wheat bran extract (WBE) were found to be similar to those of the control samples, suggesting that the presence of WBE resulted in the similar energy required to break the crystalline structure of starch granules and facilitating their swelling, likely due to the similar gelatinization temperature values (Schirmer, Jekle, & Becker, 2015), which is consistent with the result obtained from RVA analysis.

During the cooling stage, starch retrogradation occurs, leading to the re-crystallization of the amorphous structure in starch granules through hydrophobic interactions and hydrogen bonding. The addition of WBE to the flour samples had minimal impact on Delta H at 7 days, ranging from 0.36 to 0.87 J/g. However, this effect was not statistically significant in terms of inhibiting starch retrogradation. These limited inhibitory effects might be attributed to the composition and the structural properties of the WBE. Previous studies found that a lower molecular weight of dietary fiber was less likely to inhibit the cross-linking of amylose molecular chain and form double helix between amylopectin molecular chains than higher molecular weight of dietary fiber combined with phenolic content (M. Yu et al., 2022).

5.3.9. RP-HPLC of gliadin and glutenin subunits

Wheat gluten primarily consists of two components: gliadin and glutenin. Gliadin can be divided into three main subunit types of ω5 - ω1, 2- gliadin, α- and γ-gliadin, and glutenin composed of four main high molecular weight subunit types, low molecular weight B-, C-, and D-LMS (Bruneel, Lagrain, Brijs, & Delcour, 2011). The quantitative data obtained from RP-HPLC chromatograms of dough samples with wheat bran extract (WBE) added are summarized in **Table 6**. At low concentration (2.5%) of WBE, there were no significant changes observed in the proportion of gliadin and glutenin subunits. However, at higher concentration of WEB, a decrease in the proportion of glutenin subunits was observed. Specifically, the extractable glutenin subunits decreased significantly from 40.93 µg/mg (control) to 27.76 µg/mg with the addition of 7.5%

WBE. The results suggest that the hydrophilic interaction influenced by WBE may inhibit the aggregation of glutenin, leading to a decreased concentration of glutenin at higher concentrations.

The ratio of gliadin to glutenin (gli/glu) plays an important role during the formation of development of dough. Gliadin contributes to the viscosity and flowability of the dough, while glutenin contributes to the elasticity and strength through the formation of a polymeric network. In **Table 2**, as the level of WBE increases, gli/glu also significantly increased from 1.06 to 1.59 ($p < 0.05$). This change in the gli/glu is likely to impact the physical and rheological properties of the dough. Furthermore, a previous study reported that a gli/glu of 1.82 resulted in a relatively high bread volume (303 cm^3), whereas a ratio of led to a lower bread volume (162 cm^3) (Dizlek & Awika, 2023). However, researchers have not reached a consensus on the specific factors responsible for the positive effects of the gluten complex on the dough and bread characteristics. Various assumptions have been proposed, including the gli/glu, ratio of glutenin, and the composition of glutenin subunit (Barak, Mudgil, & Khatkar, 2013; Dizlek & Awika, 2023; Liwei Yu et al., 2022).

5.3.10. FTIR secondary structure

The secondary structure pattern of gluten protein plays an essential role in the formation of the gluten matrix. The α-helix and β-sheet secondary structures are more compact and ordered compared to the random coil conformation, the helix is relatively more stable than the β-sheet structure due to stronger intramolecular hydrogen bonds (Correa, Ferrer, Añón, & Ferrero, 2014). FTIR spectra of gluten samples were collected to elucidate the effect of wheat bran extract (WBE) on gluten conformation. The FTIR spectra exhibit characteristic band regions that are indicative of specific gluten secondary structures (**Figure 4**). The amide I region was deconvoluted to determine the gluten secondary structures, and the peak assignments can be found in **Table 6**. The gluten structure of doughs was not significantly affected by the addition of 2.5 to 7.5% WBE ($p > 0.05$). Earlier study reported the supplementation of dietary fiber or oat β-glucan increased α-helix and random coil while decreased β -turn and β -sheet structure (Nawrocka, Szymańska-Chargot, Miś, Wilczewska, & Markiewicz, 2017; L. Wang et al., 2017). One possible explanation for this discrepancy is that in our study, we extracted the water-soluble fraction of wheat bran, whereas earlier studies may have used water-unextractable dietary fiber. In starch-containing systems, the band at 1644 cm^{-1} is associated with hydrogen bonding within the hydroxyl group, which is influenced by the content of bound water molecules in the non-crystalline region of starch. The identical intensities observed at 1644 cm^{-1} for the samples indicate that the presence of the WBE samples had no significant impact on the hydrogen bonding within the starch hydroxyl group. This confirmed the results of the thermal properties of WBE-containing flour measured by DSC.

5.3.11. Effect of wheat bran extract (WBE) on bread quality

The specific volume (SV) of bread is a crucial characteristic that significantly influences consumer preference. **Figure 5** presents the photos of bread slices, and the addition of wheat bran extract (WBE) showed a significant facilitation effect on SV **(Table 7)**. The SV of bread with increasing WBE concentration was significantly larger (4.30 – 4.84 cm^3/g) compared to the control bread (4.18 cm^3/g). The positive effect of WBE can be mainly attributed to the interactions among WBE, gluten, and starch. These interactions involve hydrogen bonds and hydrophobic interaction, leading to the formation of network in flour samples (Peng, Wang, Zou, Zhang, & Hu, 2022). Studies on steamed bread have also shown that increased SV is closely associated with the stability of the bubble interface through the network structure of starch and protein and enhance the appearance structure (K. Xu et al., 2022).

The dough with addition of WBE resulted in increased peak viscosities as measured by RVA and Mixograph. The increase in SV with the addition of WBE can be attributed to two factors. Firstly, WBE physically facilitates the foam structure of the dough and maximizes retention of gas during fermentation, leading to a larger SV. Secondly, the larger SV can be attributed to the stronger intermolecular cross-links of gluten after addition of WBE, as measured by the G' and G'' values. Therefore, the increase in SV observed in this study is likely a combined result from the influences of WBE on physical and chemical factors. Furthermore, soluble dietary fibers such as β-glucan have been observed to promote the gluten aggregations within the gluten networks during the processes of dough mixing and heating (Z. H. Huang et al., 2017; L. Wang et al., 2017).

The color properties of breadcrumbs, as presented by the L*, a*, b* values, are shown in **Table 7**. When 0 to 5% wheat bran extract (WBE) was added to the bread formulation, no significant difference in color was observed compared to white bread. However, the addition of 7.5% WBE led to an increase in reddish and yellow tones (higher a* and b* values), while the E value shows no significant difference compared to the control bread. The color of bread crust and crumb is primarily influenced by the Maillard reaction and caramelization processes that occur during baking. These reactions involve the interaction between amino acid residues and reducing sugars, resulting in the development of a brown color. The variation in bread color may also be related to the presence of different pigment compounds in WBE, with higher levels of WBE promoting these reactions and resulting in a darker crumb color. Similar findings were reported in studies involving bran-enriched bread, where an increase in reddish and yellow was reported in bran corporation bread (C. Li et al., 2023). The intensified browning and Maillard reactions significantly impact the sensory quality of bread.

The addition of wheat bran extract (WBE) had an impact on the texture of the bread, resulting in similar or decreased values of bread hardness, gumminess, and chewiness compared to the control white bread, both on the same day and after 2 days of analysis (**Table 8**). In general, bread made with WBE had similar or better external characteristics, soft and resilient crumbs, and an evenly distributed cell structure than white bread. The Texture profile analysis (TPA) values of white bread slices showed hardness, springiness, gumminess, and chewiness were 2.55 N, 168.59%, 2.12 N, and 3.57 N, respectively. In comparison, bread with the addition of WBE showed similar hardness, springiness, gumminess, and decreased chewiness in the range of 2.45 – 2.69 N, 131.06 – 154.33%, 1.87 – 2.01 N, and 2.63 – 2.92 N on the same day. After 2 days of storage, bread with WBE addition exhibited similar or lower hardness values ranging from 6.95 to 7.75 N compared to the control whose hardness was 8.16 N. This lower hardness was related to the larger specific volume. Previous studies revealed a positive impact of both water extractable and unextractable pentosans on the crumb firmness of the bread, whereas water soluble pentosans were found to be better than insoluble pentosans reducing crumb firmness (Arif, Ahmed, Chaudhry, & Hasnain, 2018).

The results of the C-cell analysis, including cells per slice area, area of cells, wall thickness, and cell diameters determined are presented in **Table 7**. The bread with addition of WBE had small and uniform pores, similar to that the white bread. Previous studies have emphasized the small pores and a high number of cells per slice area for producing high-quality bread, as for white flour dough, the leavened gas was effectively retained and expended within the continuous viscoelastic gluten network during fermentation and baking (W. Yu et al., 2019). The incorporation of WBE significantly enhanced the gas retention capacity by promoting the gluten network, leading to larger dough and loaf volumes. Moreover, WBE promoted gluten-starch network in stabilizing the expansion of gas cells against disproportionation and coalescence (Upadhyay, Ghosal, & Mehra, 2012).

5.4. Conclusion

In this study, we have developed a straightforward method to withdraw the value of wheat bran by water extraction method. Wheat bran extract (WBE) is rich in protein, dietary fiber, and monosaccharide, which contribute to the bread quality improvement. The morphological characteristics were analyzed using SEM, revealing that WBE promoted the continuity of gluten network. At higher concentrations, WBE induced the structure of gluten to expand and promoted the connection between starch granules and the gluten network. As a result, the bread containing 7.5% WBE possesses a remarkable 16% increase in volume compared to the control bread. By gradually incorporating WBE into white flour, various viscosity parameters **including** peak, breakdown, setback, and final viscosities increased. The dough extensibility and gelatinization temperature remained unaffected by the addition of WBE. Moreover, higher WBE resulted in a significant increase in the gli/glu ratio (1.06 to 1.59) and G' values, indicating enhanced elastic interactions and a dough with improved elasticity and strength. These findings hold substantial potential for elevating the economic value of wheat bran and reducing costs in the bakery industry.

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Table 5.1. Proximate composition and sugar profile of wheat bran extract (WBE).

Proximate analysis (%)		Dietary fiber (%)		Sugar profile (%)		β-Glucan (%)
Crude protein	24.25 ± 0.05	Soluble dietary fiber	6.7 ± 0.2	Xylose	0.53 ± 0.00	0.52 ± 0.01
Ash	13.0 ± 0.0	Insoluble dietary fiber	2.9 ± 0.2	Arabinose	0.57 ± 0.02	
Moisture	8.05 ± 0.05	Total dietary fiber	9.5 ± 0.1	Mannose	0.11 ± 0.00	
Crude fat	4.35 ± 0.24			Glucose	7.09 ± 0.10	
Total carbohydrate*	50.3 ± 0.3			Galactose	1.28 ± 0.00	
				A/X	1.08 ± 0.03	

The total carbohydrate content was estimated by subtracting the crude protein, crude fat, ash, and moisture content.

Table 5.2. Dough mixing properties measured by Mixograph.

Sample	Water absorption (%)	Peak time (min)	Peak value (%)	Curve tail integral (%Tq*Min)	Peak width (%)	8 min Width (%)
Control	59.0 ± 0.0 a	5.0 ± 0.0 a	36.4 ± 0.3 d	309.8 ± 5.3 b	20.3 ± 0.6 a	13.2 ± 0.2 a
2.5%WBE	59.0 ± 0.0 a	5.3 ± 0.1 a	39.5 ± 0.1 c	334.1 ± 0.1 a	20.7 ± 0.4 a	11.1 ± 0.5 ab
5%WBE	59.0 ± 0.0 a	5.6 ± 0.1 a	41.5 ± 0.1 b	342.6 ± 4.2 a	20.3 ± 0.2 a	10.4 ± 0.1 b
7.5%WBE	59.0 ± 0.0 a	5.5 ± 0.1 a	43.0 ± 0.4 a	352.2 ± 2.4 a	30.2 ± 3.4 a	10.5 ± 0.5 b

WBE, wheat bran extract; Control, white flour dough; 2.5-7.5% WBE, control, and 2.5 – 7.5% WBE addition dough. Samples with different letters differ significantly ($p < 0.05$).

Table 5.3. RVA parameters for white flour with different addition of water-extracted wheat bran (0%, 2.5%, 5%, 7.5%).

Sample	Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Peak time (min)	Pasting temperature (°C)
Control	1239.0 ± 10.0 c	662.0 ± 24.0 b	577.0 ± 14.0 c	1508.0 ± 11.0 c	846.0 ± 13.0 c	5.5 ± 0.1 a	70.5 ± 0.3 a
2.5%WBE	1533.0 ± 11.0 b	796.0 ± 31.0 a	737.0 ± 20.0 b	1736.5 ± 21.5 b	940.5 ± 9.5 bc	5.7 ± 0.1 a	69.1 ± 0.4 a
5%WBE	1591.0 ± 15.0 b	770.0 ± 8.0 ab	821.0 ± 7.0 b	1770.5 ± 12.5 ab	1000.5 ± 4.5 ab	5.7 ± 0.1 a	69.4 ± 0.0 a
7.5%WBE	1746.0 ± 31.0 a	814.0 ± 12.0 a	932.0 ± 19.0 a	1902.5 ± 46.5 a	1088.5 ± 34.5 a	5.8 ± 0.0 a	69.0 ± 0.4 a

WBE, wheat bran extract; Control, white flour. Samples with different letters differ significantly ($p < 0.05$).

Table 5.4. Dough extensibility of flour with addition of wheat bran extract (WBE).

Sample	Force (g)	Distance (mm)
Control	15.27 ± 0.87 a	21.35 ± 3.53 a
2.5% WBE	15.04 ± 2.01 a	29.98 ± 7.62 a
5% WBE	12.98 ± 1.37 a	28.58 ± 5.82 a
7.5% WBE	14.62 ± 2.91 a	30.97 ± 7.65 a

*Control, white flour dough; 2.5-7.5% WBE, control and 2.5 – 7.5% WBE addition dough. Samples with different letters differ significantly ($p < 0.05$).

Table 5.5. Gelatinization and retrogradation of wheat flour samples with adding 0-7.5% wheat bran extract (WBE).

Sample	Onset (°C)	End (°C)	Peak (°C)	Peak height (mJ/sec)	Delta H (J/g)
Gelatinization					
Control	60.16 ± 0.45 a	71.32 ± 0.19 a	65.96 ± 0.68 a	0.28 ± 0.05 a	1.02 ± 0.21 a
2.5%WBE	58.49 ± 0.27 a	71.77 ± 0.33 a	65.19 ± 0.23 a	0.37 ± 0.00 a	1.57 ± 0.00 a
5%WBE	59.47 ± 0.18 a	71.94 ± 0.16 a	65.68 ± 0.05 a	0.38 ± 0.00 a	1.56 ± 0.01 a
7.5%WBE	59.56 ± 0.34 a	72.18 ± 0.01 a	65.78 ± 0.05 a	0.35 ± 0.02 a	1.44 ± 0.01 a
Retrogradation					
Control	32.03 ± 0.27 a	50.73 ± 1.07 a	42.24 ± 2.05 a	0.05 ± 0.01 a	0.33 ± 0.09 a
2.5%WBE	31.70 ± 0.02 a	57.47 ± 0.81 a	43.86 ± 0.11 a	0.08 ± 0.02 a	0.87 ± 0.35 a
5%WBE	32.17 ± 0.35 a	53.27 ± 0.52 a	42.56 ± 0.44 a	0.05 ± 0.00 a	0.36 ± 0.05 a
7.5%WBE	31.77 ± 0.36 a	55.73 ± 2.60 a	43.75 ± 0.65 a	0.06 ± 0.00 a	0.47 ± 0.06 a

WBE, wheat bran extract. 2.5-7.5% WBE, control flour and 2.5 – 7.5% WBE addition. Samples with different letters in the same column differ significantly ($p < 0.05$).

Table 5.6. Gliadin and glutenin concentration and secondary structure with wheat bran extract (WBE) according to Amide I region (%) of dough samples.

Sample	Gliadin (µg/mg)	Glutenin (µg/mg)	Ratio gli/glu	Helix (%)	β-Sheet (%)	β-Turn (%)	Random (%)
Control	43.20 ± 0.53 a	40.93 ± 2.97 a	1.06 ± 0.06 b	40 ± 6 a	20 ± 4 a	6 ± 0 a	34 ± 4 a
2.5%WEB	41.12 ± 0.35 a	41.99 ± 0.36 a	0.98 ± 0.01 b	53 ± 8 a	18 ± 5 a	4 ± 2 a	25 ± 3 a
5%WEB	49.59 ± 5.21 a	31.10 ± 0.93 b	1.59 ± 0.14 a	45 ± 3 a	17 ± 3 a	6 ± 1 a	32 ± 6 a
7.5%WEB	43.09 ± 1.40 a	27.76 ± 0.41 b	1.55 ± 0.06 a	43 ± 3 a	15 ± 5 a	5 ± 1 a	37 ± 5 a

Control, white flour dough; 2.5-7.5% WBE, control and 2.5 – 7.5% WBE addition dough. Samples with different letters in the same column differ significantly ($p < 0.05$).

Table 5.7. Specific volume, crust color, and C-cell properties of bread with the addition of wheat bran extract (WBE).

	Control	2.5% WBE	5% WBE	7.5% WBE
Specific volume (cm^3/g)	4.18 ± 0.00 c	4.30 ± 0.02 c	4.52 ± 0.05 b	4.84 ± 0.02 a
L*	65.57 ± 0.13 a	66.37 ± 0.68 a	64.64 ± 0.75 a	64.72 ± 0.91a
a*	1.52 ± 0.06 b	1.56 ± 0.04 b	1.73 ± 0.08 b	2.08 ± 0.10 a
b*	16.31 ± 0.06 b	16.45 ± 0.48 b	17.39 ± 0.44 b	18.69 ± 0.34 a
E	67.59 ± 0.12 a	68.39 ± 0.77 a	66.96 ± 0.75 a	67.40 ± 0.88 a
Number of cells/areas (mm^2)	0.66 ± 0.05 a	0.71 ± 0.06 a	0.67 ± 0.10 a	0.65 ± 0.04 a
Area of Cells (%)	49.53 ± 0.68 a	49.73 ± 0.57 a	49.88 ± 0.62 a	50.30 ± 0.84 a
Wall Thickness (mm)	0.45 ± 0.02 a	0.43 ± 0.02 a	0.45 ± 0.03 a	0.45 ± 0.01 a
Cell Diameter (mm)	1.88 ± 0.06 a	1.82 ± 0.19 a	1.89 ± 0.22 a	1.91 ± 0.06 a

WBE, wheat bran extract; 2.5 - 7.5% WBE, bread slices made from control flour with 2.5 – 7.5% WBE addition. Samples with different letters differ significantly ($p < 0.05$).

Table 5.8. TPA data of bread slices on the same day and after 2 days.

	Hardness (N)	Springiness (%)	Gumminess	Chewiness
On same day				
Control	2.55 ± 0.01 a	168.59 ± 2.13 a	2.12 ± 0.08 a	3.57 ± 0.10 a
2.5% WBE	2.69 ± 0.17 a	131.06 ± 24.53 a	2.01 ± 0.09 a	2.63 ± 0.48 b
5% WBE	2.45 ± 0.27 a	150.15 ± 17.19 a	1.87 ± 0.17 a	2.84 ± 0.54 ab
7.5% WBE	2.45 ± 0.15 a	154.33 ± 10.08 a	1.90 ± 0.12 a	2.92 ± 0.19 ab
After two days				
Control	8.16 ± 0.48 a	111.84 ± 20.28 a	4.74 ± 0.19 a	5.27 ± 0.72 a
2.5% WBE	7.01 ± 0.64 b	116.64 ± 19.35 a	4.22 ± 0.54 ab	4.93 ± 1.06 a
5% WBE	6.95 ± 0.27 b	97.46 ± 2.73 a	3.90 ± 0.37 b	3.81 ± 0.46 a
7.5% WBE	7.75 ± 0.16 ab	101.38 ± 6.76 a	4.38 ± 0.11 ab	4.44 ± 0.28 a

WBE, wheat bran extract. 2.5 – 7.5% WBE, bread slices made from control flour with 2.5 – 7.5% WBE addition. Samples with different letters differ significantly ($p < 0.05$).

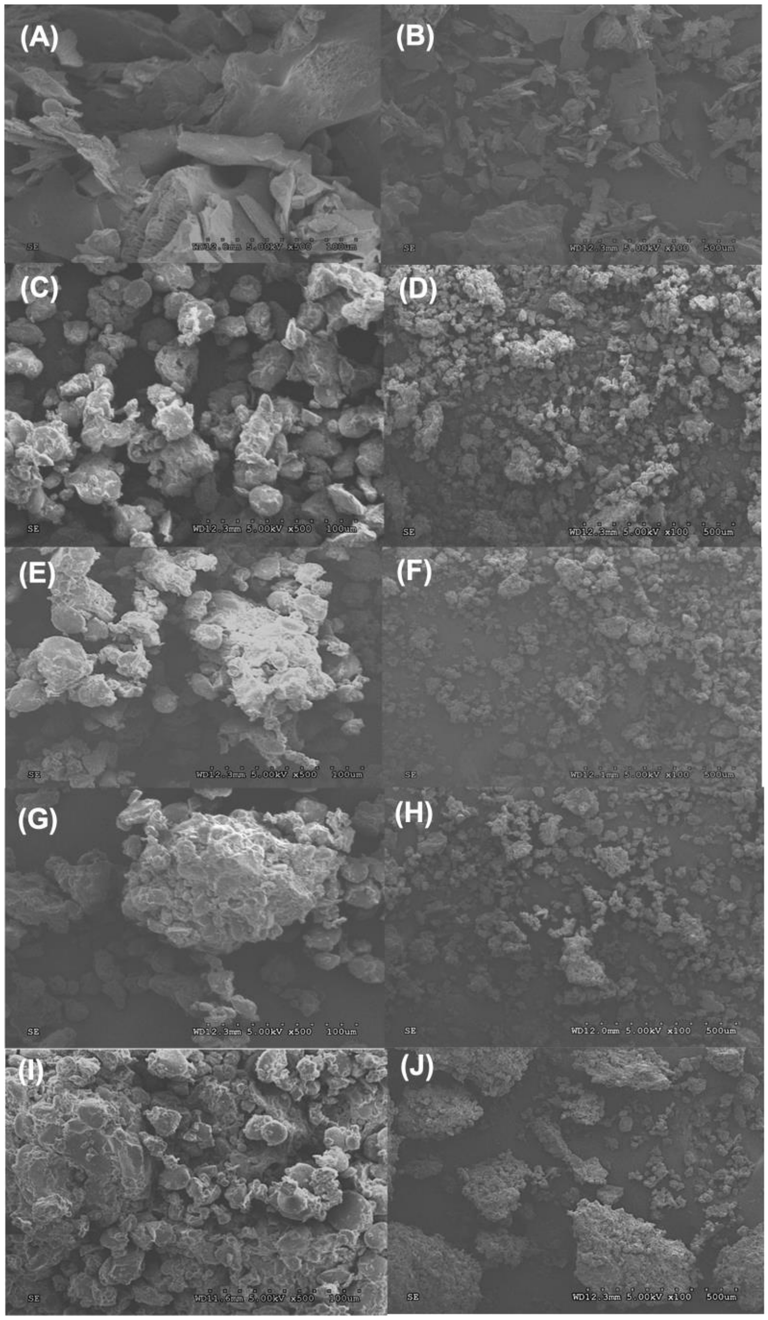

Figure 5.1. Microstructural analysis of wheat bran extract (WBE) and WBE-supplemented dough by scanning electron microscopy (SEM).

WBE (A, 500x; B, 100x), control dough (C, x500; D, x100), 2.5%WBE dough (E, x500; F, x100); 5% WBE dough (G, x500; H, x100), 7.5% WBE dough (I, x500; J, x100). Control dough was made from white flour, 2.5-7.5% WBE dough, and dough at different WBE levels (2.5 – 7.5%).

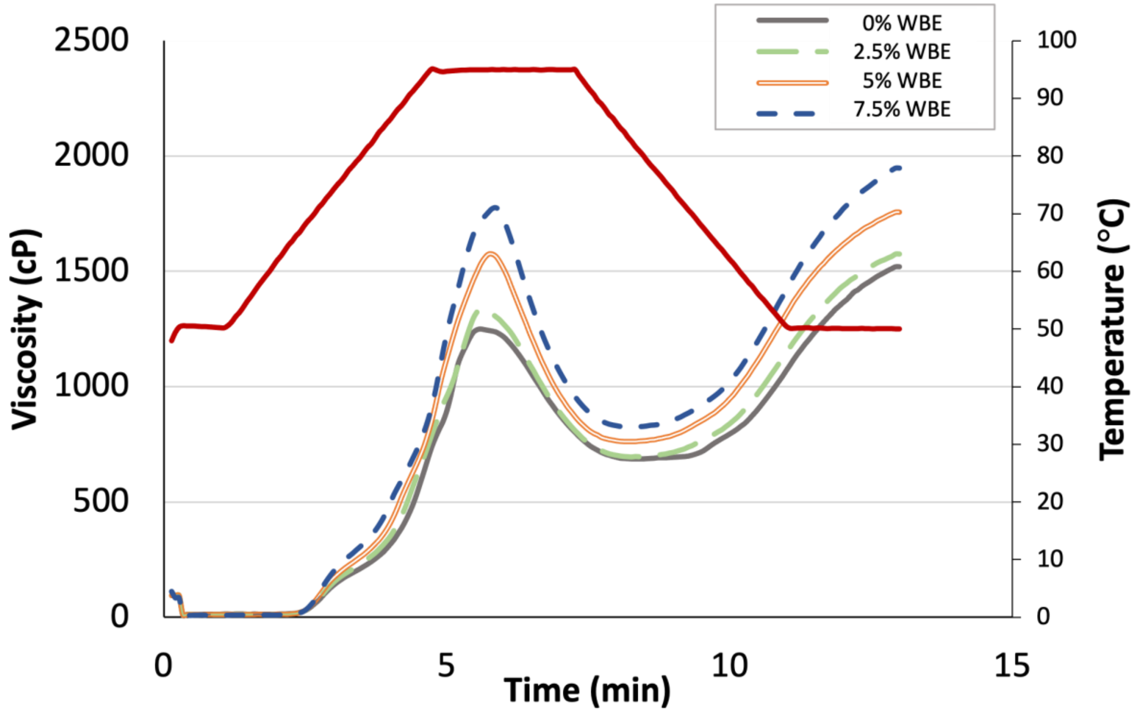

Figure 5.2. RVA curves of white flour with the addition of WBE (0, 2.5, 5, and 7.5%).

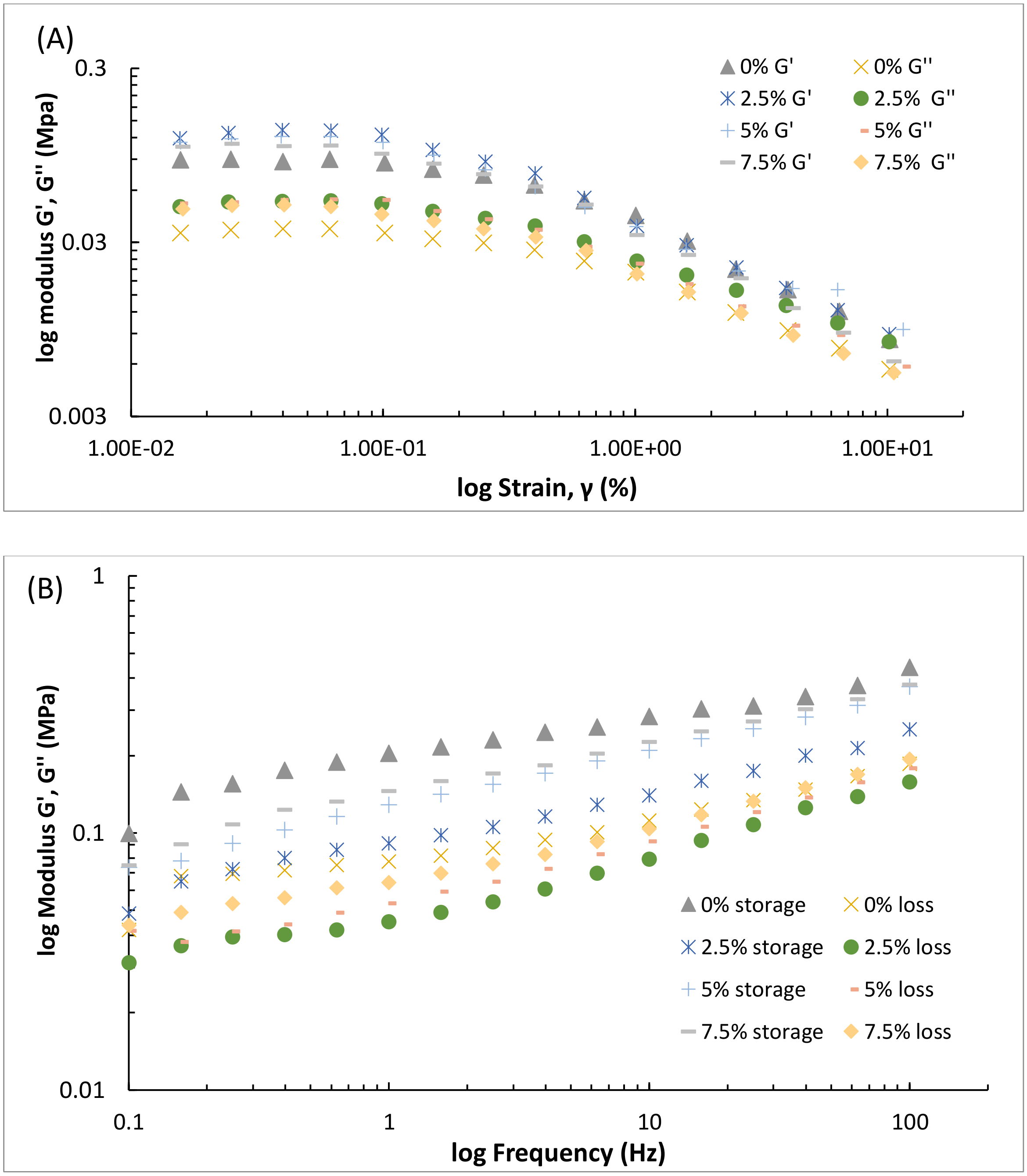

Figure 5.3. Storage modulus G' (solid symbols), and loss modulus G" (open symbols) of the doughs prepared from wheat flour with the addition of 0 - 7.5% wheat bran extract (WBE). (A) as a function of strain at a frequency of 1 Hz, (B) as a function of frequency at a strain of 0.1%.

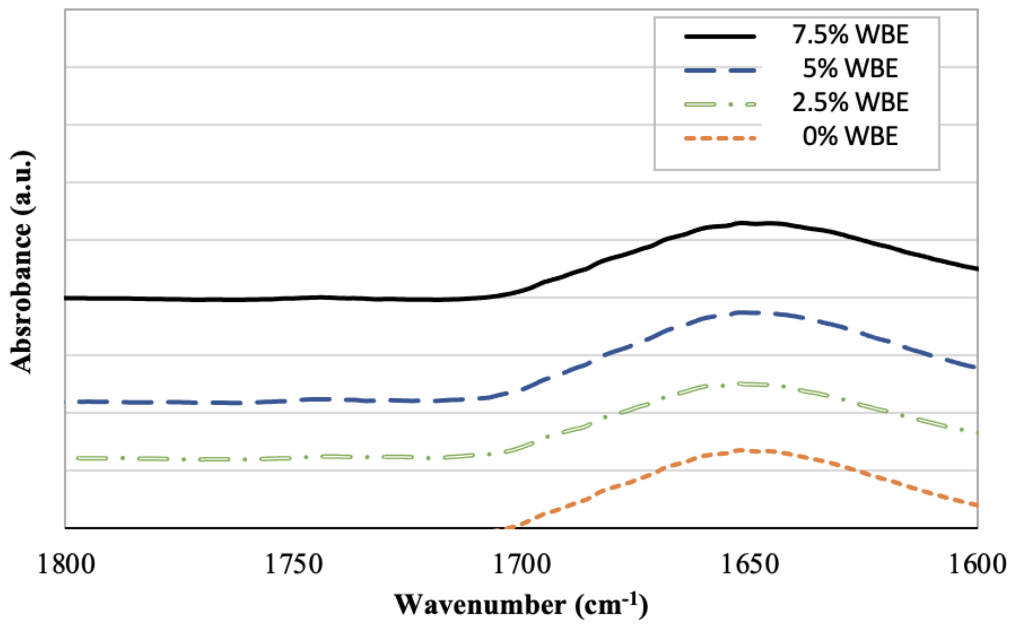

Figure 5.4. FTIR spectra of freeze-dried dough samples.

WBE, wheat bran extract; 0 -7.5% WBE, dough made with white flour and 0 – 7.5% WBE addition. The amide-I bands (1600 to 1800 cm^{-1}) is boxed.

Figure 5.5. Photos of bread slices.

Control: bread made from white wheat flour; 2.5 - 7.5% WBE, bread made from white wheat flour, and an additional 2.5 - 7.5% wheat bran extract (WBE).

Chapter 6 - Biochemical characterization, antioxidant and anti-aging potential in co-extracted xylo-oligosaccharides and peptide hydrolysates from wheat brans

Abstract

The accumulation of reactive oxygen and nitrogen species (RONS) during cellular activity leads to oxidative stress, resulting in damage to cellular macromolecules, which is closely linked to aging and diseases. Natural antioxidants, specifically phenolic acids and bioactive peptides obtained through dietary sources, have gained interest in their potential to modulate cellular oxidative status. Wheat bran, a byproduct of wheat milling, is a cost-effective and identified as a rich source of natural dietary antioxidants. Arabinoxylan (AX), a polysaccharide that serves as a carrier for phenolic acids, and proteins are major components of wheat bran, exhibiting promising antioxidant properties. However, the presence of polymer network in AX may limit the accessibility of phenolic acids, and other components such as fat and starch could significantly influence the antioxidant capacity of AX. In this study, AX and protein from different types of wheat bran were extracted and subjected to enzymatic hydrolysis after removing fat and starch, resulting in the production of xylo-oligosaccharides (XOS) and peptides, respectively. The antioxidant capacities of XOS and protein hydrolysates were evaluated, along with their inhibitory effects on collagenase, elastase, and hyaluronidase involved in aging. The results demonstrates that the XOS and protein hydrolysate, obtained after a proper extraction and enzymatic hydrolysis, exhibited significant higher antioxidant capacity, and pronounced inhibition of the aforementioned aging-related enzymes compared with previous studies. This study contributes to the evaluation

and utilization of AX and protein from wheat bran, maximizing their potential value in the production of functional ingredients for antioxidant and anti-aging purposes.

Keywords: wheat bran; oligosaccharide; peptide hydrolysate; antioxidant; anti-aging.

6.1. Introduction

Reactive oxygen and nitrogen species (RONS) produced in cellular activity result in oxidative stress, which damages DNA, proteins, and membrane lipids. It is widely known as an important inducer in aging and associated with age-related and chronic diseases, such as Alzheimer's, cancer, and cardiovascular disease (Liguori et al., 2018). Endogenous antioxidant systems in the human body, including various enzymes and nonenzymatic compounds are the first guard to eliminate the damage caused by RONS (Nimal et al., 2022). Unfortunately, endogenous antioxidant systems may be insufficient and slowing down in extreme conditions during aging (Giordano, Darley-Usmar, & Zhang, 2014). Therefore, great interest has been generated in natural antioxidants that are promoted to assist modulating cellular oxidative status (Feng et al., 2023; Gu et al., 2023; S. Wang, Melnyk, Tsao, & Marcone, 2011).

Wheat-based food was demonstrated as a good source of natural dietary antioxidants and consumable for population considering the relatively low cost (Dziki, Rózyło, Gawlik-Dziki, & Świeca, 2014). Among the fractions of wheat, wheat bran, as the byproduct in wheat milling processing, is considered to most concentration of phenolic acids that have significant antioxidant properties in vitro such as inhibition of low-density lipoprotein and DNA oxidation, and scavenging activities against peroxyl, 2,2-diphenyl-1-picryhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation (Ghamry et al., 2023; Y. Li et al., 2023; Shang et al., 2021; Yuan, Wang, & Yao, 2005).

Arabinoxylan (AX) is one of the majority components in wheat bran and associated with various health benefits not only antioxidant capacity, but microbiota regulation, short-chain fatty acid production, and antitumor activity (Mendis & Simsek, 2014). The other component, protein, exhibits a promising antioxidant and diverse bioactivates when turned into bioactive peptide after enzymatic hydrolysis (Y. Li et al., 2023). In particular, bioactive peptides have exhibited antioxidant, anti-inflammatory, and anti-microbial properties, as well as the ability to inhibit enzymes involved in aging of the skin (collagenase, elastase, hyaluronidase) which has revealed that they have great potential as active ingredients in cosmeceutical products (Aguilar-Toalá, Hernández-Mendoza, González-Córdova, Vallejo-Cordoba, & Liceaga, 2019).

Several studies have revealed that the antioxidant capacity of AX is a result from the attached phenolic acids that contain resonance-stabilized structure to trap radicals ((Dupoiron et al., 2018; Kaur et al., 2019; S. Li et al., 2021; Shang Lin, Agger, Wilkens, & Meyer, 2021; Lu et al., 2015)). However, the polymer network of AX could hide the phenolic acid attached on the side chain, reducing the ability of antioxidant. Xylo-oligosaccharides (XOS) hydrolyzed from AX by xylanase showed higher antioxidant ability than wheat bran used as a whole. While these results cannot indicate the antioxidant ability of XOS properly since other components, such as fat, starch, and protein, may have significant impact on the result. Therefore, the first aim of this project is to properly evaluate the antioxidant ability of XOS and peptide hydrolysates from hard and soft wheat bran in order to maximize its value. The composition of the extracted XOS and peptide hydrolysates will be analyzed in terms of proximate composition, microstructural characteristics using scanning electron microscopy (SEM).

In addition to oxidation damage caused by the accumulation of RONS, skin-aging is also caused by the degradation or formation prevention of collagen and elastin by enzymes, such as collagenase, elastase, and hyaluronidases. Since the hydrolysate of protein and AX are peptide and oligosaccharide respectively, and they could be the potential substrates of aging related enzymes prementioned (Aguilar-Toalá et al., 2019), the second aim of this project is to evaluate impact of protein hydrolysate and XOS on anti-aging effects through in-vitro collagenase, elastase, and hyaluronidase inhibition activates to find new functional ingredients for anti-aging.

6.2. Materials and methods

6.2.1. Materials

Hard red winter wheat bran (protein content: 16.8%) and soft red winter wheat bran (protein content: 13.3%) were obtained from Hal Ross Flour Mill (Manhattan, Kansas) as generous donations. The bran samples were collected and processed using a Pin Mill with six passes to achieve a fine particle size suitable for extraction. Following the milling process, the milled bran samples were collected and stored at refrigerator until further extraction was performed. The Econase XT xylanase powder was kindly provided from AB Vista (Plantation, FL). The chemicals and reagents were supplied from Fisher Scientific (Fairlawn, NJ) or Sigma-Aldrich (St. Louis, MO).

6.2.2. Extraction of xylo-oligosaccharide and peptide hydrolysate

Two bran samples were subjected to a series of treatments to obtain xylo-oligosaccharide and peptide hydrolysate. The extraction procedure involved the following steps: The samples were first defatted using 4 mL/g hexane extraction for one hour, followed by de-starching using α-amylase, (500U/mg) at pH 6 and deproteinization using alcalase (0.8U/g) at pH 7.5. The deproteinization was performed for 30 minutes at 60°C. The resulting peptide hydrolysate was boiled to inactivate the enzymes. Xylanase was then added on the pre-treated bran sample in 20 mM sodium acetate buffer at pH 5.5, and the mixture was incubated at 50°C for 12 hours. After that, the mixture was centrifugated at 8000g for 15 minutes to separate the supernatant. The supernatant was then dialyzed for three days in a refrigerator and lyophilized to obtain xylo-oligosaccharide.

6.2.3. Proximate composition

The protein contents of the peptide hydrolysate samples were analyzed using LECO, following the AOAC official method 990.03 with a factor of 5.7 (AOAC, 2006). The determination of crude fat, moisture, ash, crude fiber, and β-glucan contents were carried out using AOAC official methods 920.39(A), 934.01, 942.05, 978.10 and 995.16, respectively (AOAC, 2006). The calculation of carbohydrates was performed by subtracting the sum of protein %, ash %, crude fat%, and moisture content% from 100%. To obtain a complete amino acid profile of the peptide hydrolysate samples, the analysis was conducted using AOAC official method 982.30 E (a, b, c), as described in chapter 45.3.05 (AOAC, 2006). The sugar profile of the xylo-oligosaccharide sample was determined based on a previously published method (Oxley et al., 2006).

6.2.4. SDS-PAGE of peptide hydrolysate

Peptide hydrolysate samples were boiled with 2 × sample buffer for 10 min and then subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis using a 16.5% Mini-PROTEAN® Tris-Tricine Gel (Bio-Rad, CA, US) under a constant voltage of 110 V. The gel electrophoresis was performed using the Bio-Rad PowerPac 1000 system (Hercules, CA, US). To determine the molecular weight of the peptide hydrolysate samples, ultra-low range molecular-weight standards (1.06–26.6 kDa) (Sigma-Aldrich, MO, US) were used as ladder.

6.2.5. Scanning electron microscope (SEM)

The microstructure of wheat bran, xylo-oligosaccharide samples from hard and soft wheat bran were examined using a Scanning electron microscope (SEM) model S-3500N (Hitachi Co. Japan) at magnifications of 50x, 100x, and 500x. Furthermore, the residue samples obtained after the extraction were observed at a magnification of x50. The SEM analysis was performed with a sample working distance of 51 mm and an acceleration voltage (HT) of 5 kV.

6.2.6. Total phenolic contents of peptide hydrolysate and arabinoxylan

The total phenolic contents (TPC) of the peptide hydrolysate and xylo-oligosaccharide extracted from hard and soft red winter wheat bran were quantified using a previous method (Tian et al., 2021). The TPC was reported as mg gallic acid equivalents (GAE)/g weight of peptide hydrolysate and arabinoxylan sample.

6.2.7. Antioxidant DPPH radical scavenging capacity

The DPPH radical scavenging capacity was assessed using a previous reported method (Tian et al., 2021). In brief, the peptide hydrolysate and xylo-oligosaccharide samples (0.05 mL) were mixed with 3.9 mL DPPH solution in the darkness. After 25 minutes, the mixture was centrifuged at 8000g for 10 minutes, and the absorbance at 517 nm was measured. The DPPH value was expressed as micromole Trolox equivalence per gram of sample (μmol TE/ g).

6.2.8. Antioxidant ABTS radical scavenging capacity

The ABTS •+ scavenging capacities of the peptide hydrolysate and xylo-oligosaccharide were determined according to the previous method (Tian et al., 2021). Under dark conditions, 0.13 mM 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) solution (3.9 mL) was added with the sample (0.05 mL) and incubated for 10 minutes. Subsequently, the absorbance of the resulting mixture was recorded at 734 nm. Similar to DPPH assay, the ABTS value was expressed as μmol TE/ g.

6.2.9. Collagenase inhibitory assay

Collagenase inhibitory activity was evaluated following a modified method (Van Wart & Steinbrink, 1981). A 50 mM tricine buffer (pH 7.5) containing 10 mM calcium chloride and 400 mM sodium chloride was prepared. FALGPA (1 mM) and collagenase from clostridium histolyticum (0.1 units) were prepared in tricine buffer. Each peptide hydrolysate or xylo-oligosaccharide sample (1000 μg/mL, 30 uL) in distilled water was incubated with collagenase (10 μL) in the tricine buffer (60 μL) for 15 minutes before adding 20μL of FALGPA. The absorbance at 345 nm was measured for 20 minutes with distilled water as a control. The inhibition (%) was calculated using the formula ($Absorbance_{sample}$ - $Absorbance_{control}$) / ($Absorbance_{control}$) × 100.

6.2.10. Elastase inhibition assay

Elastase inhibitory activity was determined following a previous method (Ritthibut, Oh, & Lim, 2021). The reaction mixture consisted of 30 μL of the hydrolysate or xylo-oligosaccharide sample (1000 μg/mL) in distilled water, 100 μL of 2 mM Tris-HCl buffer (pH 8.0), and 10 μL of Porcine pancreatic elastase (1 U/mL) in the Tris buffer. After incubation for 15 minutes, 40 μL of

3 mg/mL N-Succ-Ala-Ala-Ala-p-nitroanilide was added. The mixture was further incubated 30°C for 20 minutes, and the absorbance was measured at 410 nm. Elastase inhibition activity was calculated using the same formula as the collagenase inhibitory assay.

6.2.11. Hyaluronidase inhibition assay

Hyaluronidase inhibitory activity was measured according to a modified method (Liyanaarachchi, Samarasekera, Mahanama, & Hemalal, 2018). Type-1-S bovine testes hyaluronidase (4000 units/mL, 10uL) prepared in 0.1 M acetate buffer (pH 3.5) was mixed with each sample (0.5 mg/mL) dissolved in distilled water and incubated at 37 °C for 20 minutes. Calcium chloride (20ul, 12.5mM) was added and incubated at 37 °C for 10 minutes, followed by the addition of 50 μL of sodium hyaluronate (5uL, 12 mg/mL) dissolved in 0.1 M acetate buffer (pH 3.5), and further incubated at 37 °C for 40 minutes. Then, 30 μL of 0.9 M sodium hydroxide were added and incubated for 10 minutes. 50 μL of 4-(Dimethylamino) benzaldehyde solution (1% w/v 4-(Dimethylamino) benzaldehyde in 87.52% of 100% acetic acid and 12.48% of 10N hydrochloric acid) was added, and the mixture to was incubated at 37 °C for 10 minutes. Absorbance was measured at 585 nm, and the inhibition (%) was calculated using the same formula as the collagenase inhibitory assay.

6.2.12. Statistical analysis

All experiments were independently performed in at least duplicate. Statistical analysis was carried out using SAS Studio 3.7 (SAS Institute Inc., NC, US). The data were subjected to one-way analysis of variance (ANOVA), and the significant differences were determined using

Tukey's test. The results were expressed as mean ± standard deviation (SD), and the statistical significance was set at $p < 0.05$.

6.3. Results and discussion

6.3.1. Chemical characteristics and amino acid profile of peptide hydrolysate

The chemical composition of peptides hydrolysates derived from hard wheat bran (HP) and soft wheat bran (SP) are shown in **Table 1**. HP exhibited higher protein content (30.49%), moisture content (10.91%), and ash content (10.28%) compared to SP. The crude fat and fiber contents were similar between HP and SP. The total amino acid profile of HP and SP is also provided in **Table 1**. Glutamic acid, aspartic acid, and arginine were the most predominant amino acids in both HP and SP, consistent with previous studies on wheat bran and germ protein isolate (Abarghoei, Goli, & Shahi, 2023; Y. Li et al., 2023). The lysine content, which is considered a limiting amino acid, was higher in HP than in SP. HP contained higher levels of both essential (EAA) and non-essential amino acids. Additionally, the hydrophobic amino acids, including Gly, Ala, Val, Leu, Pro, Met, Phe, Trp, and Ile were identified as contributors to the antioxidant activity. The total content of hydrophobic amino acids was 163.90 mg/g in HP and 134.50 mg/g in SP. The higher content of hydrophobic amino acids in HP compared to SP suggests a potential for enhanced antioxidants activity and improved interaction with free radicals, thereby mitigating oxidative damage (Terriente-Palacios, Rubiño, Hortós, Peteiro, & Castellari, 2022).

6.3.2. Sugar composition of xylo-oligosaccharides

The sugar composition of xylo-oligosaccharides derived from hard (HXOS) and soft wheat bran (SXOS) fractions are presented in **Table 2**. HXOS primarily consisted of xylose and arabinose, which accounted for up to 76.8% of the monomeric sugar units, along with minor amounts of glucose, galactose, and mannose. In comparison, SXOS exhibited a relatively lower xylose, similar arabinose, and an increased glucose content. The Ara to Xyl ratio in SXOS was significantly higher than that in HXOS, indicating greater degrees of substitution in SXOS (Pietiäinen et al., 2022). Moreover, the β-glucan content of HXOS was similar to that in SXOS. β -glucan primarily localized in the aleurone layers of wheat bran (Antoine et al., 2003b). The noticeable presence of glucose in SXOS, rather than in HXOS, can be attributed to the structure of soft wheat bran, which is more prone to partial amorphous cellulose formation under high-temperature extraction conditions and the degradation of hemicellulose, including β-glucan.

6.3.3. SDS-PAGE

The electrophoretic patterns of peptide hydrolysis extracted from hard (HP) and soft wheat bran (SP) are depicted in **Figure 1**. The molecular weight distribution of both peptide hydrolysis was found in the range of 1.06 to 17kDa, indicating the effect successfully hydrolysis of the major protein fractions by the alcalase. No significant differences were observed in the SDS-PAGE profiles when comparing HP and SP, suggesting that the use of same proteinases did not result in noticeable changes in the molecular weight distribution. This finding aligns with a previous study where alcalase wheat germ protein hydrolysate achieved a molecular weight to less than 14.4kDa (Hosseini et al., 2022). Alcalase, derived from *Bacillus licheniformis*, encompasses several proteinases with broad specificity, leading to improved wheat protein hydrolysis and increased degree of hydrolysis compared to enzymes such as Protamex, Neutrase, or Flavourzymes (F. R. Liu, Wang, Wang, & Chen, 2013).

6.3.4. Microstructural characteristics

The microstructures of the xylo-oligosaccharides extracted from hard wheat bran (HXOS) and soft wheat bran (SXOS) were investigated by SEM. The SEM images of hard and soft wheat bran, HXOS, SXOS and the residues after the extraction are depicted in **Figure 2**. Compared to the wheat bran, HXOS and SXOS displayed laminated and fragment-like structures. A previous study has reported a comparable sheet-like structure for arabinoxylan extracted from wheat bran (Shang et al., 2021). The SEM images of the residues after peptide hydrolysates and XOS extractions revealed the removal of starch, protein, and hemicellulose fractions, leaving behind residues with a semi-cylindrical morphology and a rough surface. The enzymatic hydrolysis process was observed to disrupt the structure of wheat bran, as evidenced by the distinct edges of broken cell walls, indicating possible disruption of cellulose-hemicellulose cross-linking and xylan solubilization. Similar findings have been reported on wheat bran oligosaccharides using enzymatic hydrolysis assisted by microwave treatment (T. H. Wang & Lu, 2013).

6.3.5. Antioxidant properties

As illustrated in **Figure 3**, xylo-oligosaccharides and protein hydrolysates exhibited promising total phenolic contents (TPC) and antioxidant capacities. The TPC of hard wheat bran xylo-oligosaccharides (HXOS) was determined to be 39.63 mg GAE /g, which exhibited a significantly higher value compared to soft wheat bran xylo-oligosaccharides (SXOS) (32.06 mg GAE /g). Additionally, the hard wheat bran peptide hydrolysate (HP) demonstrated a TPC of 31.76

mg GAE /g, significantly higher than soft wheat bran peptide hydrolysate SP (29.91 mg GAE /g). These findings suggest that the extraction method employed was more efficient in enhancing the extractable antioxidant components, particularly phenolics from hard wheat bran rather than soft wheat bran. It is worth mentioning that wheat bran has previously been reported to contain approximately 2 mg GAE/g TPC, which is 3-14 folds higher than the TPC in wheat flour, depending on the particle size, and the higher TPC contributed to higher antioxidant activates (Suyun Lin et al., 2022c; Lu et al., 2015). Our results indicate a significantly higher TPC in comparison to precious reports on wheat bran or wheat flour. The enzymatic hydrolysis employed has shown promise in increasing the phenolic contents. Consistent with previous findings, xylanase has been reported to degrade the xylan backbone of wheat bran, facilitating the release of phenolic acid (Faulds, Mandalari, LoCurto, Bisignano, & Waldron, 2004). Wheat bran predominately consists of arabinoxylan, different types of xylanases exhibited distinct specificities for xylan hydrolysis based on the backbone substitution, can significantly alter the effectiveness for degrading wheat bran (Faulds et al., 2004).

In terms of investigating in-vitro antioxidant activities, DPPH and ABTS assays are widely utilized. Both assays are based on and named after electron-deficient radical (D. Zhang et al., 2011). The ABTS assay, employed a higher wavelength, is less susceptible to interference from colored samples (Y. C. Huang, Chang, & Shao, 2006). Regarding DPPH radical scavenging activities, no significant difference was observed between SXOS and HXOS, while HP exhibited higher scavenging activity than SP. Moreover, HXOS demonstrated significantly stronger radical cation ABTS+ scavenging activities compared to SXOS, and HP exhibited higher activity than SP. The increased antioxidant activities in HP compared to SP can be attributed its higher amino acid and TPC. The scavenging ability of wheat bran protein hydrolysates for ABTS radicals were found to be stronger than that for DPPH radicals, which can be attributed to the release of more polar amino acids, favoring the scavenging of ABTS radicals. Overall, alcalase successfully hydrolyzed the proteins from hard and soft wheat bran into small peptides, as confirmed by the amino acid profiles and SDS-PAGE. Similar results have been previously reported, wherein the use of peptidase or pepsin significantly hydrolyzed ester or glycosidic bonds and increased the release of bran antioxidants (Y. Li et al., 2023).

6.3.6. The inhibitory effects of collagenase, elastase, and hyaluronidase

Collagenase and elastase are enzymes implicated in the degradation of collagen, elastin fibers and hyaluronan, leading to the manifestation of sagging, wrinkled and less supple skin (Castro-Jácome et al., 2021; Stern & Jedrzejas, 2006). The inhibitory effects of peptide hydrolysates and xylo-oligosaccharides on collagenase, elastase, and hyaluronidase activities are illustrated in **Fig. 4.** The results indicate that both hard wheat bran xylo-oligosaccharides (HXOS) and soft wheat bran xylo-oligosaccharides (SXOS) exhibited greater collagenase inhibition activity (41.2-49.4%) compared to hard wheat bran peptide hydrolysates (HP) and soft wheat bran peptide hydrolysates (SP) (11.2 – 12.4%) at a concentration of 1 mg/mL. However, there were no significant differences in elastase inhibition activity among HXOS, SXOS, HP and SP (20.5 – 22.9%). Notably, HXOS displayed significant hyaluronidase inhibition activities (49.9%) compared to other samples (14.7 – 22.7%) at the concentration of 0.5 mg/mL. Similarly, low molecular weight peptides derived from sorghum have been reported to exhibit modest collagenase inhibition activity (less than 10%) and elastase inhibition activity (less than 25%) (Castro-Jácome et al., 2021). Furthermore, all our samples demonstrated stronger elastase inhibition activity compared to the reported plant extracts, which only exhibited 20% inhibition at a concentration of

500 mg /mL (Liyanaarachchi et al., 2018). Therefore, the observed inhibitory effects of HXOS, SXOS, HP and SP can be attributed to their interactions with the active sites and allosteric sites of the enzymes, thereby preventing binding with collagen elastin or hyaluronan.

6.4. Conclusion

Xylo-oligosaccharides and peptide hydrolysates derived from hard and soft wheat bran demonstrated significant antioxidant potential and inhibitory effects against collagenase, elastase, and hyaluronidase. Notably, hard wheat bran xylo-oligosaccharide (HXOS) exhibited the highest hyaluronidase inhibition activity (49.9%) at a concentration of 0.5 mg/mL. HXOS comprised 76.8% xylose and arabinose as the major monomeric sugar units, which were significantly higher than those in soft wheat bran xylo-oligosaccharide (SXOS). In terms of peptide hydrolysates, hard wheat bran peptide hydrolysate (HP) exhibited higher protein content (30.49%) and ash content (10.28%) compared to soft wheat bran peptide hydrolysate (SP) (24.25% protein, 7.96% ash). The molecular weight distribution of HP and SP ranged from 1.06 to 17kDa with glutamic acid, aspartic acid, and arginine being the most abundant amino acids. HP contained higher levels of essential and non-essential amino acids, as well as hydrophobic amino acids. Furthermore, HP demonstrated higher total phenolic contents, DPPH and ABTS scavenging activities compared to SP. However, there were no significant differences between HP and SP in terms of collagenase, elastase and hyaluronidase inhibition activates. The total content of hydrophobic amino acids was 163.90 mg/g in HP and 134.50 mg/g in SP. The higher content of hydrophobic amino acids in HP suggests an enhanced potential antioxidants activity and improved interaction with free radicals, thereby offering protection against oxidative damage.

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Table 6.1. Proximate composition and amino acid composition of hard wheat bran peptide hydrolysate (HP) and soft wheat bran peptide hydrolysate (SP).

	HP	SP
Chemical composition		
Carbohydrate (%)	49.55 ± 0.24 [b]	60.66 ± 0.54 [a]
Crude protein (%) (N=5.7)	27.81 ±0.01 [a]	22.12 ± 0.03 [b]
Moisture (%)	10.91 ± 0.74 [a]	7.34 ± 1.26 [a]
Crude Fat (%)	1.30 ± 0.54 [a]	1.79 ± 0.75 [a]
Crude Fiber (%)	0.16 ± 0.01 [a]	0.14 ± 0.01 [a]
Ash (%)	10.28 ± 0.02 [a]	7.96 ± 0.01 [b]
Amino acid profiles (mg/g)		
EAA		
Threonine	9.60 ± 0.00 [a]	7.80 ± 0.00 [b]
Valine	14.50 ± 0.00 [a]	11.80 ± 0.00 [b]
Methionine	3.85 ± 0.05 [a]	3.10 ± 0.00 [b]
Isoleucine	10.05 ± 0.05 [a]	8.25 ± 0.05 [b]
Leucine	17.10 ± 0.00 [a]	14.20 ± 0.00 [b]
Phenylalanine	10.80 ± 0.00 [a]	8.90 ± 0.00 [b]
Histidine	8.05 ± 0.05 [a]	6.70 ± 0.00 [b]
Lysine	11.20 ± 0.00 [a]	9.40 ± 0.00 [b]
Tryptophan	4.15 ± 0.05 [a]	4.00 ± 0.00 [b]
Total EAA	89.30 ± 0.00 [a]	74.15 ± 0.05 [b]
NEAA		
Arginine	17.75 ± 0.05 [a]	15.20 ± 0.00 [b]
Proline	17.20 ± 0.00 [a]	14.25 ± 0.05 [b]
Aspartic Acid	24.85 ± 0.05 [a]	19.25 ± 0.05 [b]
Serine	11.40 ± 0.00 [a]	9.30 ± 0.10 [b]
Glutamic Acid	53.50 ± 0.10 [a]	43.55 ± 0.25 [b]
Glycine	14.70 ± 0.00 [a]	12.10 ± 0.00 [b]

Alanine	14.05 ± 0.05 [a]	11.20 ± 0.00 [b]
Cysteine	5.50 ± 0.00 [a]	5.15 ± 0.05 [b]
Tyrosine	4.95 ± 0.05 [a]	4.60 ± 0.00 [b]
Hydrophobic	106.40 ± 0.20 [a]	87.80 ± 0.10 [b]
Total NEAA	163.90 ± 0.00 [a]	134.50 ± 0.40 [b]
Total AA	253.20 ± 0.7 [a]	208.65 ± 0.45 [b]

Different letters in the same column indicate significant differences ($p < 0.05$). EAA, essential amino acids; NEAA, non-essential amino acids; Hydrophobic, glycine, alanine, isoleucine, leucine, methionine, phenylalanine, proline, valine, trypot=phan; EAA radio (%) is the ratio of EAA to total AA. Samples with different letters differ significantly ($p < 0.05$).

Table 6.2. Sugar composition (relative mole %) and b-glucan of xylo-oligosaccharides extracted from hard (HXOS) and soft red wheat brans (SXOS).

Sample	Xylose (%)	Arabinose (%)	Mannose (%)	Glucose (%)	Galactose (%)	Ara/Xyl	b-Glucan (%)
HXOS	46.69 ± 0.19 [a]	30.11 ± 0.22 [a]	2.27 ± 0.36 [a]	15.05 ± 0.52 [b]	5.87 ± 0.19 [a]	0.64 ± 0.01 [b]	0.12 ±0.01 [a]
SXOS	26.02 ±0.29 [b]	21.93 ± 0.29 [a]	1.90 ± 0.15 [a]	47.81 ± 0.15 [a]	2.63 ± 0.29 [b]	0.84 ± 0.00 [a]	0.13 ± 0.01[a]

Ara/Xyl, ratio of arabinose and xylose. Different letters in the same column indicate significant differences ($p < 0.05$).

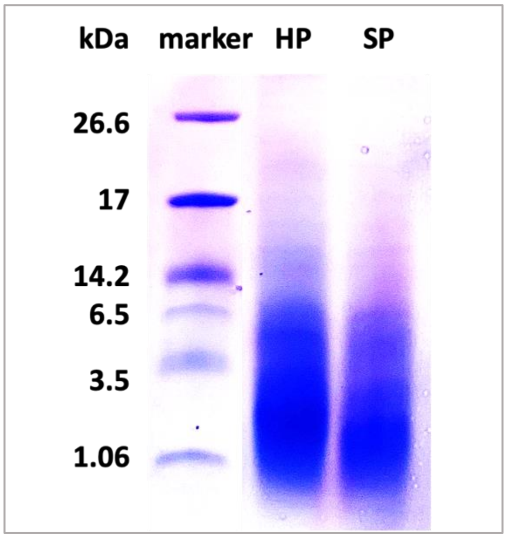


Figure 6.1. Electrophoretic (SDS-PAGE) profile of hard (HP) and soft (SP) wheat bran peptide hydrolysates.

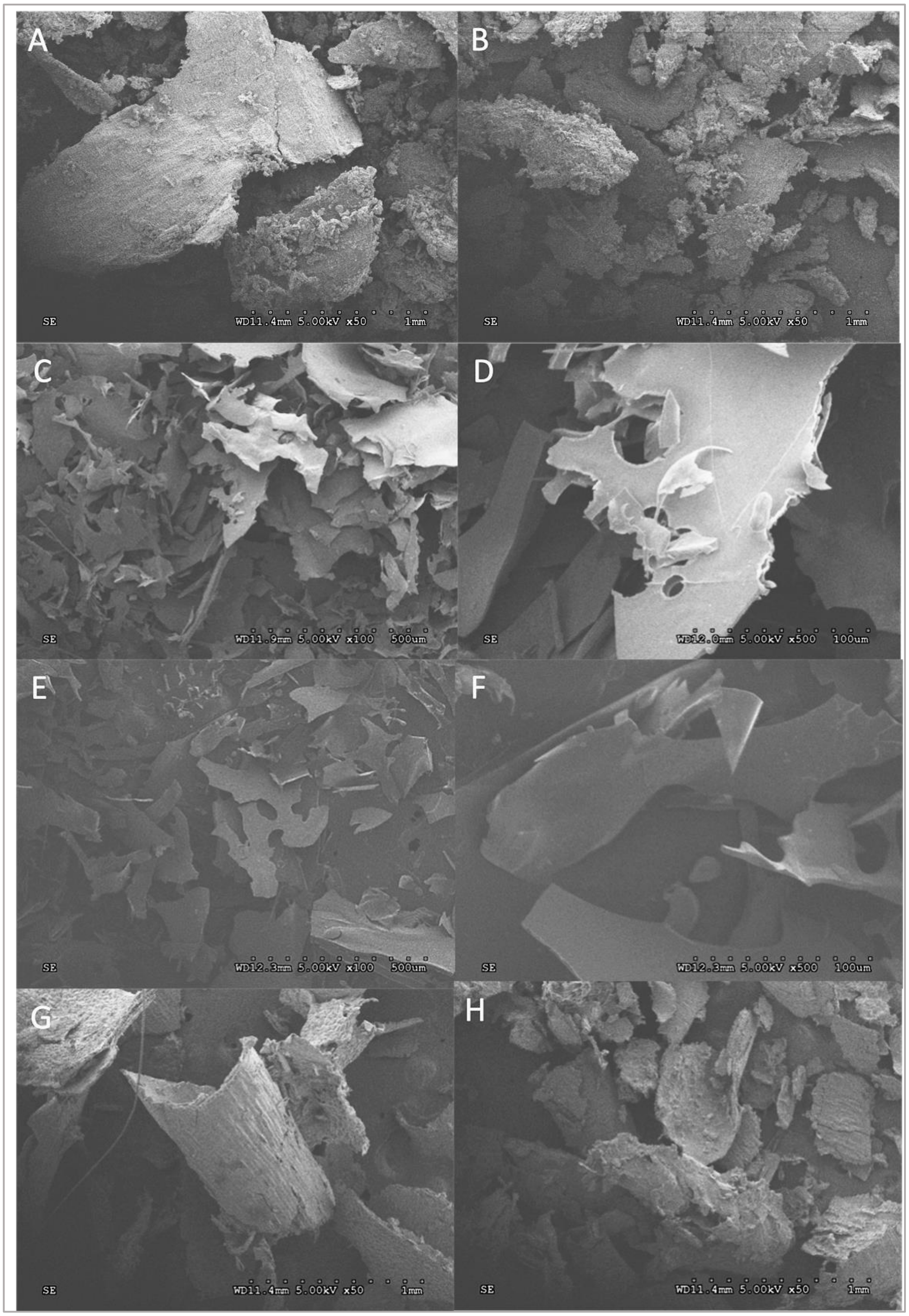


Figure 6.2. Scanning electron microscopy (SEM) images of wheat bran, xylo-oligosaccharide (XOS) and residues.

(A) hard wheat bran; (B) soft wheat bran; (C, D), HXOS; (E, F), SXOS; (G), Residue from hard wheat bran; (H) Residue soft wheat bran.

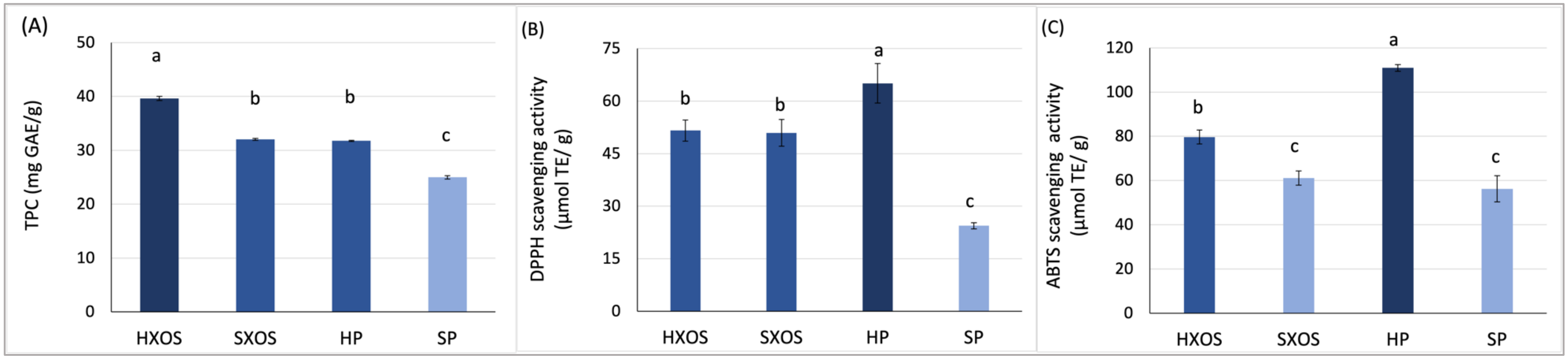


Figure 6.3. Total phenolic contents, DPPH, ABTS antioxidant activities of peptide hydrolysates and xylo-oligosaccharides. (A), Total phenolic contents (TPC); (B), DPPH scavenging activity; (C), ABTS scavenging activity; HXOS, SXOS, xylo-oligosaccharide extracted from hard and soft wheat bran, respectively; HP, SP, peptide hydrolysate extracted from hard wheat bran and soft wheat bran, separately; GAE, gallic acid equivalence; TE, Trolox equivalence. The vertical bars represent the standard deviation; Values marked by the different letter are significant differences ($p < 0.05$).

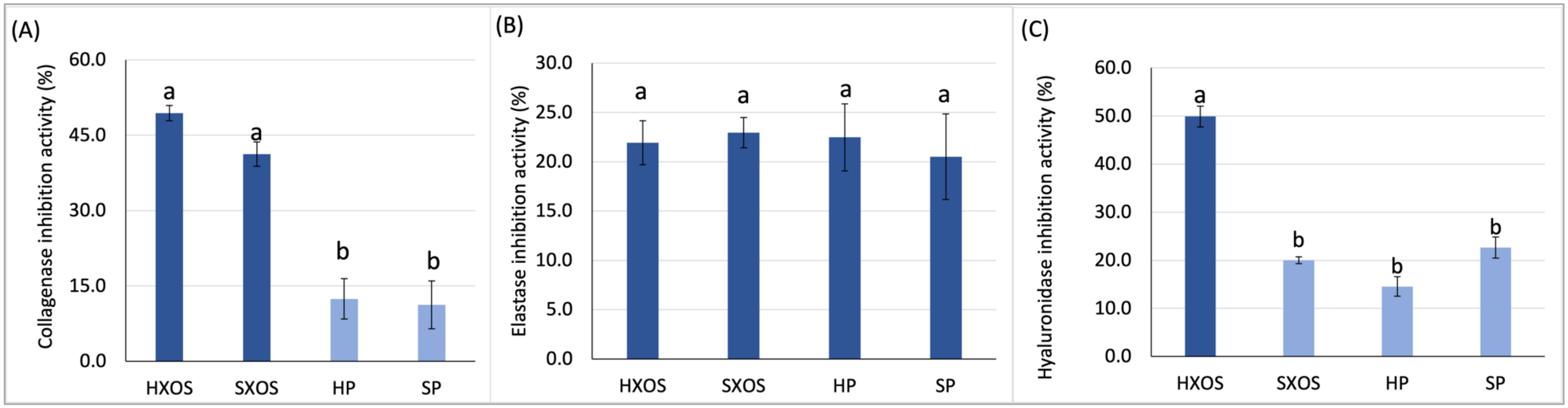


Figure 6.4. (A) Collagenase inhibition activities, (B) elastase inhibition activities, (C) hyaluronidase inhibition activities of peptide hydrolysates and xylo-oligosaccharides.

HXOS, SXOS, xylo-oligosaccharide extracted from hard and soft wheat bran, respectively; HP, SP, peptide hydrolysate extracted from hard wheat bran and soft wheat bran, separately; GAE, gallic acid equivalence; TE, Trolox equivalence. The vertical bars represent the standard deviation; Values marked by the different letter are significant differences ($p < 0.05$).

Chapter 7 - Conclusions and future prospectives

7.1. Conclusions

Wheat bran has emerged as a potential source leading to a reduced risk of obesity and colon diseases. Although there are well-documented benefits of consumption, there is still a severe deficiency in the human diet. The research aims to investigate the effects of bran particle size, assess correlations between white flour (WF) and WWF, analyze the chemical composition of WWF, and explore the potential of wheat bran extracts to optimize the production of high-quality whole wheat bread.

Previous studies related to the chemical composition, phytochemical and antioxidants of wheat bran, and bread quality were reviewed in Chapter 1. Chapter 2 describes the impact of particle size of brans from four different types of wheat, containing various protein contents, on dough rheology properties and bread quality. The results show that reducing wheat bran particle sizes to a medium size (315–363 μm) significantly improves the bread-making quality of whole wheat flour. Additionally, this process results in the addition of more nutrients, such as fiber and protein, due to the inclusion of wheat bran. These findings have the potential to facilitate the standardization of wheat bran addition in whole wheat flour (WWF) and contribute to effective industrial production. Chapter 3 described the correlation analysis between WF from 64 wheat genotypes and its corresponding WWF on dough rheology properties and bread-making performance when being used in the medium particle size acquired in Chapter 2. The results revealed a strong correlation between WF and WWF in dough rheology, which could be very useful in predicting the quality of dough-based bakery products, such as cookies and pizza, leading to economic benefits for both breeders and producers and contributing to the enhancement of the baking industry.

However, the correlation between WF and WWF in predicting bread-making properties using protein content is not strong enough. Therefore, in Chapter 4, the study was conducted to understand the impact of the components and composition of bran on bread-making properties. The physicochemical properties, including phenolics, antioxidant, and oxidation-related enzyme characteristics in wheat bran, were determined, followed by an investigation into their effect on the qualities of reconstituted whole wheat flour dough and bread-making. Notably, lipid content and lipoxygenase activity were demonstrated to be significantly related to the dough and bread properties. These findings make a valuable contribution to the assessment of whole wheat flour quality and its effective industrial application in baking.

Building upon the findings accomplished in Chapter 4, we decided to capitalize the components in bran that have positive effects on bread-making properties. Consequently, in Chapter 5, a simple and straightforward method was developed and evaluated to extract the valuable components from wheat bran. The water-soluble components extracted from wheat bran were used as additives to increase bread volume, resulting in a remarkable 16% increase in volume. These significant findings have the potential to elevate the economic value of wheat bran and reduce costs in the bakery industry.

In addition to its utilization as additives in baking, bran holds huge potential in antioxidation processes since it contains phenolic acids that have been proven to possess antioxidant activity. Chapter 6 describes another method to maximize the value of wheat bran by extracting the hydrolysates of arabinoxylan in bran, where phenolic acids are attached along with the protein hydrolysates after the removal of other interfering components. Both protein and arabinoxylan hydrolysates exhibited notable antioxidant and anti-aging properties in vitro, compared with findings from previous studies. These findings may have implications for the food and nutraceutical industries.

7.2. Future prospective

Besides topics that have been described in this thesis and many previous studies, the following fields have not been extensively studied but may worth further investigating in the future:

1. Sensory evaluation for a comprehensive assessment of the feasibility of using wheat bran extract, as described in Chapter 5, as additives to increase bread volume.
2. Characterization of the bio-active peptide possessing the antioxidant properties presented in Chapter 6 using LC/MS.
3. Apply the hydrolysates of protein and arabinoxylan on in vivo models, such as mice and rabbits, followed by the evaluation of their effect on controlling oxidative stress condition. Simultaneously, proteomics could be used to understand the antioxidation effect and mechanism of these hydrolysates in cells in order optimizing their utilization.

Appendix - Supplementary data

Chapter 3- Table S1. Flour milling yield (%) of different wheat line.

Sample No.	White flour yield (%)
1	68
2	68.8
3	68.7
4	66.8
5	66.8
6	61.5
7	67.8
8	69.5
9	67.3
10	66
11	69.2
12	66.6
13	67.7
14	67.9
15	63.6
16	69.4
17	64.3
18	68.6
19	67.6
20	66.8
21	67.7
22	65.1
23	63.5
24	65.9
25	62.6
26	64.3
27	67.8
28	68.4
29	68.2
30	69.2
31	67.5
32	69.8
33	64.5
34	68.9
35	69.1

36	68.5
37	68.9
38	70
39	69.3
40	68.7
41	70.7
42	66.7
43	70.7
44	67
45	69.4
46	66.8
47	64
48	66.9
49	65.7
50	69.3
51	65
52	66.5
53	67.6
54	69.6
55	64.6
56	67.7
57	67.4
58	65
59	67.4
60	67
61	65.6
62	65.5
63	69.1
64	66.7
Average	67.230
Std	1.993

Chapter 3- Table S2. Moisture (%), protein contents (%), and water retention capacity (WRC) (%) of white (WF) and whole wheat flour (WWF).

	White flour (WF)			Whole wheat flour (WWF)		
No.	Moisture (%)	Protein (%)	WRC (%)	Moisture (%)	Protein (%)	WRC (%)
1	13.90±0.04	8.72±0.19	54.14±0.7	11.85±0.01	10.86 ± 0.20	59.53±0.95
2	13.85±0.10	8.48±0.13	55.25±1.06	11.95±0.11	10.52 ± 0.09	60.91±0.14
3	13.29±0.04	7.93±0.08	55.17±0.68	11.4±0.11	10.00 ± 0.03	61.17±0.26
4	13.85±0.09	8.93±0.16	56.1±0.4	11.79±0.04	10.55 ± 0.11	64.33±1.25
5	14.02±0.11	8.59±0.08	54.42±0.5	11.81±0.06	10.37 ± 0.06	63.9±0.78
6	13.74±0.00	6.98±0.16	51.92±0.26	11.83±0.08	8.86 ± 0.26	64.58±0.16
7	13.74±0.06	8.08±0.11	52.93±0.13	11.77±0	9.55 ± 0.03	61.25±0.13
8	14.01±0.03	8.06±0.10	52.6±0.38	11.33±0.13	9.95 ± 0.03	59.38±0.18
9	14.06±0.03	8.56±0.05	52.1±0.12	11.66±0.08	10.29 ± 0.09	61.43±0.56
10	14.01±0.09	8.89±0.16	54.22±0.9	11.67±0	11.06 ± 0.11	63.62±0.84
11	13.83±0.05	8.54±0.20	51.25±0.06	11.94±0	10.20 ± 0.11	61.01±1.2
12	14.01±0.03	8.00±0.10	54.92±0.12	12.04±0.16	9.41 ± 0.06	63.3±0.92
13	13.88±0.03	8.60±0.02	65.25±1.4	12.23±0.1	10.20 ± 0.11	62.66±0.25
14	13.92±0.01	9.15±0.15	61.5±0.87	11.73±0.11	11.54 ± 0.20	63.41±1.06
15	13.73±0.09	8.34±0.08	56.6±0.91	12.45±0.04	10.40 ± 0.26	65.71±0.89
16	13.66±0.11	7.73±0.08	53.01±0.09	11.56±0.06	9.43 ± 0.26	59.49±0.51
17	13.38±0.05	7.12±0.08	51.06±0.51	11.26±0.05	9.52 ± 0.06	62.58±1.31
18	13.55±0.08	9.22±0.06	59.23±0.26	11.28±0.04	11.09 ± 0.14	63.74±0.55
19	13.58±0.03	8.20±0.11	52.65±0.09	11.85±0.17	10.06 ± 0.09	61.19±0.59
20	13.68±0.07	8.90±0.08	54.17±0.4	11.53±0.02	11.23 ± 0.11	61.82±0.68
21	13.63±0.06	7.97±0.08	53.91±0.27	11.74±0.08	10.17 ± 0.03	60.17±0.73
22	13.28±0.08	8.03±0.15	56.08±0.79	11.61±0.01	9.95 ± 0.14	62.99±0.05
23	13.49±0.11	8.18±0.13	53.21±0	11.48±0.10	10.40 ± 0.09	65.12±1
24	13.58±0.01	7.71±0.13	64.57±1.22	11.93±0.04	9.72 ± 0.03	61.99±0.28
25	13.69±0.04	7.42±0.14	50.83±1.04	11.73±0.16	9.92 ± 0.06	62.26±1.01
26	13.84±0.01	8.07±0.13	68.27±0.08	12.53±0.05	9.92 ± 0.06	63.57±0.5
27	13.54±0.01	8.67±0.06	54.2±0.17	11.99±0.02	10.63 ± 0.03	63.65±0.17
28	13.69±0.01	8.45±0.20	52.97±0.65	11.59±0.11	10.32 ± 0.06	61.26±0.01
29	14.07±0.08	8.95±0.12	68.16±0.39	12.18±0.07	10.66 ± 0.11	62.6±0.25
30	13.49±0.07	8.96±0.08	66.27±0.65	11.4±0.04	10.86 ± 0.20	62.78±0.85
31	13.71±0.08	8.44±0.05	55.1±0.98	11.5±0.02	10.77 ± 0.17	62.06±0.4
32	13.71±0.06	8.78±0.11	53.11±0.07	11.62±0.04	10.74 ± 0.09	61.07±0.28
33	13.98±0.03	10.9±0.01	58.03±0.39	11.60±0.06	12.43 ± 0.40	62.74±0.84

34	13.97±0.01	11.73±0.05	59.91±1.1	11.79±0.04	13.59 ± 0.03	64.4±1.36
35	14.00±0.02	10.65±0.08	55.33±0.85	11.43±0.03	12.74 ± 0.03	63.85±0.12
36	14.11±0.08	11.34±0.10	58.08±0.41	11.67±0.09	13.62 ± 0.00	63.44±0.3
37	14.31±0.03	10.84±0.04	64.54±0.43	11.86±0.02	12.68 ± 0.03	62.51±0.03
38	14.16±0.07	12.55±0.06	56.79±0.05	11.77±0.09	14.56 ± 0.03	60.7±0.8
39	13.86±0.05	13.21±0.01	57.91±0.52	10.89±0.76	15.25 ± 0.03	61.98±1.1
40	14.09±0.06	12±0.08	56.84±0.49	12.85±0.01	14.05 ± 0.09	63.92±0.42
41	14.3±0.05	11.23±0.07	57.33±0.18	12.9±0.16	13.48 ± 0.03	64.52±0.12
42	14.22±0.13	10.88±0.09	56.7±0.59	12.58±0.1	13.25 ± 0.09	64.07±0.31
43	14.45±0.02	11.29±0.06	55.67±0.09	12.61±0.01	13.68 ± 0.17	62.99±0.48
44	13.69±0.02	8.18±0.09	52.52±0.24	12.34±0.01	10.37 ± 0.40	61.98±0.82
45	13.57±0.04	9.23±0.01	53.98±0.29	12.35±0.03	11.37 ± 0.09	61.93±0.18
46	13.32±0.08	9.03±0.01	61.09±0.26	11.73±0.06	11.23 ± 0.06	66.03±0.55
47	13.34±0.09	8.39±0.04	52.77±1.66	11.62±0.1	10.66 ± 0.11	61.73±0.76
48	13.27±0.04	9.91±0.05	56.4±0.3	12.22±0.11	12.23 ± 0.03	63.31±0.07
49	13.62±0.08	9.17±0.08	65.77±1.76	12.53±0.03	11.23 ± 0.06	60.48±0.89
50	13.43±0.03	8.86±0.02	55±0.06	12.65±0.08	11.00 ± 0.06	63.4±0.57
51	13.94±0.04	9.18±0.13	56.93±0.87	12.44±0.11	11.37 ± 0.03	62.09±0.03
52	13.78±0.01	9.16±0.13	52.58±0.14	12.59±0.12	11.26 ± 0.03	59.71±0.38
53	13.94±0.09	10.21±0.04	56.25±0.17	12.19±0.13	12.23 ± 0.14	63.86±0.97
54	14.17±0.81	9.94±0.02	53.72±0.68	11.88±0.15	11.80 ± 0.00	61.94±0.06
55	13.69±0.07	8.65±0.02	56.85±0.18	11.99±0.1	11.49 ± 0.09	62.46±0.62
56	13.55±0.03	8.16±0.18	56±0.85	11.72±0.2	10.17 ± 0.14	61.82±0.06
57	13.82±0.08	8.47±0.62	53.65±0.51	12.09±0.15	10.40 ± 0.09	61.45±0.89
58	13.91±0.11	7.83±0.11	53.14±0.72	12.39±0.21	10.29 ± 0.09	61.58±0.08
59	13.84±0.04	8.34±0.11	54.5±0.59	11.89±0.16	10.03 ± 0.23	59.17±0.39
60	13.81±0.05	8.7±0.11	54.49±0.66	13.9±0.15	11.09 ± 0.03	61.83±0.03
61	13.6±0.09	7.45±0.04	52.64±1.1	13.25±0.04	10.12 ± 0.03	63.26±0.6
62	13.4±0.03	6.82±0.06	51.3±1.33	13.77±0	9.35 ± 0.06	61.91±0.32
63	13.63±0.04	8.64±0.11	55.41±0.27	13.33±0	10.83 ± 0.06	62.56±1
64	13.91±0.04	7.93±0.18	50.98±0.76	13.9±0.13	10.03 ± 0.06	57.46±0.71
Average	13.780	8.990	56.07	12.04	11.05	62.34
LSD	0.238	0.256	1.314	0.266	0.237	1.390
MSE	0.014	0.016	0.432	0.018	0.782	0.469

MSE, Mean square error. *Significant 0.05.

Chapter 3- Table S3. Mixograph properties of white flour.

Sample No.	Absorption (%)	MPT (min)	PV (%)	Curve tail Integral (%Tq*Min)	Breakdown (%)	Slope (%/min)
1	56.9±0.2	9.52±0.01	29.55±2.01	210.20±18.48	0.32±0.09	0.48±0.07
2	56.5±0.2	8.04±0.31	30.10±1.89	234.00±7.71	2.56±0.14	2.41±0.62
3	55.8±0.4	8.02±0.33	28.14±2.06	217.24±15.92	1.16±0.34	0.81±0.04
4	57.2±0.2	7.84±1.10	33.94±3.07	255.01±20.65	1.92±0.15	2.31±0.20
5	56.8±0.3	8.64±0.03	30.37±1.96	229.46±15.49	5.44±5.08	9.08±8.34
6	54.0±0.0	9.39±0.21	22.75±0.88	184.05±12.74	1.18±1.11	0.34±0.08
7	56.1±0.4	9.66±0.22	25.49±1.09	180.46±14.85	1.96±2.03	2.16±0.63
8	56.1±0.3	8.30±0.58	29.30±1.05	233.35±7.20	2.33±0.74	1.87±0.22
9	56.4±0.0	9.04±0.21	33.05±0.03	255.49±4.96	2.74±1.65	1.12±0.77
10	57.3±0.4	9.67±0.33	31.17±1.47	222.95±10.06	0.45±0.50	0.67±0.28
11	56.8±0.4	7.86±0.31	30.92±2.28	236.97±18.63	7.59±4.44	2.41±0.89
12	55.8±0.2	8.33±0.51	29.03±1.79	214.93±8.99	4.93±3.38	2.13±0.04
13	56.7±0.2	5.55±0.37	33.44±1.46	254.72±8.34	7.93±1.22	3.99±0.34
14	57.7±0.4	6.93±0.34	32.46±2.70	247.59±19.49	6.88±3.01	2.50±1.61
15	56.4±0.3	9.53±0.47	29.58±2.01	215.86±8.79	3.66±3.73	1.75±0.60
16	55.7±0.6	8.50±0.24	30.99±2.48	228.73±20.09	3.19±1.52	2.45±0.83
17	54.6±0.3	8.74±1.26	28.18±0.83	214.86±6.65	3.49±3.52	1.11±0.74
18	56.8±0.6	5.45±0.25	34.46±2.26	267.38±13.28	12.47±4.97	2.93±0.94
19	56.2±0.4	8.60±0.07	30.16±1.73	238.65±13.79	4.73±3.70	4.35±3.36
20	56.9±0.0	6.71±0.05	35.85±1.08	269.68±1.96	4.76±2.54	3.13±0.59
21	55.8±0.3	7.84±1.44	27.84±1.86	217.59±23.17	0.57±0.21	1.84±1.66
22	55.9±0.4	6.83±0.55	30.37±3.95	233.89±28.23	8.64±5.05	1.28±0.08
23	56.1±0.3	7.38±0.84	28.82±2.27	233.36±16.06	5.30±0.12	2.16±1.04
24	55.6±0.4	9.27±0.23	26.79±1.71	218.40±23.31	3.12±2.97	0.10±0.72
25	55.1±0.4	5.91±0.56	26.09±2.12	214.09±14.13	7.07±0.46	1.56±0.64
26	55.9±0.1	5.79±0.62	32.27±3.26	276.82±29.22	6.45±1.47	2.77±0.19

27	56.5±0.0	6.32±0.11	34.06±0.20	271.85±0.71	7.45±1.15	2.50±0.06
28	56.4±0.2	6.22±0.38	31.45±3.45	251.06±23.30	12.23±3.03	3.06±0.88
29	57.2±0.1	7.12±1.67	31.94±0.19	241.62±11.40	7.16±6.35	2.77±1.07
30	57.5±0.4	6.18±0.70	33.77±3.13	270.89±29.82	9.09±4.77	2.57±0.15
31	56.8±0.5	5.79±0.44	30.72±3.16	251.82±26.06	7.31±0.35	2.60±0.20
32	57.2±0.5	6.79±0.71	29.60±2.37	234.86±11.87	5.19±1.92	2.56±0.87
33	60.2±0.3	5.91±0.74	36.70±0.27	281.60±16.47	9.22±3.91	5.69±2.04
34	61.4±0.1	3.51±0.22	39.23±2.15	300.79±14.75	11.22±0.37	9.66±0.80
35	59.9±0.3	4.84±0.09	36.30±3.14	283.43±22.74	8.97±0.90	5.67±1.57
36	60.8±0.1	6.00±0.40	36.57±3.28	292.93±19.02	6.21±1.21	1.73±0.01
37	60.0±0.1	5.57±0.45	36.55±3.36	292.14±23.78	7.57±0.68	3.38±0.50
38	62.8±0.3	4.67±0.30	41.40±3.58	308.43±28.14	10.93±0.23	12.24±0.37
39	63.7±0.3	4.64±0.26	44.27±3.21	319.22±23.51	12.66±0.14	14.39±0.56
40	61.8±0.2	5.38±0.03	39.83±2.20	298.79±19.59	8.27±0.17	8.26±1.19
41	60.8±0.3	6.03±0.21	35.67±3.52	270.94±27.27	6.36±0.72	3.83±0.61
42	60.5±0.5	5.83±0.15	33.31±3.70	255.63±31.23	6.88±1.15	4.04±0.36
43	61.1±0.4	4.24±1.15	45.98±5.98	365.05±46.26	11.71±2.89	6.27±2.30
44	56.3±0.5	8.49±0.07	28.12±2.75	213.52±21.79	3.72±3.09	3.59±1.81
45	57.4±0.0	5.98±0.73	38.11±2.08	296.03±27.57	3.95±1.13	4.97±0.82
46	57.5±0.4	7.85±0.71	29.93±2.88	233.16±29.78	4.87±4.34	6.59±4.65
47	56.6±0.4	6.20±0.57	31.16±2.23	250.16±8.29	6.44±0.33	2.80±0.96
48	58.7±0.4	6.22±0.40	35.35±0.19	284.65±7.16	3.21±0.87	2.67±0.37
49	57.7±0.4	5.21±0.68	38.19±1.02	312.43±13.85	6.00±0.52	3.64±1.44
50	57.2±0.4	9.58±0.00	32.40±2.04	239.57±3.09	0.38±0.18	1.26±0.28
51	57.8±0.4	6.07±0.04	34.03±1.62	278.72±22.29	9.67±4.99	1.78±0.17
52	57.7±0.4	5.05±0.19	32.68±2.92	243.88±24.77	10.60±1.54	6.72±0.20
53	59.4±0.4	6.86±0.21	34.66±1.77	267.86±15.75	4.96±2.07	1.68±0.60
54	58.7±0.1	6.90±0.10	34.56±2.90	264.37±24.94	8.31±3.35	3.54±0.68
55	57.0±0.5	5.14±0.74	34.65±1.97	283.36±11.38	7.71±1.67	2.24±0.24

56	56.1±0.3	7.58±0.09	29.17±1.04	230.57±13.63	0.59±0.44	1.02±0.64
57	56.3±0.0	9.15±0.17	29.32±3.50	204.35±16.31	4.02±3.59	10.43±8.68
58	55.6±0.3	4.81±0.17	28.04±1.84	203.89±15.34	12.24±0.50	5.09±1.41
59	56.5±0.3	5.24±0.26	31.59±2.58	244.06±25.73	10.04±0.99	5.09±0.39
60	57.1±0.4	5.51±0.13	32.70±4.45	249.15±40.94	12.80±0.85	3.78±0.65
61	55.3±0.5	4.77±0.27	27.78±2.73	198.82±31.14	13.72±1.61	4.43±0.37
62	54.3±0.5	4.60±0.33	25.31±3.44	200.19±30.36	10.16±0.40	1.68±0.07
63	57.0±0.5	7.71±0.04	30.62±3.40	239.82±15.34	3.54±2.80	1.89±1.46
64	55.9±0.4	4.18±0.67	28.35±3.46	212.47±17.07	10.45±0.87	3.04±0.26
Average	57.348	6.802	32.276	249.185	6.355	3.544
LSD	0.997	1.513	7.213	57.750	7.018	5.259
MSE	0.249	0.573	13.038	835.672	12.341	6.930

*MPT, dough development time; PV, peak value (%); MSE, Mean square error.
*Significant 0.05.

Chapter 3- Table S4. Mixograph properties of whole wheat flour.

Sample No.	Absorption (%)	MPT (min)	PV (%)	Curve tail Integral (%Tq*Min)	Breakdown (%)	Slope (%/min)
1	58±0	8.47±0.29	30.57±0.20	233.42±7.27	2.832±0.483	4.380±0.509
2	61±0	7.20±0.04	24.22±0.07	195.94±1.98	4.517±1.100	4.452±0.184
3	58±1	7.47±0.19	25.66±0.02	205.78±0.66	2.601±0.205	0.943±0.789
4	60±0	7.81±0.22	28.41±0.38	218.27±0.23	3.605±0.143	2.624±1.225
5	59±0	8.45±0.43	26.85±0.25	206.38±3.84	2.439±1.607	3.190±1.123
6	57±1	9.13±0.35	23.51±2.03	186.94±14.30	2.365±1.157	1.467±0.966
7	58±0	9.61±0.33	27.87±0.38	210.83±1.00	0.836±0.954	1.110±0.325
8	58±0	6.42±0.18	26.52±0.27	214.38±3.63	3.885±0.079	3.475±1.478
9	58±0	7.39±0.26	30.87±0.16	249.94±4.35	2.972±1.762	2.700±1.135
10	58±0	7.69±0.28	32.77±0.00	255.57±1.45	3.920±0.095	3.695±2.603
11	58±0	6.56±0.22	30.42±0.08	243.79±5.28	5.331±0.015	3.806±0.085
12	58±0	6.31±0.22	29.72±0.08	244.70±0.16	4.913±0.182	4.342±0.665
13	58±0	5.02±0.15	31.46±0.41	246.41±2.30	16.362±0.785	5.563±0.034
14	58±0	5.49±0.10	34.86±0.32	277.76±1.50	11.547±0.162	4.330±0.787
15	64±3	9.28±0.15	26.78±0.79	206.98±6.61	4.059±1.262	1.408±0.336
16	58±0	7.41±0.19	24.81±0.11	194.05±2.43	3.404±0.022	3.921±0.034
17	57±0	7.88±0.19	24.68±0.16	198.01±0.43	5.621±1.614	2.494±0.822
18	60±0	4.72±0.16	26.79±0.16	186.70±5.35	25.292±1.712	4.660±0.169
19	59±0	7.51±0.10	27.22±0.59	215.73±11.90	4.562±0.162	4.185±2.020
20	60±0	7.12±0.13	27.68±0.55	214.48±2.70	6.788±2.198	5.732±0.763
21	63±0	8.55±0.07	20.95±0.26	157.83±0.34	2.618±0.778	2.911±0.963
22	59±0	6.67±0.06	24.56±0.50	198.27±2.30	8.485±3.362	2.920±1.462
23	59±0	6.77±0.11	24.57±0.05	197.32±1.39	7.000±0.765	2.306±0.674
24	59±0	9.01±0.05	23.09±0.04	187.11±1.15	0.196±0.141	2.462±1.038
25	58±0	6.47±0.12	22.39±0.47	193.92±1.80	5.789±0.237	1.555±1.363
26	59±0	7.24±0.02	27.93±0.19	227.02±0.97	7.637±0.992	3.736±0.457
27	60±0	4.94±0.08	27.85±0.21	220.77±1.99	16.769±0.664	3.460±0.101
28	60±0	6.36±0.21	23.70±0.35	193.57±0.60	6.116±0.998	2.127±0.068
29	60±0	7.53±0.58	27.80±0.00	221.52±2.30	4.420±0.704	2.265±0.733

30	61±0	6.88±0.20	25.83±0.57	198.42±7.45	7.671±2.762	2.765±0.835
31	60±0	5.55±0.30	24.21±0.25	176.90±4.04	22.936±0.649	3.231±0.645
32	59±0	5.52±0.52	26.35±0.71	219.62±6.05	5.902±0.577	2.989±0.020
33	62±0	6.24±0.22	30.24±0.44	217.03±0.32	23.427±2.362	5.454±0.652
34	65±1	3.63±0.01	31.76±0.53	167.77±0.86	31.650±0.417	7.430±0.126
35	63±0	5.35±0.21	28.23±0.13	177.81±3.30	28.242±0.120	5.396±0.790
36	64±0	6.13±0.21	32.59±0.47	244.65±0.52	14.374±2.422	5.456±0.607
37	63±0	6.14±0.11	30.85±0.37	227.65±5.96	17.990±5.011	6.511±1.106
38	66±0	5.59±0.05	34.23±0.54	246.23±4.03	9.913±0.153	8.927±0.269
39	63±1	4.81±0.18	40.40±0.40	298.11±5.05	14.674±1.827	11.436±0.559
40	65±0	5.89±0.21	35.04±0.09	259.19±1.05	10.350±0.627	8.158±0.149
41	64±0	6.27±0.09	32.06±0.58	242.89±3.17	16.183±0.522	4.044±0.373
42	64±0	4.86±0.11	29.79±0.07	179.86±0.80	29.798±0.069	5.943±0.292
43	64±0	5.22±0.04	31.22±0.33	212.95±2.64	30.765±0.414	5.657±0.736
44	60±0	8.89±0.17	26.40±0.10	204.88±0.47	0.288±0.003	1.609±0.965
45	60±0	5.88±0.02	28.66±0.61	213.64±5.10	23.935±1.448	6.084±0.402
46	61±0	8.19±0.24	26.12±0.25	202.54±0.27	5.347±1.366	4.295±0.438
47	62±0	7.10±0.08	24.07±0.28	186.31±0.94	5.963±1.073	4.174±0.094
48	60±1	6.32±0.01	35.96±0.25	285.65±7.19	14.427±4.356	5.959±1.003
49	62±0	5.44±0.15	30.05±0.47	213.04±9.00	27.062±1.909	5.334±0.891
50	60±0	8.31±0.34	29.85±0.23	234.55±5.52	2.786±0.337	4.079±0.408
51	60±0	4.92±0.09	30.62±0.34	220.43±4.66	29.988±0.313	6.355±0.578
52	60±0	4.83±0.34	27.03±0.67	176.69±8.75	27.055±0.691	4.425±0.095
53	62±0	7.01±0.27	28.50±0.22	226.75±1.32	6.116±0.984	3.201±0.075
54	58±1	5.50±0.03	33.70±0.79	249.47±8.01	31.939±0.076	5.191±0.712
55	60±0	4.56±0.09	28.07±0.54	174.15±1.26	27.961±0.677	5.502±0.459
56	61±0	8.84±0.22	23.26±0.38	179.33±1.41	1.982±1.474	3.202±2.655
57	60±1	8.91±0.07	23.51±1.13	187.23±12.93	2.656±2.407	5.840±4.428
58	60±0	4.85±0.46	23.22±0.38	146.67±3.59	23.220±0.381	3.657±1.158
59	60±0	5.06±0.43	21.72±0.41	138.70±0.88	19.170±0.257	3.699±0.190
60	59±0	4.80±0.04	29.31±0.63	204.63±7.67	29.329±0.571	2.654±0.788
61	58±0	4.27±0.17	30.91±1.10	234.92±1.87	12.576±0.321	5.836±3.132

62	62±0	3.99±0.56	21.08±0.75	155.27±3.04	20.910±0.919	1.182±0.362
63	60±0	7.06±0.15	29.74±0.07	238.19±1.51	5.237±0.250	4.302±0.019
64	58±0	2.29±0.21	26.18±0.77	180.14±0.42	26.198±0.775	13.645±4.608
Average	60.245	6.491	28.05	211.338	12.266	4.31
LSD	1.370	0.640	1.434	13.340	3.974	3.526
MSE	0.470	0.103	0.516	44.581	3.958	3.115

*MPT, dough development time; PV, peak value (%); MSE, Mean square error.
*Significant 0.05.

Chapter 3- Table S5. Pasting properties of white flour measured by RVA.

WF No.	PV (cP)	Trough (cP)	BKD (cP)	FV (cP)	Setback (cP)	PKT (Min)	TEMP (°C)
1	3876.5±9.5	2540.0±21.0	1336.5±30.5	3915.0±22.0	1375.0±1.0	6.40±0.00	69.38±0.03
2	3307.5±43.5	2395.5±22.5	912.0±21.0	3960.5±61.5	1565.0±39.0	6.33±0.07	69.00±0.40
3	3354.5±0.5	2483.0±5.0	871.5±5.5	3939.0±9.0	1456.0±14.0	6.40±0.00	69.78±0.48
4	3664.0±18.0	2366.0±28.0	1298.0±10.0	3771.0±13.0	1405.0±15.0	6.30±0.03	70.20±0.05
5	3661.5±28.5	2366.0±29.0	1295.5±57.5	3754.5±18.5	1388.5±10.5	6.37±0.03	69.43±0.08
6	3695.5±25.5	2584.5±13.5	1111.0±12.0	4180.5±28.5	1596.0±15.0	6.47±0.00	70.08±0.02
7	3745.5±40.5	2425.0±35.0	1320.5±5.5	3732.5±15.5	1307.5±19.5	6.40±0.07	69.70±0.35
8	3424.0±20.0	2451.5±25.5	972.5±5.5	4045.0±31.0	1593.5±5.5	6.33±0.00	69.38±0.03
9	3197.0±19.0	2338.5±3.5	858.5±15.5	3885.5±19.5	1547.0±16.0	6.33±0.00	68.98±0.32
10	3164.0±1.0	2398.5±44.5	765.5±43.5	3958.5±0.5	1560.0±45.0	6.43±0.03	69.73±0.38
11	3540.5±23.5	2389.0±4.0	1151.5±19.5	3840.0±3.0	1451.0±1.0	6.37±0.03	69.70±0.40
12	3072.5±5.5	2254.0±22.0	818.5±16.5	3788.0±9.0	1534.0±13.0	6.37±0.03	68.95±0.45
13	3711.0±1.0	2355.0±3.0	1356.0±4.0	3693.0±5.0	1338.0±2.0	6.33±0.00	70.08±0.02
14	3163.0±12.0	2532.5±3.5	630.5±15.5	3995.0±20.0	1462.5±23.5	6.57±0.03	69.38±0.08
15	3396.0±23.0	2703.5±24.5	692.5±47.5	4233.5±17.5	1530.0±42.0	6.57±0.03	69.73±0.38
16	3193.0±15.0	2390.5±21.5	802.5±6.5	3857.5±5.5	1467.0±16.0	6.47±0.00	70.05±0.00
17	3056.5±9.5	2239.5±3.5	817.0±6.0	3865.0±16.0	1625.5±12.5	6.40±0.00	70.15±0.00
18	3831.0±24.0	2584.5±24.5	1246.5±0.5	4104.0±30.0	1519.5±5.5	6.30±0.03	69.25±0.00
19	3347.5±20.5	2332.5±18.5	1015.0 ± 2.0	3956.5±33.5	1624.0±15.0	6.23±0.03	68.93±0.48
20	3624.0±32.0	2703.0±19.0	921.0±13.0	4369.0±45.0	1666.0±26.0	6.40±0.00	68.98±0.38
21	3325.5±3.5	2667.0±15.0	658.5±18.5	4245.0±6.0	1578.0±21.0	6.50±0.03	68.95±0.35
22	3445.5±36.5	2728.5±35.5	717.0±1.0	4325.0±22.0	1596.5±13.5	6.57±0.03	68.53±0.02
23	3362.0±23.0	2766.5±6.5	595.5±16.5	4222.5±26.5	1456.0±20.0	6.70±0.03	71.38±1.18
24	3277.5±29.5	2590.0±21.0	687.5±8.5	4269.5±35.5	1679.5±14.5	6.43±0.03	68.20±0.40
25	3583.5±65.5	2988.0±47.0	595.5±18.5	4522.5±60.5	1534.5±13.5	6.70±0.03	70.13±0.08
26	3546.5±32.5	2490.0±18.0	1056.5±14.5	4002.0±25.0	1512.0±7.0	6.37±0.03	70.15±0.00
27	3437.5±12.5	2680.0±12.0	757.5±0.5	4205.0±5.0	1525.0±7.0	6.57±0.03	69.45±0.05
28	3394.0±1.0	2614.5±32.5	779.5±31.5	4276.5±10.5	1662.0±43.0	6.47±0.07	69.73±0.43
29	3365.0±0.0	2608.5±2.5	756.5±2.5	4161.5±5.5	1553.0±8.0	6.50±0.03	69.75±0.35
30	3541.0±40.0	2352.0±63.0	1189.0±23.0	3807.5±56.5	1455.5±6.5	6.27±0.00	68.13±0.42
31	3016.0±37.0	2357.0±11.0	659.0±26.0	3829.5±38.5	1472.5±27.5	6.50±0.03	68.95±0.35
32	3250.5±3.5	2394.5±17.5	856.0±14.0	3998.5±13.5	1604.0±4.0	6.43±0.03	69.33±0.08
33	3580.0±34.0	2342.5±30.5	1237.5±3.5	3841.5±30.5	1499.0±0.0	6.20±0.00	70.15±0.10
34	3387.0±18.0	2523.0±30.0	864.0±48.0	4113.0±1.0	1590.0±31.0	6.37±0.03	68.60±0.00
35	3286.0±23.0	2471.5±75.5	814.5±52.5	4108.0±41.0	1636.5±34.5	6.37±0.03	69.33±0.03
36	3478.0±4.0	2590.5±13.5	887.5±17.5	4168.5±9.5	1578.0±4.0	6.37±0.03	69.78±0.38
37	3839.5±24.5	2462.5±49.5	1377.0±25.0	3862.0±48.0	1399.5±1.5	6.37±0.03	69.35±0.00
38	3633.0±6.0	2087.5±3.5	1545.5±2.5	3456.0±6.0	1368.5±2.5	6.07±0.00	70.13±0.03
39	3595.0±28.0	2095.0±7.0	1500.0±21.0	3438.0±6.0	1343.0±1.0	6.07±0.00	70.08±0.02
40	3141.5±8.5	2084.0±33.0	1057.5±24.5	3638.0±20.0	1554.0±13.0	6.10±0.03	69.43±0.02
41	3575.0±1.0	2133.5±18.5	1441.5±17.5	3554.5±9.5	1421.0±9.0	6.03±0.03	69.00±0.50
42	3061.0±11.0	2307.0±39.0	754.0±28.0	3774.5±8.5	1467.5±47.5	6.40±0.07	69.78±0.43

43	3244.0±3.0	2313.5±4.5	930.5±1.5	3969.0±15.0	1655.5±10.5	6.27±0.00	68.58±0.02
44	3407.5±11.5	2354.0±29.0	1053.5 ± 17.5	3984.0±15.0	1630.0±14.0	6.23±0.03	69.75±0.45
45	3679.5±20.5	2722.5±7.5	957.0±13.0	4334.0±17.0	1611.5±9.5	6.47±0.00	68.55±0.00
46	3108.0±19.0	2278.0±2.0	830.0±17.0	3732.0±19.0	1454.0±17.0	6.33±0.00	69.30±0.00
47	3529.0±24.0	2649.5±46.5	879.5±22.5	4151.5±41.5	1502.0±5.0	6.50±0.03	69.30±0.00
48	3281.5±6.5	2479.0±5.0	802.5±11.5	4058.5±4.5	1579.5±9.5	6.47±0.00	68.88±0.38
49	3305.0±12.0	2623.0±38.0	682.0±26.0	4057.5±3.5	1434.5±34.5	6.67±0.07	69.85±0.40
50	3365.5±12.5	2477.0±24.0	888.5±36.5	4098.5±6.5	1621.5±30.5	6.40±0.00	69.40±0.00
51	3364.0±15.0	2676.5±28.5	687.5±43.5	4113.5±20.5	1437.0±49.0	6.67±0.07	69.78±0.38
52	3111.0±14.0	2159.0±2.0	952.0±12.0	3777.0±3.0	1618.0±1.0	6.27±0.00	69.35±0.05
53	3317.0±16.0	2490.0±23.0	827.0±7.0	4094.5±1.5	1604.5±21.5	6.47±0.00	69.35±0.05
54	3263.0±12.0	2407.5±23.5	855.5±11.5	3980.0±13.0	1572.5±10.5	6.47±0.00	68.93±0.48
55	3309.5±11.5	2522.0±12.0	787.5±23.5	4020.0±2.0	1498.0±10.0	6.57±0.03	68.25±0.45
56	3267.0±37.0	2293.0±36.0	974.0±1.0	4044.5±20.5	1751.5±15.5	6.30±0.03	68.60±0.10
57	3230.0±2.0	2293.5±27.5	936.5±29.5	4024.5±9.5	1731.0±37.0	6.30±0.03	68.15±0.35
58	2797.0±5.0	1958.0±3.0	839.0±8.0	3571.5±1.5	1613.5±4.5	6.23±0.03	68.98±0.38
59	2882.5±2.5	2022.5±3.5	860.0±1.0	3634.5±2.5	1612.0±6.0	6.27±0.00	67.68±0.08
60	2918.5±42.5	2069.0±27.0	849.5±15.5	3623.0±52.0	1554.0±25.0	6.33±0.00	67.80±0.05
61	2878.5±4.5	2002.0±1.0	876.5±3.5	3604.5±24.5	1602.5±23.5	6.23±0.03	69.00±0.45
62	2746.5±15.5	1888.5±7.5	858.0±23.0	3479.5±35.5	1591.0±43.0	6.20±0.07	68.88±0.38
63	3575.0±49.0	2546.0±22.0	1029.0 ± 27.0	4409.0±27.0	1863.0±5.0	6.27±0.00	68.55±0.00
64	2826.0±3.0	1930.5±9.5	895.5±12.5	3551.0±3.0	1620.5±12.5	6.20±0.00	68.53±0.07
Average	3355.0	2415.6	939.4	3957.3	1541.7	6.4	69.3
LSD	66.190	131.800	135.900	94.600	76.020	0.154	0.991
MSE	1103.1	4373.6	4652.6	2255.5	1455.3	0.0	0.2

*PV, peak viscosity (cP); Trough, Trough viscosity (cP); BKD, Breakdown viscosity; FV, final viscosity (cP); Setback, setback viscosity (cP); PKT, peak time (Min); TEMP, pasting temperature (°C); MSE, Mean square error. Significant 0.05.

Chapter 3- Table S6. Pasting properties of whole wheat flour (WWF) measured by RVA.

WWF No.	PV (cP)	Trough (cP)	BKD (cP)	FV (cP)	Setback (cP)	PKT (Min)	TEMP (°C)
1	2573.0±21.0	1641.5±20.5	931.5±0.5	2915.5±16.5	1274.0±4.0	6.13±0.00	70.98±0.02
2	2042±33	1413±40	629±7	2934±0	1521±40	5.87±0.1	87.15±0.02
3	1950.5±2.5	1390.5±10.5	560±13	2623±5	1232.5±5.5	6.1±0.03	87.25±0.1
4	2347±5	1462.5±2.5	884.5±7.5	2680±2	1217.5±4.5	6±0	71.78±0.07
5	2261.5±1.5	1355.5±8.5	906±10	2653.5±8.5	1298±17	5.97±0.03	87.25±0.1
6	2118.5±29.5	1490±8	628.5±21.5	2944.5±6.5	1454.5±1.5	6.1±0.03	87.15±0
7	2450±9	1463±1	987±8	2696±9	1233±8	6.07±0	71.85±0
8	2085±7.5	1438±2.5	647±5	2879±9.5	1441±7	6±0.03	87.25±0.42
9	1825.5±7.5	1314.5±15.5	511±8	2631.5±3.5	1317±12	6.13±0.07	88.85±0.1
10	1583.5±0.5	1158.5±9.5	425±9	2418±0	1259.5±9.5	6±0.07	89.18±0.42
11	2271±18	1473.5±42.5	797.5±24.5	2841±30	1367.5±12.5	6.13±0.07	87.95±0
12	1735.5±10.5	1230.5±26.5	505±16	2538±29	1307.5±2.5	5.97±0.03	87.2±0.05
13	2446±27	1456±30	990±3	2794±34	1338±4	6.07±0	72.6±0.05
14	1858.5±4.5	1359.5±12.5	499±8	2704.5±5.5	1345±7	6.1±0.03	88.43±0.33
15	1887±27	1394.5±28.5	492.5±1.5	2752±29	1357.5±0.5	6.1±0.03	88.85±0.05
16	1960±16	1389±1	571±15	2760±3	1371±2	6.13±0	88.38±0.38
17	1747.5±5.5	1304±19	443.5±13.5	2651.5±4.5	1347.5±14.5	6.13±0.07	88.45±0.4
18	2227±7.5	1415±15.5	812±23	2683±4.5	1268±11	6±0.03	86.4±0.38
19	2142±30	1501±24	641±6	2916.5±30.5	1415.5±6.5	6.03±0.03	71.38±0.42
20	2039±7.5	1457±8.5	582±1	2838±11.5	1381±3	6.07±0	87.95±0.07
21	1976.5±9.5	1485.5±5.5	491±4	2835.5±7.5	1350±2	6.1±0.03	87.98±0.07
22	1967±8	1363±5	604±3	2938±20	1575±15	6.1±0.03	87.63±0.38
23	1763±5	1338±6.5	425±1.5	2694±4.5	1356±2	6.13±0.03	89.65±0.03
24	1928±33	1469±9	459±24	3025±10	1556±1	6±0	88±0.38
25	2025±12	1551±12	474±0	2951±9.5	1400±2.5	6.2±0.03	88±0.02
26	2250.5±3.5	1537.5±20.5	713±17	2961±1	1423.5±19.5	6.13±0	87.98±0.02
27	2120±11	1500±7	620±4	2937±17	1437±10	6.13±0	87.18±0.02
28	1973±5	1448±11	525±16	2870±4	1422±15	6.07±0	87.58±0.42
29	2099±25	1533.5±13.5	565.5±11.5	3095.5±3.5	1562±10	6.13±0	88.05±0.05
30	2473±27	1486±30	987±3	2828±34	1342±4	6.07±0	72.65±0.05
31	1637±1	1249±5.5	388±4.5	2475±2	1226±3.5	6.13±0.03	88.8±0.05
32	1912.5±5.5	1363±9	549.5±14.5	2774.5±17.5	1411.5±8.5	6.07±0	88±0.8
33	2127±24	1445.5±8.5	681.5±15.5	2737±18	1291.5±9.5	5.93±0	72.15±0.4
34	1933.5±8.5	1412±13	521.5±4.5	2758±6	1346±19	5.93±0.07	87.2±0.05
35	1764.0±11.0	1333.5±9.5	430.5±1.5	2563.5±20.5	1230.0±11.0	6.10±0.03	87.18±0.08
36	1935±2	1377±12	558±10	2762±21	1385±33	6.03±0.03	72.53±0.02
37	2365.5±11.5	1414.5±8.5	951.0±3.0	2643.5±5.5	1229.0±3.0	6.03±0.03	71.03±0.02
38	2214.5±9.5	1272.5±3.5	942±6	2486.5±11.5	1214±8	5.8±0	72.1±0.4
39	2050.0±3.0	1192.5±5.5	857.5±2.5	2373.5±9.5	1181.0±4.0	5.73±0.00	72.58±0.02
40	1954±18	1316±16	638±2	2649.5±15.5	1333.5±0.5	5.77±0.03	71.35±0.45
41	2391±34	1416±4	975±30	2756.5±24.5	1340.5±20.5	5.73±0	70.18±0.08
42	2054.5±36.5	1577.5±22.5	477±14	2906±45	1328.5±22.5	6.2±0	87.28±0.02

43	1855±2	1293±6	562±4	2777±4.5	1484±10.5	5.93±0.03	87.2±0.07
44	2015.5±0.5	1353.5±2.5	662±3	2747±4	1393.5±6.5	5.9±0.03	86.83±0.48
45	2281.5±35.5	1559±17	722.5±18.5	3081.5±33.5	1522.5±16.5	6.03±0.03	88.43±0.42
46	1832.5±0.5	1291.5±5.5	541.0±6.0	2658.0±6.0	1366.5±0.5	5.93±0.00	87.58±0.48
47	2091±19	1477±35	614±16	2832.5±4.5	1355.5±39.5	6.07±0.07	87.25±0.05
48	1783.5±1.5	1212.5±1.5	571±0	2700±2	1487.5±3.5	5.93±0	88.8±0.05
49	1937±15	1358.5±5.5	578.5±9.5	2701.5±7.5	1343±2	6.1±0.03	89.13±0.42
50	2141.5±5.5	1411.5±3.5	730±2	3038.5±9.5	1627±6	5.9±0.03	87.18±0.02
51	1892.5±4.5	1289±7	603.5±2.5	2603±3	1314±4	6.03±0.03	89.58±0.02
52	1863±10	1258.5±0.5	604.5±9.5	2728.5±17.5	1470±18	5.9±0.03	87.65±0.4
53	1885±21	1278±10	607±11	2886.5±6.5	1608.5±3.5	5.8±0	88.8±0.05
54	1856.5±1.5	1297±3	559.5±4.5	2791±29	1494±32	5.97±0.03	88.88±0.03
55	1777.5±23.5	1255±10	522.5±13.5	2463.5±35.5	1208.5±25.5	6.07±0	89.18±0.42
56	1849.5±4.5	1293±11	556.5±15.5	2794.5±5.5	1501.5±5.5	5.9±0.03	87.18±0.02
57	1890±6	1316.5±3.5	573.5±2.5	2840±3	1523.5±0.5	5.93±0	87.28±0.02
58	1475.5±4.5	973.5±8.5	502±13	2200.5±1.5	1227±7	5.77±0.03	87.25±0
59	1887.5±15.5	1283.5±18.5	604±3	2653.5±4.5	1370±14	6.07±0	88.45±0.35
60	1676.5±8.5	1133.5±4.5	543±4	2447±10	1313.5±14.5	5.93±0	87.15±0
61	1534.5±2.5	1055.5±7.5	479±10	2339±5	1283.5±2.5	5.87±0	71±0.1
62	1634.5±14.5	1090.5±16.5	544±2	2455.5±17.5	1365±1	5.87±0	71.23±0.48
63	1981.5±2.5	1393±4	588.5±6.5	2913.5±1.5	1520.5±5.5	5.97±0.03	70.18±0.02
64	1635.5±17.5	1106.5±10.5	529±7	2482±23	1375.5±12.5	5.8±0.07	70.58±0.33
Average	1988.9	1362.9	626.0	2734.3	1371.4	6.0	83.6
LSD	101.1	91.8	65.8	105.1	63.7	0.094	0.893
MSE	2561.8	2114.2	1085.8	2769.6	1016.0	0.002	0.200

*PV, peak viscosity (cP); Trough, Trough viscosity (cP); BKD, Breakdown viscosity; FV, final viscosity (cP); Setback, setback viscosity (cP); PKT, peak time (Min); TEMP, pasting temperature (°C); MSE, Mean square error. Significant 0.05.

Chapter 3- Table S7. Specific volume of bread made with white flour (WF) or whole wheat flour (WWF).

No.	WF specific volume (cm^3/g)	WWF specific volume (cm^3/g)
1	4.48±0.01	1.87±0.07
2	3.91±0.03	2.16±0.01
3	4.00±0.00	1.19±0.01
4	4.53±0	2.15±0
5	4.43±0.10	2.09±0.02
6	3.74±0.02	1.76±0.02
7	3.53±0.01	1.84±0.01
8	3.84±0.01	2.11±0.03
9	4.23±0.02	1.95±0.02
10	4.23±0.01	2.17±0.02
11	3.94±0.04	2.22±0.11
12	3.84±0	1.84±0.02
13	3.97±0.07	1.83±0.03
14	4.19±0.02	2.03±0.02
15	4.31±0	1.64±0.02
16	4.04±0.07	2.14±0.02
17	3.65±0.02	2.05±0.01
18	4.3±0.09	2.33±0.03
19	3.95±0.03	2.44±0.03
20	4.28±0.02	2.50±0.01
21	3.67±0.10	2.32±0.01
22	3.52±0.04	1.68±0.03
23	3.44±0.02	2.36±0.03
24	3.75±0.04	1.67±0.06
25	3.28±0.01	1.63±0.03
26	3.75±0.09	2.07±0.01

27	3.7±0.03	1.84±0.02
28	4.16±0.03	1.86±0.07
29	3.90±0.03	2.06±0.05
30	4.31±0.03	2.32±0.02
31	4.13±0	2.08±0.04
32	3.80±0.01	2.20±0.03
33	4.33±0.07	1.89±0.00
34	4.01±0.01	2.56±0.01
35	4.56±0.02	2.16±0.01
36	4.39±0.06	2.6±0.03
37	4.65±0.01	2.82±0.03
38	4.71±0.05	3.04±0.06
39	5.21±0.07	2.65±0.07
40	5.08±0.01	3.76±0.06
41	4.66±0.02	2.57±0.03
42	4.34±0.03	2.66±0.01
43	3.58±0.03	2.41±0.01
44	4.15±0.11	2.04±0.02
45	4.62±0.04	1.93±0.04
46	4.26±0.07	1.79±0.03
47	3.82±0.07	1.88±0.00
48	4.16±0.01	1.65±0.03
49	4.08±0.11	2.02±0.04
50	4.11±0.03	1.92±0.04
51	4.52±0.05	2.17±0.09
52	4.18±0.06	2.01±0.05
53	4.27±0.06	1.83±0.03
54	4.83±0.03	2.11±0.02
55	4.29±0.07	1.9±0.03

56	4.23±0.01	2.23±0
57	3.69±0.01	1.99±0.01
58	3.92±0.03	1.51±0.01
59	3.97±0.07	2.72±0.01
60	3.95±0.11	1.67±0.03
61	3.55±0.05	1.6±0.02
62	3.34±0.04	1.84±0.01
63	3.43±0.02	1.79±0.07
64	3.87±0.05	1.50±0.01
Average	4.096	2.088
LSD value	0.139	0.101
MSE	0.005	0.003

*WF, white flour; WWF, whole wheat flour; MSE, Mean square error. Significant 0.05.

Chapter 3- Table S8. Textural properties of bread made with white flour.

Sample No.	Hardness (g)	Springiness (%)	Gumminess	Chewiness
1	717.68±57.82	98.47±2.41	415.33±40.05	409.33±43.86
2	595.04±64.37	154.97±10.53	389.02±37.58	601.14±55.31
3	702.51±32.78	99.34±1.35	353.58±20.74	351.44±24.04
4	701.22±62.64	96.52±0.35	432.44±41.72	417.35±39.89
5	511.19±38.88	106.33±15.52	322.66±22.29	345.38±71.82
6	858.07±79.94	97.20±2.05	411.89±34.21	400.43±35.41
7	649.55±70.34	97.5±1.81	394.11±34.11	384.37±34.92
8	798.22±134.19	120.55±20.57	470.73±60.46	564.85±112.7
9	716.37±38.5	98.21±1.51	428.6±36.98	421.02±38.14
10	602.23±34.33	97.5±2.44	368.3±16.86	359.25±21.23
11	667.66±41.00	99.65±0.20	356.61±26.84	355.40±27.08
12	731.19±43.87	99.69±1.33	443.67±22.89	442.23±21.93
13	611.55±13.6	97.03±1.21	342.48±15.95	332.39±17.7
14	625.43±38.8	97.83±2.03	369.53±26.48	361.85±31.37
15	692.97±65.67	96.02±1.59	377.34±17.14	362.15±13.11
16	659.99±42.60	101.37±7.12	350.33±31.64	356.20±50.86
17	693.49±20.08	99.35±1.92	349.45±17.02	347.26±19.79
18	615.68±13.14	98.01±1.91	387.81±25.86	379.86±22.94
19	712.81±6.92	100.87±1.63	426.97±9.26	430.69±11.41
20	661.95±50.22	100.3±0.7	447.15±22.61	448.46±22.14
21	891.96±71.53	146.27±22.38	552.54±29.89	805.94±118.6
22	722.13±49.45	100.07±1.85	416.51±37.97	416.86±39.77
23	705.96±64.89	103.67±5.42	451.98±23.56	468.4±32.4
24	515.58±21.19	118.31±17.43	360.83±20.41	427.6±72.11
25	926.74±43.85	96.33±1.74	473.44±34.07	455.81±30.15
26	756.53±65.62	98.58±2.21	409.34±34.26	403.32±31.92
27	651.12±29.9	99.52±1.38	409.72±24.87	408.01±29.09
28	505.78±42.41	120.02±15.60	335.59±24.74	401.46±51.10
29	769.31±61.92	99.35±1.55	428.22±35.13	425.48±36.27

30	576.36±57.50	101.97±5.00	355.89±16.21	362.58±18.65
31	625.78±37.58	101±5.44	396.22±12.48	400.28±26.79
32	769.31±66.34	99.35±1.70	428.22±38.41	425.48±39.66
33	608.40±123.74	106.07±11.94	380.36±57.57	400.61±54.89
34	738.93±37.88	99.35±2.18	434.87±19.47	431.92±19.15
35	565.98±20.28	103.27±7.38	367.12±13.74	378.68±23.49
36	614.69±24.29	100.17±3.22	395.14±15.28	395.76±18.27
37	568.26±68.38	141.24±16.26	394.10±42.51	551.63±42.86
38	394.03±19.63	118.04±14.58	271.5±14.16	318.96±28.55
39	428.1±21.16	114.03±14.68	299.64±11.62	341.68±46.2
40	567.7±24.57	101.99±6.63	384.35±15.1	392.23±33.4
41	496.52±31	111.4±13.31	341.56±17.4	379.13±36.26
42	568.26±68.38	141.24±16.26	394.10±42.51	551.63±42.86
43	754.67±30.26	100.15±2.06	430.13±24.28	430.83±26.89
44	729.79±35.55	98.79±1.74	427.58±18.79	422.32±18.34
45	627.93±39.29	106.87±12.61	392.22±15.17	418.02±39.54
46	761.92±50.95	100.13±0.5	474.42±29.56	475.12±30.97
47	763.17±37.70	110.62±10.71	459.93±25.06	508.20±50.15
48	563.29±23.09	100.45±0.73	364.36±13.95	366.01±14.62
49	492.76±31.69	100.42±3.45	314.30±15.22	315.11±6.02
50	678.89±66.6	98.49±1.7	406.91±34.51	400.71±33.51
51	595.77±21.66	100.01±2.20	375.38±18.22	375.38±19.49
52	741.23±56.50	97.46±1.98	431.98±38.37	420.96±38.15
53	586.06±36.25	108.68±8.55	382.62±19.66	414.66±23.14
54	540.59±34.57	100.24±0.68	346.66±15.52	347.49±15.76
55	586.67±26.56	99.11±2.16	379.44±9.37	376.16±14.9
56	800.2±25.75	99.07±1.51	437.61±19.37	433.49±19.7
57	982.15±43.91	114.28±15.09	651.48±38.20	745.29±112.9
58	761.70±38.68	144.54±3.92	447.21±30.02	646.13±43.22
59	758.96±16.64	97.61±2.48	450.77±21.33	440.3±28.88
60	659.46±24.85	101.89±1.99	383.56±14.56	390.94±19.59

61	874.09±37.72	98.3±1.51	459.67±15.96	451.93±18.67
62	1179.71±167.7	95.71±2.11	579.89±101.7	555.22±98.27
63	1007.88±136.3	106.01±13.74	557.70±63.47	585.72±54.89
64	922.79±19.56	99.51±2.34	491.29±29.69	489.07±34.91
Average	683.66	105.90	407.53	430.92
LSD value	67.31	11.18	38.48	56.78
MSE	3496.50	96.44	1142.71	2488.11

*MSE, Mean square error. Significant 0.05.

Chapter 3- Table S9. Textural properties of bread made with whole wheat flour.

Sample No.	Hardness (g)	Springiness (%)	Gumminess	Chewiness
1	2031.38±143.78	87.35±1.42	1057.8±68.28	923.59±55.28
2	2058.73±150.73	86.17±1.94	1016.80±60.48	875.74±49.58
3	6228.11±418.49	84.43±5.17	2824.51±184.35	2384.59±209.55
4	1886.38±118.14	88.81±1.58	1059.8±70.15	941.75±72.45
5	1703.25±55.20	83.75±1.61	905.74±44.11	758.63±41.41
6	3410.24±191.43	87.83±1.57	1641.60±86.37	1441.56±74.52
7	2731.78±228.74	82.62±1.88	1192.9±100.17	985.69±86.77
8	2265.99±118.48	86.97±2.69	1133.63±53.65	985.77±51.77
9	2263.43±95.66	83.86±2.4	1106.75±49.13	927.32±28.13
10	2056.04±98.18	85.08±2.4	1048.75±46.44	892.19±44.79
11	2274.38±144.21	87.06±2.54	1046.17±45.17	911.18±53.49
12	2031.99±47.9	83.29±1.85	985.86±28.32	821.03±26.84
13	2113.32±47.69	78.53±3.38	1001.63±20.99	786.91±43.75
14	2355.01±132.28	82.5±1.6	1176.46±72.19	969.63±46.26
15	3090.65±210.2	85.48±1.41	1501.68±82.58	1283.43±69.69
16	1435.38±82.07	94.28±0.96	925.27±49.44	872.24±45.80
17	1625.31±61.78	88.97±3.02	950.92±20.04	846.07±34.84
18	1782.03±61.5	81.62±1.42	917.32±35.10	748.76±33.53
19	1258.49±108.34	93.23±1.29	764.95±47.5	712.96±42.41
20	1391.49±125.5	90.54±1.82	787.99±72.99	713.97±74.14
21	1345.04±84.51	91.92±1.07	746.79±56.55	686.73±56.44
22	2761.63±145.86	83.18±1.95	1260.45±31.6	1048.25±28.48
23	1763.00±74.38	85.87±1.96	917.76±38.28	788.22±39.77
24	2492.77±130.08	81.71±3.31	1185.08±7.72	968.13±35.48
25	3260.36±71.17	86.63±3.76	1460.97±96.11	1263.14±57.86
26	2323.54±239.64	83.8±1.44	1123.24±89.01	940.27±62.24
27	2441.32±143.79	83.68±1.6	1207.96±56.09	1010.98±53.76

28	1905.21±75.19	89.3±1.87	970.46±34.7	866.71±38.27
29	1510.35±89.16	93.74±2.10	863.28±48.99	809.06±45.93
30	1447.43±89.63	89.24±1.87	834.97±51.55	744.89±43.98
31	2124.31±64.64	85.29±2.36	1127.72±32.06	962.21±46.15
32	1679.72±115.50	92.59±1.77	1031.21±66.01	954.19±52.60
33	2034.15±97.55	86.30±2.46	1120.99±48.96	967.45±50.67
34	1130.67±37.2	93.46±1.12	658.99±26.52	616.11±30.69
35	1640.86±143.36	90.53±2.09	919.18±72.97	830.86±51.46
36	1137.48±449.41	77.76±33.91	686.27±293.58	637.52±272.99
37	930.21±71.89	94.47±1.49	586.62±56.81	554.27±55.24
38	1002.28±97.44	93.23±2.27	620.57±55.74	578.02±47.70
39	1027.9±62.51	93.25±1.49	656.21±43.88	612.36±47.89
40	851.69±24.28	98.16±1.74	547.96±12.91	537.77±12.31
41	1251.54±46.77	94.8±1.21	795.3±27.42	753.71±20.09
42	1216.74±59.92	94.2±0.74	764.96±40.83	720.44±36.11
43	1458.45±89.22	92.51±1.31	878.67±39.19	812.60±31.30
44	2113.21±87.39	87.26±3.18	1110.28±37.33	969.62±62.75
45	2423.61±105.25	86.67±2.88	1254.34±70.22	1086.93±67.80
46	2142.86±135.7	87.89±1.86	1208±83.23	1060.74±60.7
47	2764.48±86.15	82.82±2.13	1362.85±64.35	1128.94±66.61
48	2967.97±142.1	83.44±1.44	1554.27±70.91	1296.72±61
49	3462.49±338.91	87.77±3.29	1567.06±141.78	1376.44±142.43
50	2562.14±118.5	83.05±2.06	1283.67±58.52	1065.56±42.76
51	1776.03±45.41	88.16±1.60	1059.81±11.23	934.29±18.16
52	1821.47±48.33	90.68±1.51	1120.62±25.25	1016.34±35.48
53	2191.52±86.66	85.5±1.58	1254.5±48.64	1072.28±37.85
54	2046.51±104.54	82.93±3.39	1086.03±82.85	901.58±88.40
55	1951.69±133.14	84.73±1.33	1021.44±76.39	864.99±59.77
56	1895.71±39.27	90.03±2.53	1024.96±29.98	922.8±37.83

57	2216.37±78.84	88.27±1.73	1173.03±49.64	1035.77±55.02
58	4402.11±180.14	83.19±3.05	1880.04±90.51	1565.52±114.55
59	1526.78±124.28	89.88±1.19	774.86±72.54	695.92±61.07
60	3425.89±156.98	80.59±2.49	1592.75±66.30	1282.45±41.28
61	5798.35±377.99	84.83±5.5	2359.33±128.4	2003.2±186.88
62	4059.93±456.6	88.17±2.48	1874.76±173	1652.34±150.85
63	2495.34±154.3	87.12±2.45	1346.34±66.86	1172.14±50.99
64	3763.52±334.22	86.23±4.04	1791.46±215.54	1548.58±231.24
Average	2227.14	87.29	1136.12	985.94
LSD	193.3	2.886	87.6	86.3
MSE	28822.36	6.428122	5927.071	5744.948

*MSE, Mean square error. Significant 0.05.

Chapter 3- Table S10. C-cell properties of white flour bread.

Sample No.	Number of cells/slice areas (mm $^{-2}$)	Cells area / %	Wall thickness / mm	Cell diameter / mm
1	0.89±0.01	49.73±0.33	0.4±0	1.53±0.05
2	0.84±0.00	50.50±0.34	0.40±0.00	1.57±0.02
3	0.87±0.05	48.80±0.21	0.40±0.01	1.46±0.05
4	0.87±0.04	49.65±0.17	0.4±0.01	1.49±0.04
5	0.87±0.02	50.25±0.21	0.40±0.00	1.52±0.05
6	0.88±0.02	48.83±0.29	0.40±0.01	1.45±0.05
7	0.89±0.01	49.9±0.49	0.41±0.01	1.53±0.05
8	0.84±0.01	50.70±0.64	0.40±0.00	1.63±0.06
9	0.85±0.02	50±0.52	0.4±0	1.55±0.01
10	0.86±0.01	50.28±0.22	0.4±0	1.58±0.05
11	0.92±0.02	49.28±0.46	0.38±0.00	1.40±0.04
12	0.9±0.02	48.63±0.25	0.4±0.01	1.41±0.03
13	0.85±0.07	49.33±0.29	0.4±0.01	1.52±0.07
14	0.95±0.01	48.8±0.53	0.39±0	1.39±0.04
15	0.91±0.03	49.03±0.23	0.39±0.01	1.43±0.05
16	0.78±0.06	50.10±0.70	0.41±0.02	1.60±0.10
17	0.74±0.11	49.73±0.72	0.43±0.03	1.61±0.13
18	0.91±0.02	49.43±0.11	0.4±0	1.51±0.04
19	0.88±0.05	49.48±0.52	0.4±0.01	1.51±0.07
20	0.85±0.03	49.5±0.48	0.4±0	1.52±0.05
21	0.81±0.03	50.10±0.78	0.41±0.00	1.52±0.05
22	0.84±0.05	49.25±0.26	0.41±0.01	1.55±0.1
23	0.85±0.02	49.15±0.64	0.4±0	1.5±0.05
24	0.86±0.05	50.15±0.53	0.4±0.01	1.55±0.07
25	0.75±0.05	49.48±0.04	0.43±0.02	1.66±0.09
26	0.89±0.07	48.48±0.25	0.4±0.02	1.41±0.07

27	0.88±0.03	49.63±0.58	0.4±0	1.5±0.06
28	0.90±0.02	50.18±0.35	0.39±0.00	1.49±0.04
29	0.87±0.05	49.53±0.32	0.39±0.01	1.47±0.02
30	0.92±0.01	49.75±0.22	0.39±0.00	1.43±0.01
31	0.9±0.02	49.48±0.18	0.4±0	1.49±0.03
32	0.90±0.02	48.83±0.22	0.39±0.00	1.40±0.03
33	0.83±0.01	50.33±0.23	0.40±0.00	1.58±0.01
34	0.86±0.04	49.28±0.48	0.41±0.01	1.53±0.05
35	0.96±0.01	49.58±0.19	0.38±0.00	1.39±0.02
36	0.92±0.04	49.33±0.43	0.4±0.01	1.49±0.08
37	0.85±0.08	50.13±1.12	0.40±0.01	1.58±0.15
38	0.79±0.03	51.48±0.49	0.41±0.01	1.72±0.03
39	0.82±0.03	50.83±0.33	0.4±0	1.67±0.07
40	0.89±0.03	49.9±0.3	0.39±0.01	1.52±0.08
41	0.83±0.53	49.83±0.13	0.41±0	1.58±0.02
42	0.82±0.05	50.88±1.00	0.41±0.01	1.68±0.12
43	0.90±0.01	49.50±0.71	0.39±0.00	1.48±0.03
44	0.93±0.01	49.4±0.6	0.39±0	1.45±0.03
45	0.91±0.04	50.13±1.00	0.38±0.00	1.45±0.04
46	0.89±0.01	49.45±0.17	0.4±0	1.52±0.02
47	0.90±0.03	49.45±0.22	0.39±0.00	1.43±0.01
48	0.88±0.07	48.93±0.53	0.4±0.02	1.48±0.05
49	0.93±0.04	49.98±0.73	0.39±0.01	1.45±0.05
50	0.88±0.06	49.28±0.44	0.39±0.01	1.44±0.09
51	0.88±0.02	49.45±0.47	0.40±0.00	1.52±0.03
52	0.89±0.02	49.50±0.78	0.39±0.00	1.47±0.08
53	0.90±0.01	49.6±0.14	0.39±0	1.53±0.03
54	0.87±0.02	49.78±0.56	0.4±0	1.52±0.04
55	0.84±0.03	49.95±0.25	0.41±0	1.6±0.05

56	0.88±0.02	49.4±0.45	0.4±0	1.5±0.02
57	0.85±0.05	50.20±0.60	0.39±0.01	1.52±0.08
58	0.82±0.03	50.53±0.43	0.41±0.01	1.67±0.07
59	0.91±0.01	48.53±0.33	0.4±0	1.47±0.03
60	0.90±0.00	49.38±0.08	0.39±0.00	1.43±0.03
61	0.85±0.02	48.73±0.29	0.41±0	1.54±0.03
62	0.81±0.05	48.8±0.16	0.42±0.01	1.55±0.04
63	0.84±0.03	49.80±0.25	0.41±0.01	1.51±0.02
64	0.86±0.03	49.33±0.38	0.40±0.01	1.50±0.06
Average	0.90	49.61	0.40	1.52
LSD value	0.107	0.774	0.014	0.096
MSE	0.003	0.308	0.000	0.005

*MSE, Mean square error. Significant 0.05.

Chapter 3- Table S11. C-cell properties of whole wheat bread.

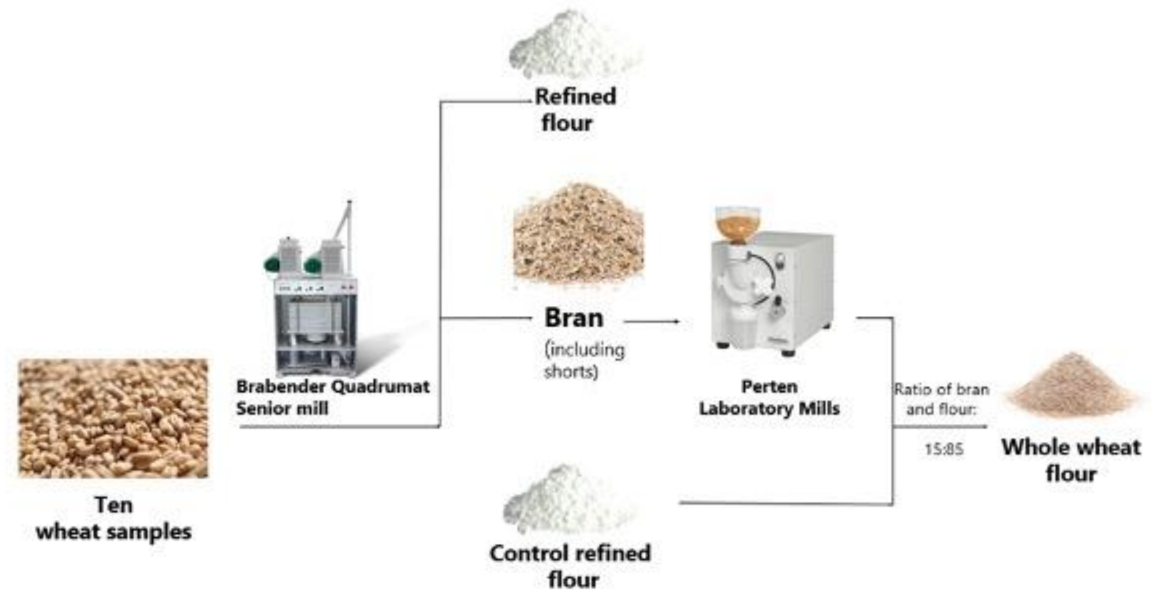
Sample No.	Number of cells/slice area (mm^{-2})	Cells area (%)	Wall thickness (mm)	Cell diameter (mm)
1	0.92±0.03	48.75±0.35	0.4±0.01	1.48±0.04
2	0.84±0.03	49.08±0.29	0.41±0.01	1.56±0.03
3	0.92±0.06	48.35±0.47	0.40±0.01	1.47±0.07
4	0.89±0.03	48.75±0.34	0.4±0	1.48±0.04
5	0.88±0.04	49.00±0.46	0.40±0.01	1.52±0.11
6	0.97±0.03	47.70±0.39	0.39±0.00	1.38±0.07
7	0.79±0.05	49.8±0.52	0.42±0.01	1.67±0.04
8	0.88±0.02	49.28±0.22	0.40±0.00	1.50±0.04
9	0.85±0.03	49.05±0.47	0.41±0.01	1.58±0.08
10	0.91±0.04	48.83±0.04	0.4±0.01	1.47±0.02
11	0.84±0.07	49.45±0.32	0.40±0.01	1.55±0.11
12	0.83±0.03	49.15±0.59	0.42±0.01	1.61±0.1
13	0.83±0.05	49.53±0.23	0.41±0.01	1.66±0.1
14	0.91±0.03	48.75±0.52	0.4±0.01	1.5±0.07
15	0.95±0.03	48.53±0.41	0.39±0.01	1.41±0.05
16	0.84±0.03	49.60±0.30	0.41±0.00	1.62±0.06
17	0.80±0.04	49.88±0.48	0.42±0.01	1.75±0.04
18	0.86±0.04	48.98±0.77	0.40±0.01	1.50±0.10
19	0.76±0.03	50.28±0.18	0.42±0.01	1.74±0.02
20	0.87±0.03	49.48±0.26	0.40±0.01	1.51±0.05
21	0.82±0.02	49.55±0.15	0.41±0	1.6±0.06
22	0.89±0.03	48.98±0.43	0.4±0	1.49±0.02
23	0.87±0.02	49.65±0.09	0.40±0.00	1.56±0.05
24	0.83±0.06	48.5±0.31	0.4±0	1.47±0.03
25	0.83±0.07	48.03±0.04	0.42±0.02	1.56±0.1
26	0.83±0.03	49.58±0.69	0.41±0	1.57±0.04

27	0.86±0.04	49.18±0.43	0.41±0.01	1.56±0.09
28	0.81±0.04	49.98±0.48	0.42±0.01	1.65±0.08
29	0.80±0.09	48.35±0.30	0.42±0.02	1.57±.0.09
30	0.87±0.01	49.35±0.15	0.4±0	1.54±0.02
31	0.82±0.08	49.38±0.29	0.41±0.02	1.59±0.08
32	0.84±0.02	49.95±0.43	0.41±0.01	1.64±0.04
33	0.81±0.07	48.90±0.35	0.41±0.01	1.55±0.08
34	0.8±0.03	50.35±0.11	0.42±0.01	1.71±0.07
35	0.9±0.03	49.2±0.27	0.4±0	1.5±0.02
36	0.95±0.06	48.83±0.68	0.39±0.01	1.4±0.05
37	0.81±0.04	50.13±0.38	0.41±0.01	1.63±0.05
38	0.86±0.02	50.00±0.31	0.40±0.00	1.54±0.04
39	0.86±0.05	50.35±0.43	0.4±0.01	1.58±0.07
40	0.75±0.04	50.43±0.19	0.42±0.01	1.68±0.04
41	0.95±0.05	48.88±0.44	0.39±0.01	1.44±0.09
42	0.87±0.02	49.68±0.28	0.4±0.01	1.55±0.03
43	0.82±0.07	49.33±0.28	0.41±0.02	1.56±0.06
44	0.87±0.03	49.53±0.35	0.4±0.01	1.55±0.06
45	0.88±0.03	48.98±0.37	0.40±0.01	1.49±0.02
46	0.9±0.02	48.55±0.15	0.4±0.01	1.45±0.02
47	0.89±0.02	48.70±0.21	0.40±0.01	1.48±0.02
48	0.93±0.02	48.75±0.29	0.39±0	1.4±0.03
49	0.86±0.06	48.28±0.69	0.40±0.01	1.43±0.04
50	0.95±0.04	48.43±0.39	0.39±0.01	1.37±0.05
51	0.78±0.05	49.58±0.56	0.42±0.01	1.67±0.09
52	0.76±0.02	49.38±0.39	0.43±0.01	1.74±0.10
53	0.93±0.03	48.88±0.26	0.39±0.01	1.44±0.01
54	0.94±0.02	49.00±0.45	0.39±0.00	1.42±0.02
55	0.78±0.06	49.65±0.5	0.42±0.01	1.67±0.07

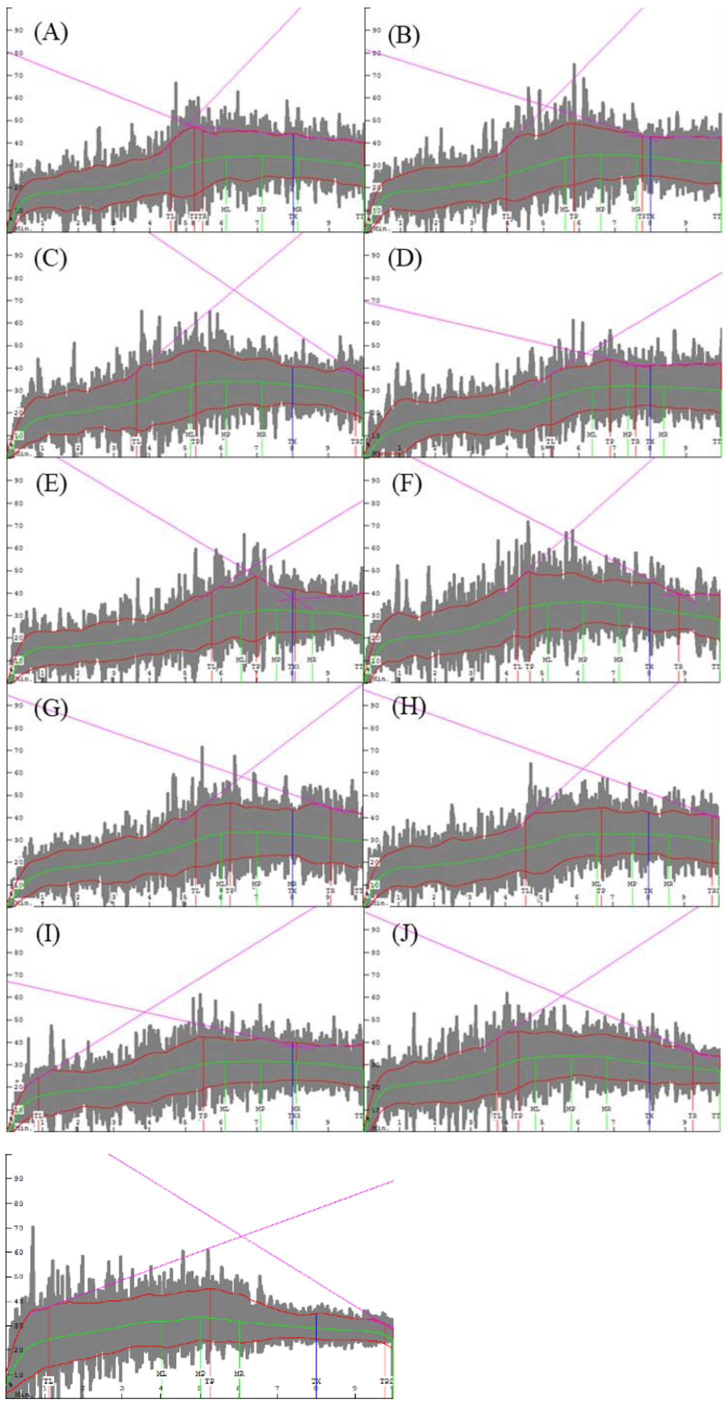
56	0.85±0.01	49.23±0.18	0.4±0	1.52±0.06
57	0.86±0.07	48.7±0.31	0.4±0.02	1.51±0.06
58	0.87±0.01	48.85±0.27	0.40±0.00	1.55±0.02
59	0.86±0.02	48.78±0.50	0.40±0.00	1.49±0.01
60	0.86±0.04	49.00±0.37	0.41±0.01	1.49±0.05
61	0.89±0.02	49.03±0.39	0.4±0	1.56±0.07
62	0.81±0.04	49.58±0.66	0.42±0.01	1.66±0.06
63	0.89±0.02	48.75±0.45	0.4±0	1.49±0.09
64	0.78±0.04	48.63±0.30	0.43±0.02	1.67±0.11
Average	0.86	49.15	0.40	1.54
LSD value	0.064	0.638	0.014	0.099
MSE	0.002	0.209	0.000	0.005

*MSE, Mean square error. Significant 0.05.

Chapter 4- Figure S1. Flowchart of preparation of the reconstituted whole wheat flour.



Chapter 4- Figure S2. Mixograms of reconstituted whole wheat flour made from 85% control white flour and 15% each bran, and the control white flour. (A-J, sample label A-J).



Control white flour