WHOLE WHEAT MILLING AND BAKING STUDIES OF HARD RED SPRING WHEAT

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

End product quality of whole wheat bread is affected by many complex parameters. The milling method and chemical composition of bran both affect whole wheat bread quality. When using a centrifugal mill, the combination of low tempering moisture level and high rotor speed produced whole-wheat flour with fine particle size, desirable whole-wheat flour quality, manageable dough, and high loaf volume. Fine bran powder was produced with the combination of low tempering moisture level, low feed rate, and high rotor speed. It was also determined that flour attached to bran affects the bran powder's temperature, protein content, and starch content. Study of the impact of bran components on whole-wheat bread revealed that fiber (FB) highly impacted gluten quality, farinograph parameters, gassing power, oven spring, loaf volume, and bread crumb protein solubility. FB interacted with other bran components (oils, extractable and hydrolysable phenolics) to increase polymeric protein solubility in bread crumb. Hydrolysable phenolics (HP) improved the farinograph stability. However, the interaction of FB with other components decreased bread loaf volume, especially for the interaction of FB-HP.

The baking method and the type of wheat used for whole-wheat bread are also important factors to evaluate whole-wheat bread quality. Sponge-and-dough (SpD), straight dough (StD), and no-time dough (NoD) methods were compared. StD had the highest variation in baking mix time, baked weight, crumb grain score, and symmetry score compared to other baking methods. The StD method was the most sensitive method to distinguish variation in whole-wheat flour samples. Location and cultivar effects were investigated for whole-wheat bread quality. Twenty-one hard red spring wheat cultivars grown at 6 locations across North Dakota were evaluated for whole-wheat bread quality. Location contributed 89% to the variability in whole-wheat baking absorption. Cultivar contributed 47% and 41% to the variability in whole-wheat loaf volume and

loaf symmetry, respectively. Loaf volume and crumb color were largely under genetic control, and breeders can aim at high loaf volume in whole wheat bread made from hard spring wheat. Overall, whole-wheat flour and bread quality are greatly affected by: milling method, bran composition, baking method, as well as the environment and genotype.

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DEDICATION

To all the dreamers who have ventured the path to treasure their own special vastness. To those who have been a shining example on my route to help me accomplish my improbable.

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GENERAL INTRODUCTION

Bread is one of the most popular wheat-based food products, and is a staple food in many countries. Bread is made by adding basic ingredients, such as water, wheat flour, yeast, sugar, milk powder, improver, shortening, and salt; although only flour, water and yeast are required. Flour and water are the most important ingredients in a bread recipe, as they affect the crumb texture (Zanoni et al. 1993). Refined flour of hard red spring (*Triticum aestivum* L.) wheat is traditionally used to measure quality due to hard red wheat breeding programs primary objective, which is to produce good bread quality (Bruckner et al. 2001). Standard methods for measuring the important parameters (including high flour protein, high water absorption, good dough extensibility and tolerance to mixing, and high loaf volume) were developed (by AACC-I Approved Methods) and based on the use of white flour or refined flour. However, there is increasing demand in the domestic market as well as the world market for whole grain bread products (Slavin 2004).

In 1999, American Association of Cereal Chemists International (AACC-I), through its Board of Directors, has defined whole grains as "whole grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis" (AACCI 1999). Whole grain flour contains vitamins, minerals, antioxidants, and other nutrients that are absent from refined flour, since these compounds are concentrated in the outer portions of the grain (Weaver 2001).

As a result of awareness and trends in fitness, whole wheat products have been gaining popularity. This has increased the demand and consumption of these types of products (Kapsak et al. 2011). In the US, the increase in whole grain food production nearly tripled whole wheat

flour production from 2002 to 2011: 3.13 x 10^8 kg in 2002–2003 compared with 9.33 x 10^8 kg in 2010–2011 (Sosland 2011).

Over the past 20 years, more than a dozen governmental, non-profit health, industrial and trade groups have encouraged the increase of whole-grain consumption (Slavin et al. 2001). Developing a food product with added benefits does not simply mean incorporating the nutritional ingredient in it at the appropriate physiological level, but also supplying a product which meets the consumer's requirements in terms of appearance, taste, and texture (Siro et al. 2008). Whole wheat flour possesses several unique challenges to the milling and baking industries. Whole wheat flour contains more lipids (Chung et al. 2009), enzymatic activity (Every et al. 2006), antioxidants (Adom et al. 2005), and dietary fiber (Slavin 2004) than refined flour. These compounds can affect the end-use products as reported by various studies such as low loaf volume and dense crumb structure (De Kock et al. 1999; Gan et al. 1992), grainy, nutty and bitter flavors (Chang and Chambers 1992; Heiniö et al. 2003), and darker crumb and crust color (Lebesi and Tzia 2011; Wang et al. 1993).

Bran represents 10-15% of the wheat grain and is a composite multi-layered material made of several tissues and some attached endosperm residues (Brouns et al. 2012). Wheat bran contains minerals, vitamins, and bioactive phytochemicals, such as antioxidant compounds and lignins (Antoine et al. 2003). Milling techniques uses to produce whole wheat flour may affects whole wheat bread quality (Kihlberg et al. 2004a). High temperature during milling can cause protein degradation and produced high starch damaged (Prabhasankar and Rao 2001). Particle size could impacts water absorption and retention, dough handling properties, as well as aesthetic appearance (Al-Saqer et al. 2000; Noort et al. 2010; Sidhu et al. 1999). There are two methods available to produce whole wheat flour. There are: 1) milling the whole kernel directly into flour;

and 2) recombine all milled fractions at the end of roller milling (Doblado-Maldonado et al. 2012). Genotype and location could also have an important role on whole wheat flour production as they might affect the bioactive phytochemicals availability. There is limited information about variation of these compounds among genotypes and how they might be affected by environment. Although some have reported that environmental factors gave greater impact than genotype on the phenolic yield (Menga et al. 2010), but little to no evidence have been found on whole wheat bread flour production. A study by Finney et al. (1985) has found that the bran of different varieties had varying effects on bread properties.

There are many challenges associated with the production of high-quality whole wheat bread. It is necessary to investigate the genetic and environmental effects on whole wheat bread quality since they play a role in the wheat quality and composition. Bread baking involves complex biochemical reactions between the constituents of the wheat flour and the addition of bran in whole wheat bread increase the number of these components. Because of this, it will be important to extract the major fractions of the bran for reconstitution studies to determine their effects on whole wheat bread quality. There are many bread baking methods cited in the literature for producing whole wheat bread, and there is no standard method used across baking laboratories and so the baking method must also be optimized to produce high-quality whole wheat bread. Given that many wheat quality labs may test wheat quality by baking white bread only, it will be essential to evaluate the correlation between white bread and whole wheat bread made from the same wheat sample.

Overall Objectives

The current research was carried out with four specific goals in mind.

1) Production and characterization of whole wheat flour through whole grain milling and bran milling.
2) Evaluate the individual bran components and their interaction towards flour, dough, and bread quality.
3) Optimization of baking method for whole wheat bread.
4) Understand the effect of location and cultivars on whole wheat bread quality.

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LITERATURE REVIEW

Wheat Kernel Structure

Wheat is among the dominant grains produced in the world. Bread wheat, belonging to the grass family Poaceae (syn. Gramineae), genus *Triticum* and species *aestivum*, is hexapoloid and accounted for more than 90 % of the world wheat production (Gooding 2009). The wheat kernel consists of three main parts; each anatomically and chemically differentiated from the others. These are the embryo or germ, the outer seed coats, and endosperm. The embryo or germ is situated at one end of the kernel as a small, yellow mass, easily distinguished from the rest of the seed. The endosperm forms much of the greater part of the entire kernel and furnishes food for the embryonic plant when the seed germinates. The outer seed coats (underlying layer) cover the entire seed and protect the embryo and endosperm from damage during the resting period of the seed's existence (Osborne and Mendel 1919). The wheat grain and its component tissues are shown in Figure 1.

Based on the magnitude of force required during milling operations and endosperm texture, wheat can be classified as hard wheat (needed higher milling force and hard endosperm) or soft wheat (required less milling force and soft endosperm) (Gooding 2009). Hardening of the wheat endosperm has be associated with absence of friabilin (puroindoline-a and -b) which weakens the interaction between gluten and starch granules (Gooding 2009). Hard wheats had high water adsorption and this characteristic is preferred for breadmaking (Gooding 2009).

Another classification of wheat grain is based on color of seed coat as a result of intensity of the red-pigment (phlobaphene) present. Red wheat contained higher phlobaphene than white wheat and were more suitable in environments where pre-harvest sprouting is likely to occur (Gooding 2009). The other form of classification is based on the flowering responses to cold

temperatures. Unlike for winter wheat, cold temperature exposure is unnecessary for normal development of spring wheats (Gooding 2009). Floral initiation for spring wheats is warmer (7-18°C for 15 days) than winter wheats (0-7°C for 30-60 days) (Gooding 2009).

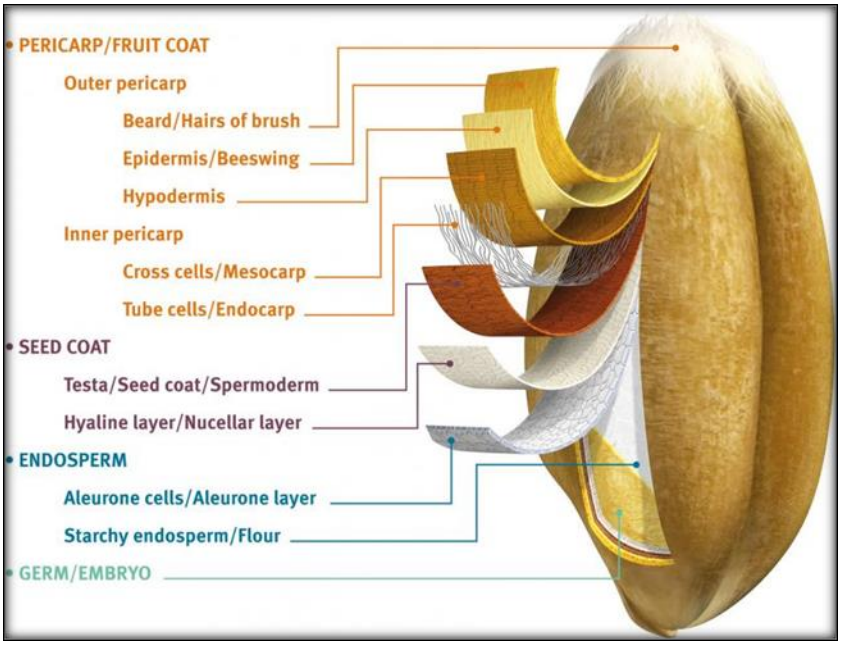

Figure 1. Wheat grain showing its component tissues.
Source: GoodMills Innovation (2016)

Whole Grain History and Definitions

The origin of wheat goes back to more than 10,000 B.C., where the consumption of whole-wheat bread started. A brief history of wheat, flour, and whole-wheat bread is summarized in Figure 2. The first flour production was attempted by the Stone Age man using rocks. Around 3,000 B.C. the first leavened and oven baked bread was produced by the Egyptians. Since then, milling technology progressively developed from watermills (85 B.C.) to windmills (1190 A.D.) and to modern roller mills (1873). Consumption of refined flour-based products was overwhelming since the invention of roller mill, as it provided affordable and efficient way to

separate the wheat fractions (Anson 2010). In the nineteen seventies, when the 'fiber hypothesis' was published by Trowell (1972), wholegrain consumption started to rise slowly. The study suggested that dietary fiber is beneficial for health by protecting against serum cholesterol and heart disease such as cardiovascular disease (CVD).

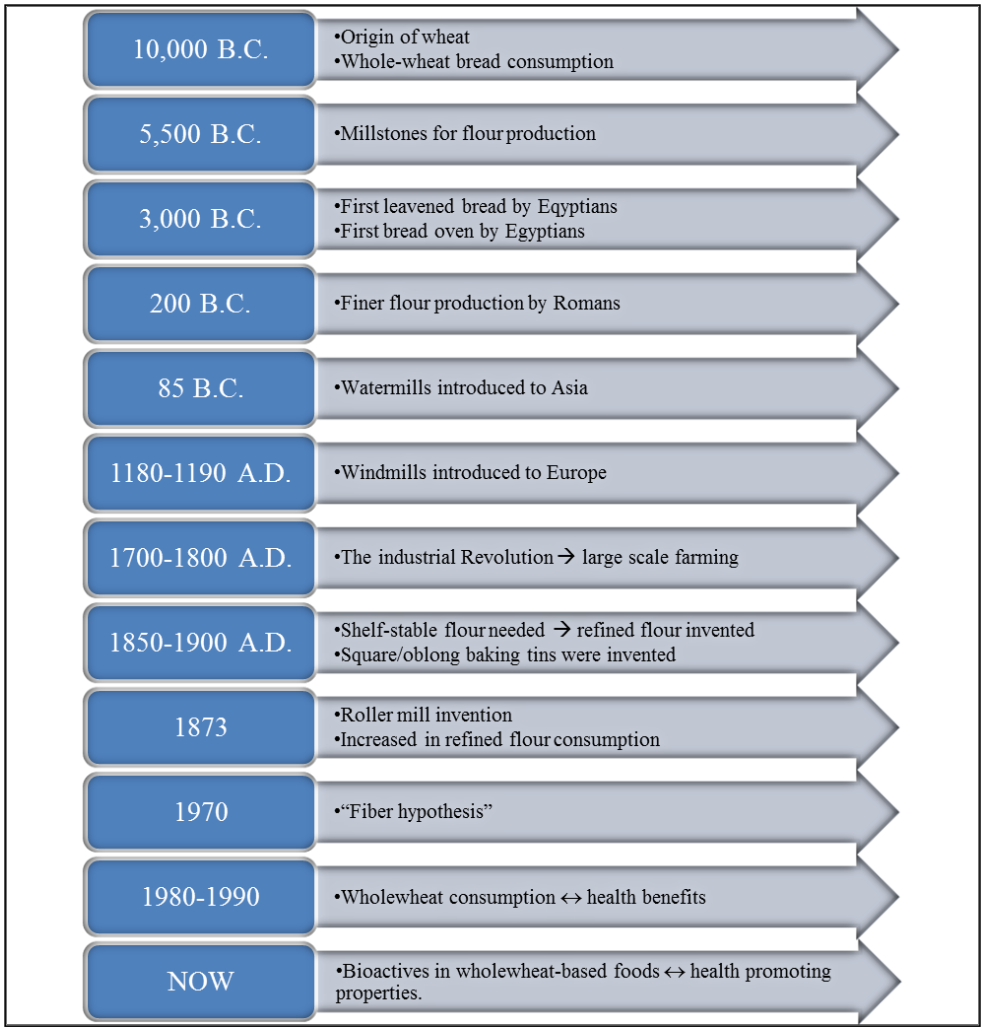

Figure 2. Timeline of wheat and bread consumption.
Source: (Anson 2010; JohnInnesCentre-and-InstituteOfFoodResearch 2016; Trowell 1972; Whitney 2013)

Since that time, additional research in the 1980's and 90's has provided strong evidence for the health benefits of whole-wheat products (Anson 2010). Abundant evidence published has led to greater popularity of whole-wheat products (Anson 2010) thus increasing the varieties of whole-wheat products on the market shelves (Whitney 2013). Recent studies have shown that the components in whole grains associated with improved health status include lignans, tocotrienols, phenolic compounds, and antinutrients including phytic acid, tannins and enzyme inhibitors (Slavin 2004). However, consumer acceptance of whole-wheat products were still lower than recommended due to low loaf volume dense crumb structure (De Kock et al. 1999), grainy, nutty and bitter flavors (Chang and Chambers 1992), and darker crumb and crust color (Lebesi and Tzia 2011).

"Whole grain" is an American term that is an abbreviation for "whole cereal grain" (Jacobs Jr and Gallaher 2004). The European use 'wholemeal' phrase; describes a finely ground wholegrain flour or a wholegrain bread (Slavin 2004). The American describes 'whole grain' as food products made from whole grain flour, both finely and coarsely ground (Slavin 2004). Therefore, to provide a more mutual understanding of whole grains, whole-grain definitions have been developed. In 1999, American Association of Cereal Chemists International (AACCI) through its Board of Directors defined whole grain as "shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis"(AACCI 1999). However the whole grains council put out its definition in 2004 as "Whole grains or foods made from them contain all the essential parts and naturally-occurring nutrients of the entire grain seed in their original proportions. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver the same rich balance of

nutrients that are found in the original grain seed". This definition means that 100% of the original kernel – all of the bran, germ, and endosperm – must be present to qualify as a whole grain" (Whole-Grains-Council 2004).

Whole grain can be a food on its own such as oatmeal, brown (red or black) rice or popcorn. Alternatively, it can be processed and used as an ingredient in a product (van der Kamp et al. 2014). When whole grain ingredients are used to make breads, pasta, crackers, breakfast cereals, and other grain-based foods, inconsistency exists between countries as to what qualifies as a whole grain food product (Slavin et al. 2014). Following the earlier stated definition of whole grain by AACCI, any food containing 100% whole grain is considered as whole grain food. Other categories of foods that have been considered as whole grain foods in USA include: (i) those food that has ≥51% of their ingredient made of whole grain; (ii) food with ≥16 g of whole grain/serving; and (iii) food that provide ≥8 g of whole grain/serving (Slavin et al. 2014). In Europe, definitions of whole grain food includes: (i) wheat or rye bread containing 90% (Baker's percentage) of whole grain; (ii) ≥50% of whole grain (and 30% of total weight) for bread; (iii) ≥60 % of whole grain for crisp bread, breakfast cereal and pasta; (iv) ≥15% of whole grain for pizzas, pierogis and other savory pies; and (v) ≥25% of whole grain for bread, sandwiches and wraps (Slavin et al. 2014). Table 1 summarizes the whole grains food definitions across USA and Europe.

Table 1. Examples of whole grain food definitions[a]

Definition	What Foods Qualify
United States	
FDA whole grain health claim (1999, 2003, 2008)	≥51% of total product weight is whole grain
Whole Grains Council Whole Grain Stamp (2005, 2006)	≥8 g of whole grain/serving (Basic Stamp); ≥16 g of whole grain/serving; all the grain is whole grain (100% Stamp)
USDA/FNS Women, Infants and Children (WIC) Program (2007, 2012)	In general, whole grain must be the first ingredient and the food must qualify for the FDA whole grain health claim (i.e., ≥51% of total product weight is whole grain)
USDA/HHS Dietary Guidelines for Americans (2010)	Several definitions qualify: 100% whole grain foods; Foods in which is the first ingredient; ≥51% of total weight is whole grain; ≥8 g of whole grain/ounce-equivalent
USDA/FNS school food programs (2012)	"Whole grain-rich" indicates ≥50% of grain is whole grain; foods also qualify if they contain ≥8 g of whole grain/serving, if they qualify for the FDA whole grain health claim, or if the first ingredient is whole grain
Europe	
Germany	Baker's percentage of whole grain required to say "whole grain": 90% whole grain for wheat and rye bread; 100% whole grain for pasta
Sweden, Keyhole Symbol (1989)	Percentage of grain as whole grain (dm): 100% for flour, meal, and grains; ≥50% for crisp bread, porridge, and pasta (unfilled); ≥25% for bread, sandwiches, and wraps; ≥15% for pizza, pierogis, and other savory pies
United Kingdom, IGD Grocers' Association (2007)	≥8 g of whole grain/serving
Denmark, Danish Wholegrain Campaign (2007)	Percentage of grain as whole grain (dm): 100% for flour, grains, and rice; ≥50% for bread (and 30% of total weight); ≥60% for crisp bread, breakfast cereal, and pasta

[a] this is not a comprehensive list. FDA=US Food and Drug administration; USDA=United States Department of Agriculture; FNS=Food and Nutrition Service; HHS=Health and Human Service; IGD=Institute of Grocery Distribution. Source: Slavin et al. 2014

Bran Structure, Composition and Its Effects on Whole-wheat Bread

Wheat bran composed of grain's outmost layers: outer and inner pericarp, testa, hyaline and aleurone layers with remaining adherent starchy endosperm. Since attention of researchers towards the nutritive value of bran, bran is now considered as a co-products as against its previous description as by-products (Zhang and Moore 1999). Compositionally, wheat bran contains protein (9.6 – 17.1 %), ash (4.0 – 6.5 %), fat (2.9 – 4.8 %), dietary fiber (48.0 %) and carbohydrate (50.7 – 59.2 %). Furthermore, wheat bran consists of important nutritional biomolecules including phenolic compounds (1.07 %), phytic acid (3116 – 5839 mg/100g of dry weight) (Chinma et al. 2015; Stevenson et al. 2012).

Fibers

Dietary fibers are a group of carbohydrate polymers that are resistant to digestion and absorption in the human small intestine, but could be hydrolyzed by gut microflora in human large intestine (AACCI 2001). Dietary fibers in wheat bran comprise of soluble or insoluble form that constitutes 2.4 and 45.6 % respectively (Chinma et al. 2015). Dietary fibers have been stated to possess prebiotic effect, anti-cancerogenic effect, regulation of blood glucose level, lowering blood cholesterol and anti-inflammatory effect (Mendis and Simsek 2014). Numerous dietary fibers have been identified including fructan fructo-oligosaccharides, oligofructose, inulin, β-glucan, and arabinoxylan. Arabinoxylan is the most abundant noncellulose dietary fiber in cereals and grasses. Structurally, arabinoxylan is a polymer of xylose (β-(1-4)-linked xylose backbone residues) with substitutes of arabinofuranosyl (Mendis and Simsek 2014).

Dietary fibers have known to be beneficial to human health; soluble fiber for its hypocholesterolemic effect and insoluble fiber for its risk reduction of colon cancer effect (Slavin et al. 2014). However, it possesses detrimental effect to whole-wheat bread quality such

as low loaf volume and dense crumb texture (Park et al. 1997; Pomeranz et al. 1977). SEM images of wholewheat bread provided by Gan et al. (1989) indicated that the bran components can disrupt the gluten matrix network; thus affecting its functionality to retain loaf structure during fermentation and baking. Rosell et al. (2010) found that fibers disrupts the viscoelastic properties and leads to weaker doughs; and fiber also greatly competes for water. Later, two published articles explained on how fiber disrupts gluten network (Bock and Damodaran 2013; Nawrocka et al. 2016). Details on that will be discussed later in 'weakening of dough strength' section of this literature review.

Phenolics

Phenolics are compounds with one or more aromatic rings with one or more hydroxyl groups. Generally, phenolics are categorized as phenolic acids, flavonoids, stilbenes, coumarins, and tannins (Liu 2007). Phenolic compounds are classified into different groups and their occurrence in plants primarily depends on the plant species. The concentrations in whole grains is affected by grain types, varieties, and the part of grain sampled (grain anatomy) (Adom et al. 2003). The most common phenolic compounds found in whole grains are phenolic acids and flavonoids. Two major groups for phenolic acids are hydroxybenzoic acid and hydroxycinnamic acid (Figure 3). Their derivatives were given in Figure 4, mainly present in the bound form, linked to cell wall structural components such as cellulose, lignin, and proteins through ester bonds. The bran/germ fraction contributed 3% of total phenolic content, 79% of total flavonoid content, 78% of total zeaxanthin, 51% of total lutein, and 42% of total β-cryptoxanthin (Liu 2007). Wheat kernels contain a number of phenolic compounds, namely ferulic, vanillic, caffeic, salicylic, syringic, p-coumaric and sinapic acids (Krygier et al. 1982; McKeehen et al. 1999). Ninety percent of total phenolic acids in grain was predominantly accounted by ferulic acid

(Adom et al. 2003; McKeehen et al. 1999), and it is esterified to arabinose (Faurot et al. 1995; Izydorczyk et al. 1991), stanols and sterols (Seitz 1989) and glucose (Herrmann and Nagel 1989).

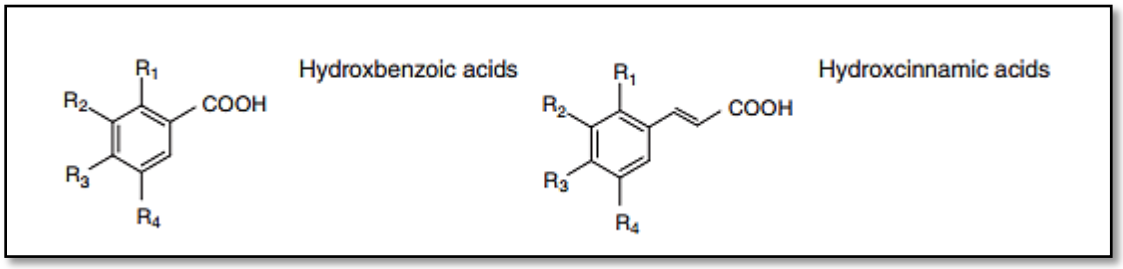

Figure 3. Chemical structures of phenolic acids (Ragaee et al. 2014)

Phenolic acids in cereals are present in free and conjugated forms (Liu 2007). Hydroxybenzoic acid derivatives include ρ-hydroxybenzoic, protocatechuic, vannilic, syringic, and gallic acids. They are commonly present in the bound form and are typically components of complex structures such as lignins, hydrolyzable tannins, derivatives of sugars and organic acids in plant foods. Hydroxycinnamic acid derivatives include ρ-coumaric, caffeic, ferulic, and sinapic acids. These derivatives are mainly present in the bound form, linked to cell wall structural components such as arabinoxylan (Figure 4). Wheat bran is a good source of ferulic acids, which are esterified to hemicellulose of the cell walls (Naczk and Shahidi 2006). These bound phenolic acids can be released during food processing steps, such as thermal processing, pasteurization, fermentation, and freezing (Dewanto et al. 2002).

Presence of phenolic acids in whole wheat bread impacted the dough (Koh and Ng 2008) and end-product quality (Han and Koh 2011b). Some phenolic compounds, such as fumaric acid and ferulic acid, carry out their reducing reaction on gluten disulfide crosslinks (Sidhu et al.

1980b). Interruption of disulfide crosslinks within gluten matrix induces dough breakdown and ultimately reduces the dough's stability (Koh and Ng 2009; Koh and Ng 2008). The phenolic acids affect breadmaking quality by altering the flour protein properties (Han and Koh 2011b). Han and Koh (2001) added different phenolic acids on wheat flour and evaluate the rheological properties of dough and bread. Addition of phenolic acids resulted in shorten dough's mixing time and tolerance, increased the dough's extensibility, and reduced loaf volume (Han and Koh 2011b). Some studies had shown that phenolic acids altered the high-molecular-weight SDS-soluble protein in breadmaking. Phenolic acids involved in altering the protein crosslinking (in gluten matrix) and also increase the solubility of high-molecular-weight SDS-soluble proteins (Han and Koh 2011b).

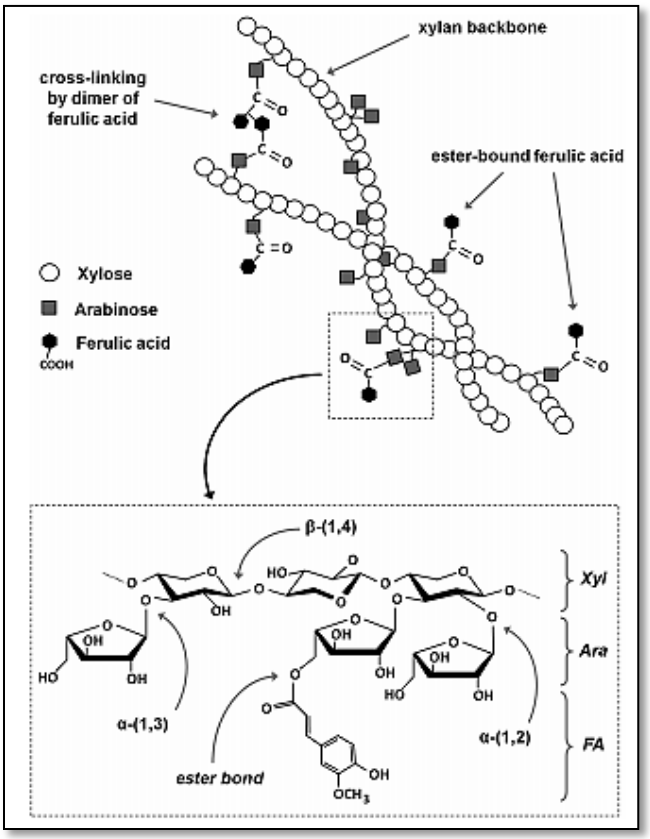

Figure 4. Ferulic acid bound to arabinoxylan structure in wheat bran.
Source: (Anson et al. 2012)

Fats and Oils

As used by baking industry, the term "fat" refers to triglycerides (three fatty acids attached to a glycerol backbone) that are semisolid at room temperature, while "oils" describes triglycerides that are liquid under the same conditions (Pyler and Gorton 2009). In general, lipids help to improve textural properties of bread crumb, mouth-feel, dough handling, loaf volume, and increase shelf life (Ponte and Baldwin 1972). Chung et al. (1978) demonstrated that lipids help to stabilize the air cells and prevent coalescence during the growth and expansion of the dough. Bakers expect their fats to provide plasticity to dough and coat the gas cells; thus stabilizing the foam structure during expansion of the loaf (MacRitchie and Gras 1973; Pyler and Gorton 2009).

In the wheat kernel, most of the lipids are located in the germ (8-15 %), bran (6 %) and endosperm (8 %) (Pomeranz 1973). Attention has been focused on endosperm lipids rather than whole-wheat lipids, which mostly were found at the germ part. Tait et al (1988) studied about lipid changes on whole-wheat flour during storage and its bread quality (Tait and Galliard 1988). The presence of oleic and linoleic acids (fatty acid) produced bread with much lower volume and texture scores compared to freshly-milled whole-wheat flour, whereas palmitic acid had no effect on either parameter. The crumb texture of the oleic acid treated bread was described as very open, 'weak', and irregular. While, the crumb texture of linoleic acid treated bread was very 'solid' with an irregular cell structure.

Whole Grain and Human Health

Research has shown that whole grain consumption has been associated with reduced the plasma total cholesterol and LDL-cholesterol concentration (Tong et al. 2014), reduced risk of cardiovascular disease (Mellen et al. 2008), heart disease (Jacobs et al. 1998), obesity (Pauline

and Rimm 2003), diabetes (Slavin 2004), and certain types of cancer (Schatzkin et al. 2008). The fermentable carbohydrates (including dietary fiber, resistant starch, and oligosaccharides) contains in whole grains is associated with lowering cholesterol level, improved glucose response, and improved laxation (Slavin 2004). Also, consumption of whole grains could improve in weight management via delays gastric emptying (McIntyre et al. 1997; Vincent et al. 1995). Jenkins et al. (1988) stated that whole grains have low glycemic index (GI). Consuming a low-GI diet (containing whole grains) exhibited in lower blood glucose levels and decreased insulin secretion for both normal and diabetic subjects (Jenkins et al. 1988). Pereira et al. (2002) concluded that wholegrain foods reduce the risk of type 2 diabetes mellitus (DM) and heart disease when conducted a study on hyperinsulinaemic adults.

Strong evidence were exist to conclude that wholegrain products may reduce the risk of coronary heart disease (CHD) (Truswell 2002). Jacobs et al. (2004) reviewed 13 prospective studies and concluded that daily intake (habitually) of whole grains may reduce the risk of CHD by 20-40% compared to subjects who rarely consume whole grains. Other studies (Humble et al. 1992; Todd et al. 1999) also concluded that consumption of dietary fiber has associated with reducing risk of CHD. Bran contains high in dietary fiber. Numerous studies have shown that inclusion of wheat bran in meal exhibited anti-cancer potentials. Food research in fiber has been reported to have lower fecal bile acid concentration, thus, decrease the risk of colorectal cancer. Wheat bran has equally showed a protective effect on colon carcinogenesis. Anticarcinogenic effect of wheat bran has been partially associated with low fermentation process in the large intestine (Kroon et al. 1997).

Wheat bran-derived arabinoxylan oligosaccharides have exhibited prebiotic properties by selectively stimulating the growth of Bifidobacterium species in in-vitro and in-vivo studies (Van

Craeyveld et al. 2009). Increase in bifidobacteria (short chain fatty acids producers) population in the intestinal result in a reduction in pH which inhibits the growth of pathogenic bacteria (Wang et al. 2010). Other reports have shown AX exhibited prebiotic effect by promoting the proliferation of probiotic bacteria like lactobacilli and bifidobacteria in the large intestine (Grootaert et al. 2007; Zhou et al. 2010). Several reports have shown that interaction of dietary fiber with the gut has exhibited significant alteration of secretion of immune related hormones and cytokines (Mikkelsen et al. 2014).

Phenolic acid, tocopherol and carotenoid compositions in acetone extract of wheat bran have displayed antioxidant functions such as scavenging of hydroxyl radical, 2,2-diphenyl-1-picryhydrazyl radical and superoxide radical anion, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid, oxygen radical absorbing capacity and chelating capacities against Cu2+ and Fe^{2+} (Zhou et al. 2005). Antioxidant properties of ferulic acid have been associated with other health beneficial effects against cancer, cardiovascular disease, diabetes and Alzheimer's disease (Zhao and Moghadasian 2008). Positive result of antioxidant potentials on human LDL oxidation and free radicals was obtained from of wheat bran extracts of Akron and Trego in three locations in Colorado (Yu et al. 2005).

Whole Wheat Bread Challenges in Food Processing and Industry

Interferences with Sensory Acceptability

One of the challenges faced during the milling of whole wheat flour is the maintenance of quality of flour. The kernel pericarps are of different colors which affect both the physical appearance of whole wheat flour and the quality of the final products (Doblado-Maldonado et al. 2012). The color of pericarp varies from white to black or from red to blue. Consumers favor lighter colored bread with a less bitter flavor that was made from white whole wheat flour

compared to red wheat flours (McGuire and O'Palka 1995). However, nutritional assessment by consumers favors whole grain muffins made from red wheat than that of white wheat, even though both muffins are of similar nutritional composition (Camire et al. 2006). Similar to high fiber rich product, baked product from whole wheat flour exhibit properties, such as reduced loaf volume, hard crumb, bitter flavor and dark color, that affect consumer sensory evaluation as well as their acceptability (Ktenioudaki and Gallagher 2012).

Handling During Processing

Product handling during processing has also been an issue which whole wheat flour. Increase in dough stickiness has been observed in high fiber dough. High stickiness might display some level of handling challenges such as difficulties in machinability during automated bread-making process (Hammed et al. 2016). Also, it is likely that development time and mixing stability of whole wheat flour/dough will be high, similarly to high fiber flour/dough thus, increase in processing time and mixing challenges. Previous results have shown that dough extensibility was reduced in whole wheat flour compared to refined flour. Dough with reduced extensibility (a measure of dough expansion during fermentation) has a negative effect on baking performance and final product quality (Ktenioudaki and Gallagher 2012).

Product Shelf Life

Presence of phytochemicals and lipids in whole wheat flour and products have been reported to influence the storability of the whole wheat flour and its products. Due to the general belief that whole wheat flour are less stable during storage, whole wheat flour has usually been stamped with 3 – 9 months shelf life unlike wheat flour with 9 – 15 months shelf life (Doblado-Maldonado et al. 2012). Reduction in shelf life can be associated with occurrence of side reactions and interaction among whole wheat flour constituents (Doblado-Maldonado et al.

2012). Several biochemical changes (with possible negative effect on quality) are distinguished in whole wheat flour compared to wheat flour (Tait and Galliard 1988). Lipid has been recognized as the most unstable composition in whole wheat flour. Degradation of lipid during storage of whole wheat flour affect gluten functionality, bread palatability, and nutritional properties (Doblado-Maldonado et al. 2012). The presence of fatty acid (as a result of lipid oxidation during storage) resulted in bread with much lower volume and denser crumb texture compared to freshly-milled whole-wheat flour (Tait and Galliard 1988). Endogenous lipid in whole wheat flour has been reported to play a significant role in flour functionality. Non-starch lipids (NSL)-gluten interaction affect dough rheological properties and bread crumb color (Goesaert et al. 2005).

Lipid oxidation decreases the nutritional quality and consumer acceptability of whole wheat flour and its end products. Nutrition qualities of whole wheat flour are lost due to loss of essential amino acids (lysine, cysteine, methionine, and tryptophan) (Pokorny et al. 1995). Poor gluten functionality could occur as a result of co-oxidation with lipids. Interactions between protein and lipid radicals would be the cause for poor gluten functionality during long-term storage of whole-wheat flour (Doblado-Maldonado et al. 2012). Lipid oxidation leads to the production of undesirable odor components thus affect the sensory acceptability of whole-wheat products (Heinio et al. 2002). Lipoxygenase activities also cause loss of carotenoid and vitamin E (Leenhardt et al. 2006; Lehtinen et al. 2003).

Weakening of Dough Strength

Unlike refined flour, whole wheat contains numerous bioactive compounds present in bran and germs. These bioactive compounds have been recognized to exert certain effect on gluten-strength, thus, affect the dough strength and ultimately impacted the end product quality

such as loaf volume and crumb texture. There are two schools of thought regarding the basic mechanism by which bran components affects the dough and bread quality. The first implicates presents of bran's fiber in dough and bread systems. The second hypothesis contends that bran's phenolic compounds impacted the dough system.

The first theory is about fiber. Bran causes the "dilution of gluten" in dough system and affected the gas-holding capacity of the dough (Pomeranz et al. 1977). Bran particles mechanically interfere with the organization of gluten network and also known to compete with gluten for water, thus, reduce water available for gluten development (Salmenkallio-Marttila et al. 2001). Underdeveloped gluten leads to low loaf volume and less favored crumb texture. Another explanation is bran particles affects loaf volume and internal crumb structure by physically disrupting the gas cells and gluten network. The evidence was shown from scanning electron micrographs (SEM) by Gan et al.(1989). Bock and Damodaran (2013) and Nawrocka (2016) conducted a study at a molecular level on how the fiber affected dough system. Both articles concluded that fiber disrupted the secondary protein structures network; especially gluten forming protein, via induced the changes of α-helix to β-structures (Figure 5) (Bock and Damodaran 2013; Nawrocka et al. 2016). Changes in protein secondary structure may leads to loss of functionality.

Second argument on whole-wheat dough weakening was present of phenolic acids. There are various classes of phenolic compounds present in whole grains (Fardet 2010; Slavin et al. 2014); however, the common phenolic acids in wheat include ferulic acid, vanillic acid, caffeic acid, syringic acid, and ρ-coumaric acid, with ferulic acid (FA) being predominant (Liu 2007). Free FA, low molecular weight conjugates, and FA-covalently bound to macromolecules have been found in wheat flours and glutens (Sosulski et al. 1982).

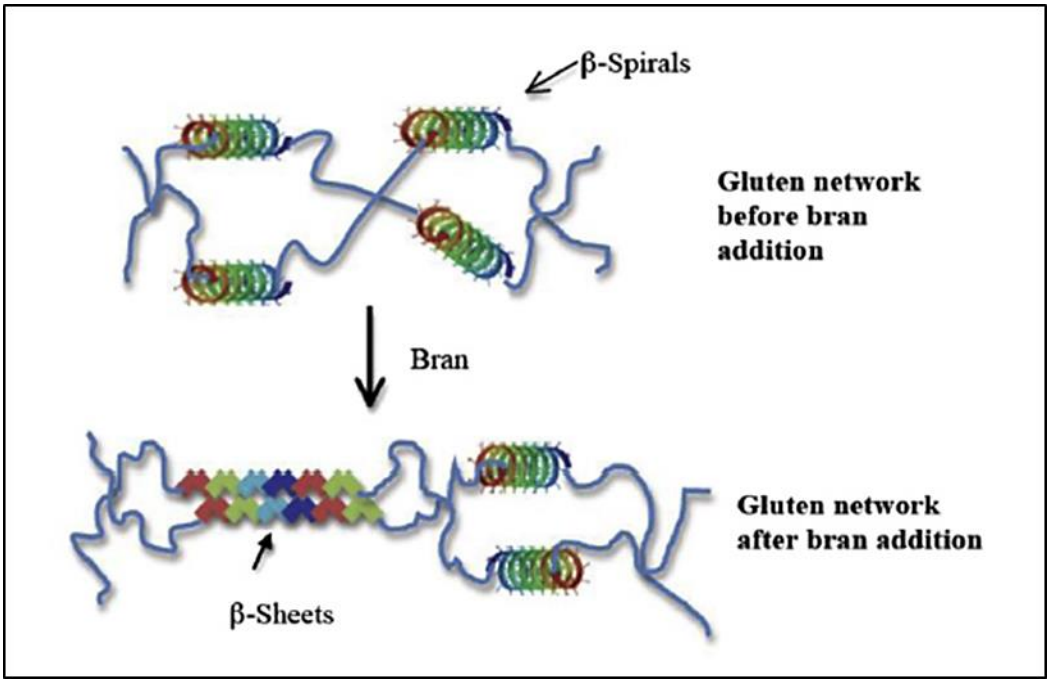

Figure 5. Schematic description of bran-induced conformational changes in gluten network. Source: (Bock and Damodaran 2013)

Gluten strength in whole-wheat dough system was reportedly weakened due to present of phenolic acids from bran (Labat et al. 2000a). Jackson and Hoseney (1986a) suggested that the dough breakdown was provoked by the reaction of FA, especially the soluble-bound form. Series of experiment were reported to confirm this theory. Labat et al. (2000) measured the FA content in fully developed dough and overmixed dough. The amount of total phenolic acids decreased (up to 46% of the total amount) in the overmixed dough, indicating the FA was linked/bound with some components in gluten (Jackson and Hoseney 1986a; Labat et al. 2000a). Koh and Ng (2008) proved that phenolic acid resulted in dough with softer texture, increased extensibility and decreased elasticity in soft wheat. Ferulic acid reduced the mixing time and mixing tolerance in hard wheat flour (Koh and Ng 2009). However, the addition of transglutaminase enzyme (TG) in the dough system, which creates non-disulfide crosslinks, resulted in the reversal of these effects after 90 min of fermentation, indicating that TG restores the fermented dough quality. Interestingly, although TG restored the dough quality after fermentation, it did not improve the

quality of end product, which were small loaf volume and increased crumb firmness (Koh and Ng 2009). Another experiment was conducted by Han and Koh (2011) to investigate the effect of phenolic acids on dough and bread characteristics and to identify the change in protein structure. Same phenomenons, which were decreased mixing time, mixing tolerance, maximum resistance to extension of dough and bread loaf volume were observed by Han and Koh (2011) when compared with other studies (Jackson and Hoseney 1986a; b; Koh and Ng 2009; Koh and Ng 2008; Labat et al. 2000a; Labat et al. 2000b). Additionally, Han and Koh (2011) found that phenolic acids reduced the high-molecular-weight proteins and increased the extractable proteins in SDS-solution, indicating that the wheat proteins are rearranged during breadmaking with the present of phenolic acids. The only explanation offered by them was the phenolic acid disrupt the gluten matrix in dough system via preventing the crosslinking between proteins and increase the solubility of protein (Han and Koh 2011b).

Milling and Mill Description

In milling, energy is expanded to break apart the bran and endosperm and reduce the endosperm into flour (Posner and Hibbs 2005). It involves the application of a force to reduce the average size of the particles. Milling converts cereals into more-palatable, more-desirable food ingredients (Delcour and Hoseney 2010). As early as Stone Age era, humans used two flat stones to reduce the wheat kernel into flour for making a bread (Figure 2) (JohnInnesCentre-and-InstituteOfFoodResearch 2016). The principle forces for size reduction are 1) compression; 2) shear; 3) friction/abrasion; and 4) impact (Posner and Hibbs 2005). Most size reduction machines combine these principles. Stone mill combines the forces of compression, shear, and abrasion. Hammer mill applies the impact forces between hammers and the wall.

Roller Mill

Roller mill applies shearing and compression as their primary forces. Roller mills (Figure 6) commonly are used in the grain milling industry because of the wide range in grinding action and economy option. Roller mill includes two compartments: 1) break section; and 2) reduction section. The basic designed of roller mill has two rolls positioned together, separated by small gap, and rotating in opposite directions. The roller mill system has two objectives: 1) remove the bran from endosperm (accomplished by the break section); and 2) reduce the endosperm to the desired particle size (accomplished by the reduction section). The break section consists of corrugated rolls, where the slow moving roll holding the material while it is being scrapped by the fast moving roll. The reduction section is mainly comprised of smooth surface rolls. The purpose is to reduce the midlings (large pieces of endosperm) to a finer particle size (i.e. pass through 132µm screen openings) (Posner and Hibbs 2005).

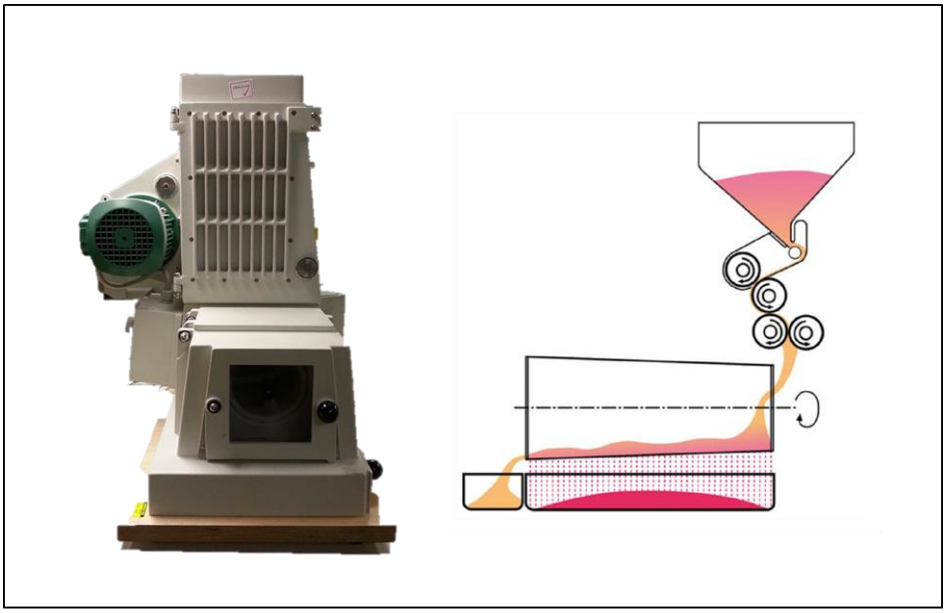

Figure 6. Roller mill and schematic picture showing the set of paired rolls.
Source: (Brabender 2016a)

Centrifugal Mill

Centrifugal mills apply impact and shearing forces for size reduction. The size reduction takes place between the rotor and the fixed ring sieve. The centrifugal mill has three compartments: 1) feeding; 2) grinding; and 3) air-cooling system (Figure 7). Grains pass through the vibratory feeder and fall onto the rotating rotor. The rotating rotor throws the grain outward (with splash-back protection) with great energy. The grains then will be precrushed due to impact with rotor teeth. Finally, the precrushed grain will be finely ground between the rotor and the ring sieve (Retsch 2015).

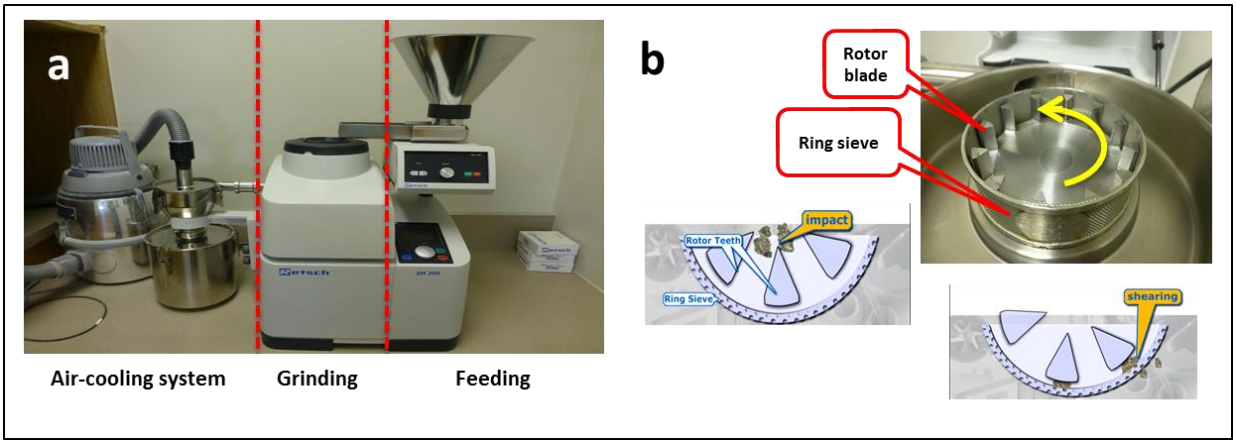

Figure 7. Centrifugal mill (a) and the rotor with the screen (b).
Source: (Retsch 2015)

Whole Wheat Milling

Whole wheat milling faces different challenges compared to normal traditional milling because the separation of kernel components is not needed. However, milling process for whole wheat is critical as this will affect the quality of the whole wheat flour as well as end product. The study of the effect of different milling technique on quality of whole wheat flour and its bread revealed that milling technique has a greater impact than did the farming system and baking technique (Kihlberg et al. 2004a). Damaged starch, extensograph parameters and

farinograph parameters (water absorption, dough development time and dough stability) were higher in roller-milled samples than for the stone milled samples. Milling process causes greater effect on whole wheat bread than the quality of wheat used for flour production or the formulation of the bread itself (Kihlberg et al. 2004a). The study also showed that roller-milled flour were sweet, juicy and compact attributes while stone-milled flour are salty, deformed and roasted cereal attributes.

Currently, the most widely use milling process for whole wheat flour production are stone and roller mills, while impact or hammer mill are rarely utilized (Doblado-Maldonado et al. 2012). There is not much difference in the application of stone mills for the production of whole wheat flour because it does not involve extra cost to the milling industries. However, roller mills might necessitate additional steps and cost when it is used for whole wheat flour production. Normally, bran and germ are reintroduced into the milling stream to produce whole wheat flour in a roller mill industry. Sometimes, bran is subjected to post-milling treatments such as steaming, thermal treatment, or ultra-fine grinding. Bran post-milling treatments lead to addition capital cost for post-milling operation and equipment for recombining the fractions (Doblado-Maldonado et al. 2012).

While tempering or conditioning is a necessary step in wheat milling for flour to achieve proper separation of endosperm and bran, tempering is not needed for whole wheat flour production in roller milling operation. However, 1 – 2 % moisture is usually added to whole wheat grain at industrial level basically to soften the grain and achieve energy efficiency for flour production. Another approach to achieve efficiency is by tightening the roll gap and using more open scalp covers to increase the break release and changing some of the smooth rolls to corrugated during reduction. Another adjustment required when using roller mills for whole

wheat flour production is the purifier air valves in other to accommodate bran and germ back into the reduction system (Kent 1994).

According to Kent (1994), roller mills has some advantages for whole wheat flour production when compared to the stone mill. Such advantages include:

(i) Variation in raw materials could be accommodated by adjusting the amount of grinding and reduction at each roll.

(ii) Less thermal damage to endosperm fraction can be achieved by selective corrugations and differential speeds to minimize shear and compressive forces during grinding and reduction.

(iii) It is easy to separate bran and germ for possible post-milling treatment that might be required.

Bran Milling

Most bran milling studies have been done to reduce the particle size and aid the separation of functional compounds, also known as dry fractionation (Antoine et al. 2004a; Hemery et al. 2009a; Hemery et al. 2009b; Rosa et al. 2013; Seyer and Gélinas 2009; Zhu et al. 2010). Van Craeyveld (2009) in his study has successfully produced nanoscale level of arabinoxylan-oligosaccharides (AXOS) from bran via ball-milling. Optimum milling condition can be achieved via controlling these factors: degree of filling of the milling jar and milling time. Ball milling makes upgrading of low-value bran feasible, and the resulting fine bran particles showed an increase in water extractable arabinoxylan (Van Craeyveld et al. 2009).

Another attempt on bran fractionation was done via ball-milling (Antoine et al. 2004a) and pin-milling (Antoine et al. 2004b) of wheat bran obtained after roller milling. It was noted that “when bran particle size was reduced below the aleurone cell dimensions, there was a

moderate increase in the extractability of the cell content marker" (Antoine et al. 2004a). Fractures in walls of cells of aleurone layer during ball-milling, resulted in increased water-extractable phytates and ρ-coumaric acid (Antoine et al. 2004a). A decrease in particle size also results in an increase in particle surface area, which can result in a higher release of bioactive compounds from the food matrix due to higher solvent-compounds interactions, and can therefore increase the bioaccessibility and/or bioavailability of these compound (Hemery et al. 2011).

Another potential dry fractionation method of wheat bran was investigated using the electric forces (Hemery et al. 2009b). Hemery and the team (Hemery et al. 2009a; 2009b) found that medium sized aleurone-rich and pericarp-rich fractions displayed different charging characteristics. Therefore, with this findings they suggested that aleurone cell walls and pericarp layers might be sorted out using appropriate electric field forces, as both layers exhibited distinct electrostatic properties (Hemery et al. 2009b).

Centrifugal impact milling was used by Chen et al. (2013) as alternative method for dry fractionation. Based on the mechanical properties of wheat bran tissues, the outer pericarp exhibited elasticity,whereas the intermediate and aleurone layers both exhibited elasto-plastic rheological properties (Antoine et al. 2003; Greffeuille et al. 2007), the wheat bran were mainly broken by impact force generated by the rotating blade tip (Chen et al. 2013) (Figure 7). Chen et al. (2013) explained further on the fate of intermediate and aleurone layers after the impact force was introduced to the bran layers, "When the impacting was ended, the outer pericarp recovered to original status for its elasticity, while the intermediate and aleurone layers might still remain bent due to their better plasticity. It caused the detachment of the outer pericarp and the other

layers". The schematic of wheat bran tissues detachment using centrifugal impact milling is shown in Figure 8.

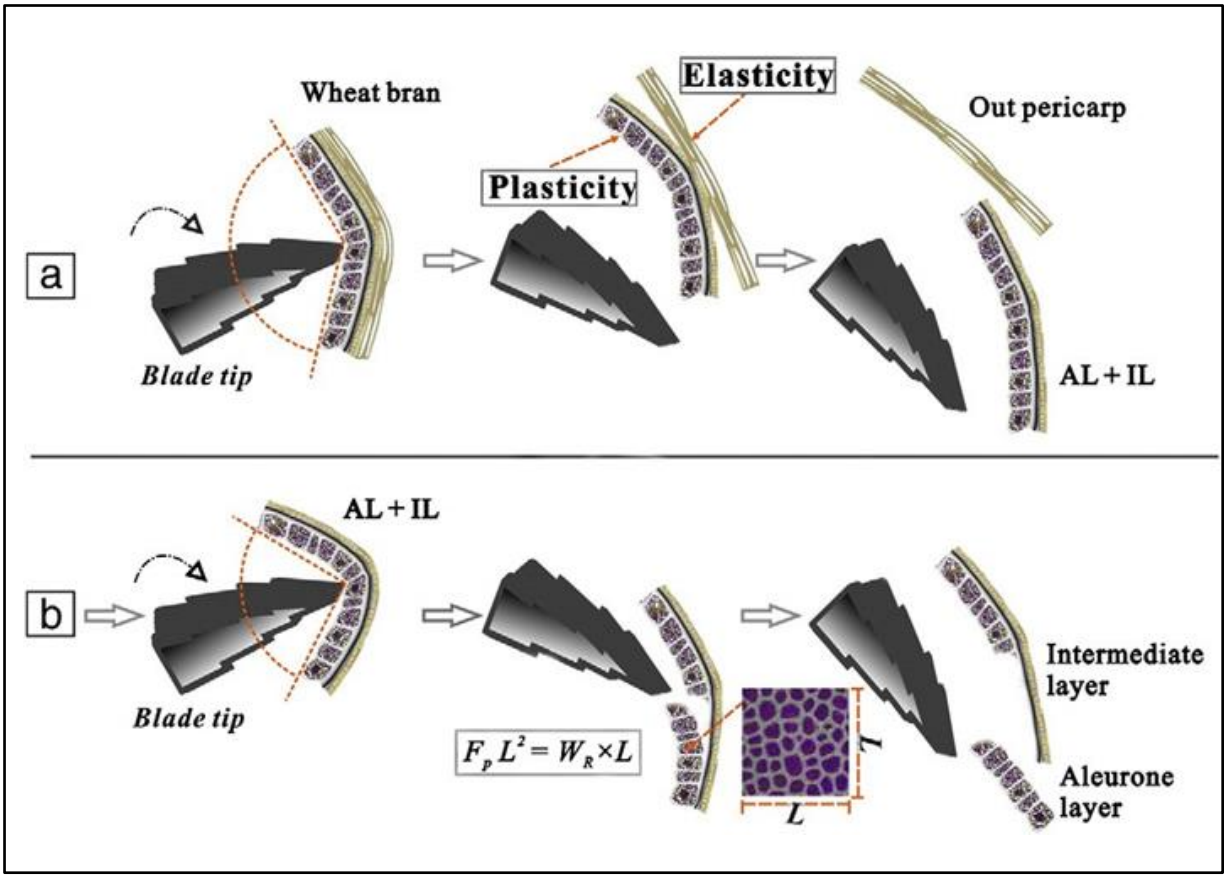

Figure 8. Schematic of wheat bran tissues detachment on centrifugal impact milling. AL=aleurone layer; IL=intermediate layer; Fp=peel force between the aleurone layer and intermediate layer; WR=rupture energy of aleurone layer; L=the length of peeled aleurone cell cluster. Source: (Chen et al. 2013).

Particle Size Distribution/Effects

One of the problems associated with whole wheat flour is its bran's particle size. Various studies have been reported the effects of bran particle size on dough rheology and bread quality (Galliard and Gallagher 1988; Khalid and Simsek 2015; Zhang and Moore 1999). Some studies reported that fine bran particle size produced better baking performance (Khalid and Simsek 2015; Lorenz 1976; Moder et al. 1984b; Shetlar and Lyman 1944; Zhang and Moore 1997),

while other studies claimed that fine bran particle size gave a detrimental effect on bread quality (Galliard and Gallagher 1988). The technique used to prepare the bran sample may contribute to the variances of the results. Most researchers prepare their different particle size fractions by sifting the whole bran. This may lead to major differences in chemical composition of bran fractions (Antoine et al. 2003; Hemery et al. 2009a; Hemery et al. 2007); such as large flakes fraction (pericarp-rich fraction) may be abundant in fiber (Antoine et al. 2003); and small particles fraction (aleurone-rich fraction) may be abundant in vitamins, minerals and antioxidant compounds (Brouns et al. 2012).

Bran particle size produced through grinding affect dough rheological properties as measured by farinograph. Fine particle size of wheat bran decrease dough mixing tolerance and reduced dough mixing requirement compared to coarse bran (Zhang and Moore 1997). Also, extensigraph reading showed that dough with fine particle size of wheat bran showed more strength than dough containing coarse bran after a 180 min rest period (Zhang and Moore 1997). Particle size of bran was reported to affect the sensory parameters, most especially the flavor, of end product. Increase off-flavor was observed in bran water mixtures possibly as a result in increase in lipase activities (Galliard and Gallagher 1988).

Depending on which method was used to effect size fractionation of raw materials, the resulting product's qualities are usually affected. For instance, wheat bran particle size was reportedly negatively correlated with loaf volume when sifting was used instead of grinding (Shetlar and Lyman 1944). It was reported that granulation produced during sifting might have resulting in composition differences between the particle size fractions (Shetlar and Lyman 1944). However, when grinding was used, variation in composition of different wheat particle

sizes was minimal (Zhang and Moore 1997), and that particle size exhibited positive correlation with specific loaf volume (Khalid and Simsek 2015; Zhang and Moore 1999).

Fine bran resulted on lower specific loaf volume and darker crumb color than bread containing coarse or medium size bran (Zhang and Moore 1999). Also, finely ground bran (<0.5 mm) produced lower loaf volume than coarse bran (Galliard and Gallagher 1988). Another report stated that both dough-mixing properties and bread-making quality were negatively influenced as wheat bran particle size was reduced (Noort et al. 2010). In contrast, Moder et al. (1984a) reported that finely ground bran (red and white) exhibited in higher loaf volume than coarsely ground bran. Furthermore, the authors also found that the bread crumb made from finely ground bran was superior than the bread crumb made from coarsely ground bran (Moder et al. 1984a). Negative effect of fiber was due to fiber-gluten interaction that resulted into weakening of dough strength. Reduction in bran particle size caused increased in interaction surface and liberation of reactive components due to cell breakage (Noort et al. 2010). It was also found out that alteration in water status, water activity and frozen water content in high fiber breads samples was as a result of influence of bran on starch-gluten-water interaction (Curti et al. 2013).

Ultra-fine grinding has been reported to increase 3-fold the surface area of wheat bran, thus resulted in increase the antioxidant capacity from 30 to 45 mmol Trolox equivalent antioxidant capacity/kg (Rosa et al. 2013). Ultra-fine grinding of wheat bran has been used by Hemery et al. (2010) to reduce the wheat bran particle size. The authors noticed that the reduction of particle size was correlated with an increase in bioaccessible phenolic acids (mainly sinapic and ferulic acid). Reduction of particle size to nanoscale level for wheat bran has been explored by Van Craeyveld et al. (2009). The authors applied extensive lab-scale ball mill treatment (120 h, 50% jar volume capacity) to increase the wheat bran water-extractable

arabinoxylan (WE-AX) level from 4% to 61% and produce arabinoxylan oligosaccharides (AXOS). It is possible that high-energy impact of ball milling process and heat development in the milling jar combined with extended milling times caused breaking of covalent bonds in AX thus resulted in increase in WE-AX level (Van Craeyveld et al. 2009).

Another study showed that the particle size of wheat bran affect its phytochemical concentration and antioxidant activities. The coarse treatment exhibited higher antioxidant properties than fine treatment in ferric reducing/antioxidant power assay, radical scavenging activity and total antioxidant capacity, except in oxygen radical absorbance capacity, in which fine treatment was higher (Brewer et al. 2014). Phytochemicals (beta carotene, zeaxanthin, lutein, anthocyanin, flavonoid and catechin) extractability in fine treatment of wheat bran were higher compared to coarse treatment sample (Brewer et al. 2014).

Bread Baking Methods

Bread baking process involves series of the interactions of bread raw materials, equipment and people in a certain environment. There are numerous activities taking place during bread making process. Such activities can be chemical, physical and biological. Chemistry of dough has shown that there are interaction between carbohydrates, lipids and proteins. The physical science in dough making is rheology and the biological activities involve the fermentation process by yeast. Over the years, bread baking methods of different pros and cons (Table 1) has been developed and improved to achieve production of breads that meet consumers' quality requirements and industrial needs (Pyler and Gorton 2009). Figure 3 summarized to most common of bread baking method.

Straight-Dough Method

This is a one-step process where by all the dough ingredients are added together and mixed in a single batch. At the initial mixing stage, the mixture matrix lack high cohesion while wet mass chumps appear. As mixing continues, the elastic properties of the dough start to increase causing the chumps start to pull away from the mixer walls. Adequate mixing is achieved when the dough exhibit smooth appearance, dry surface and optimum elastic character. Over mixed dough exhibits sheen characteristic and stickiness thus becomes difficult to handle. Usually, mixing temperature during straight dough method is 26 – 28 ^{o}C. Although higher temperature will accelerate the rate of yeast fermentation, control of fermentation become more difficult and may result to fermented dough that lacks adequate stability. Compared to sponge-and-dough process, straight-dough method is advantageous because of lower processing time, power, equipment and labor. Also, losses during fermentation are reduced since shorter fermentation time is required. Product's flavor is also enhanced as dough ingredient undergoes fermentation treatment (Pyler and Gorton 2009).

Sponge-and-Dough

Sponge-and-dough process was basically developed to ensure homogenous ingredient dispersion and flour hydrations. It involves two mixing stages, one for the sponge and the other for the dough. In the first step, leavening agent is prepared by mixing certain quantity of water, flour and yeast together and allowed to develop for few hours. Sponge mixing equally allowed formation of enough gluten to retain a sufficient amount of CO_2 produced during fermentation process. In the second step, other ingredients are added to sponge and subjected to final physical development during the dough remix stage. Compared to straight dough method, sponge-and-dough offer some advantages are: requirement of slightly lower yeast, production of good flavor

of breads, achievement of optimum loaf volume, superior grain and texture, retention of softness and process is flexible giving room for adaptability to minor schedule delay. However, sponge-and-dough method requires greater equipment demands, high labor cost, greater fermentation losses and increased processing time (Pyler and Gorton 2009).

Continuous Mixing Method

This method involves mixing of ingredients in a continuous high speed mixer within 1 to 2 minutes. Bread ingredients are first allowed to go through first stage continuous mixer (pre-mixer) and then proceed to second- and final-stage continuous mixer, or developer, and immediately extruded and discharged into the pan. This bread making method demands that ingredients are carefully measured in order to ensure correct product consistency. Ingredients are combines, blended and degassed to form uniform dough in the pre-mixer. Then the raw dough moves to the developer mixer where gluten is conditioned at high-speed mixing for protein cross-linking. High energy mixing cause an increase in dough temperature, as a result of friction within the dough, thus enhances yeast activity. Continuous mixing method is well suited for long production runs of same products but not suitable for open-grain products or short-run items. Continuous mixing method was development to automate dough preparation; however, it lost popularity when consumer demands for bread varieties increased (Pyler and Gorton 2009).

Chorleywood Method

The Chorleywood method is similar to straight-dough method, where all ingredients are mixed at once, except that ultrahigh mixing ($\geq$ 600 rpm) is done for short time (2 to 5 minutes) and partial vacuum condition (Giannou et al. 2003). The high intense mixing requires about 5 to 7.5 Watt-hours per lbs of dough and causes the dough temperature to increase, a condition that hastens fermentation. The overall bread making operation is reduced to around 3.5 hours or less,

saving 1.5 to 2 hours compared to conventional methods. This method is more suitable for low-protein wheat flours (10.5 to 11 % protein) better than high protein flours (Pyler and Gorton 2009).

No-Time Dough Method

This method allows for elimination of bulk fermentation time in a batch system of operation. It is similar to straight dough methods involving addition of all ingredient at ones in a bowl, it involve use of high speed mixer to impact necessary physical energy for proper dough development. Compared to straight dough method, a slightly warmer temperature is employed during mixing of No-time dough. It enables increase in production of bread when demand is high and supply can not be met using sponge-and-dough and straight-dough methods. No-time dough method saves from 1 to 3 hours of processing time and equally require small space. High amount of yeast is required because of the less time available for fermentation (Pyler and Gorton 2009).

Sour Dough Method

Sour dough method has been used in bread making for over 5000 years ago for texture and flavor improvement of baked cereals products (Hansen and Schieberle 2005). Instead of baker's yeast, naturally occurring lactobacilli and yeast are being used for fermentation process in sour dough method. The word sour refers to the sour taste associated with product due to presence of lactic acid produced from activities of lactobacilli (Kinsella 1993). Use of sour dough method for preparation of bread from whole wheat has shown some nutritional advantages. For instance, level of phytic acid was lower and availability of phosphorus and magnesium was higher, when sour dough method was used compared to yeast fermentation. This is possible because sour dough enhanced of acidification thus increased phosphorus and magnesium solubility and lowering effect on phytic acid – a known inhibitor of mineral availability (Lopez

et al. 2001). Also, at acid condition, lipase is inhibited thus oxidation and degradation of tocols and carotenoids are eradicated (Hammed and Simsek 2014). Sour dough technique has been reportedly to impact richer and more aromatic flavor in wheat bread, possibility due to prolong fermentation. The nature of aromatic compounds depends on the starter culture used, the length of fermentation period, as well as the presence of other ingredients (Hansen and Schieberle 2005).

Numerous studies have adopted some of these baking methods for production of whole wheat bread. For instance, Lopez et al. (2001) and Lopez et al. (2003) used sourdough method, Bruckner et al. (2001), Lai et al. (1989a) and Guttieri et al. (2000) used straight-dough method, Ranhotra et al. (1995) used sponge and dough method, and Shogren et al. (2003) used no-time dough method. However, the qualities of whole wheat bread have always been lower than bread from refined wheat flour. This observation is not unexpected because the baking methods are originally developed for refined wheat flour. It has been suggested that different processing steps and/or conditions might be required for production of whole wheat bread with improved qualities. These aspects of baking studies can be looked into in future works.

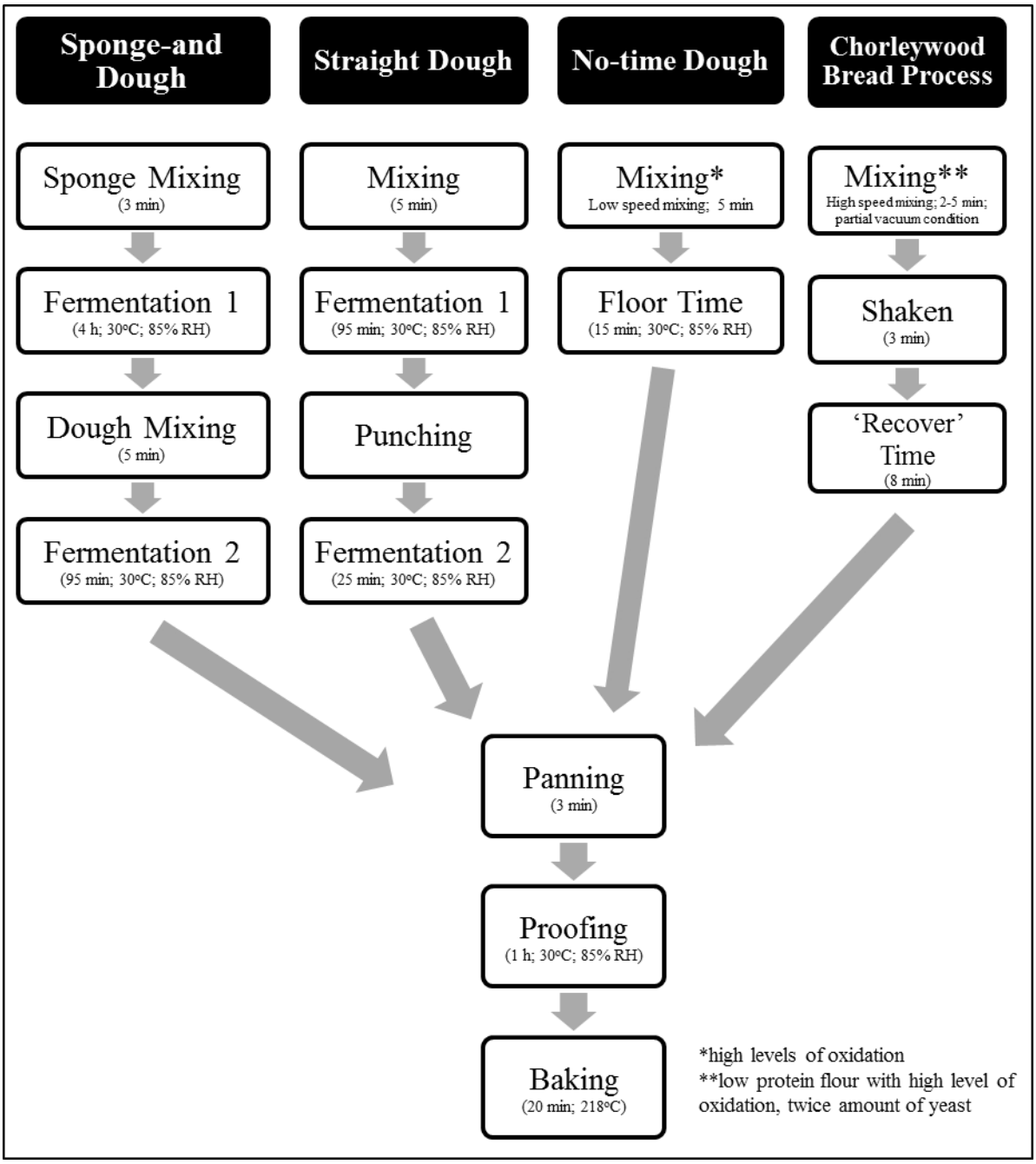

Figure 9. Common bread-baking methods.
Source: (Britannica 2016; Doves-Farm-Foods 2016; Pyler and Gorton 2009).

Table 2. Score card depicting the pros and cons of different bread baking methods (adapted from Pyler and Gorton (2009)

	Straight Dough	Sponge and Dough	No-time dough	Chorleywood process
Dough Handling	-	+		
Product's Flavor	+		-	-
Processing time	+	-	+	+
Mixing Time	-			
mixing tolerance	+	-		
fermentation tolerance	-	+		
product score		+		
equipment cost	+	-		-
shelf life		+	-	-
labor cost	+			
space requirement		-		
Process Flexibility			+	+
Ingredient cost			-	
Floor Time			-	+
Product Consistency				
Flour in sponge				
Energy cost				-
Crumb strength				
Yeast Survival			+	
Product's Appearance and Texture				

+: Advantageous, -: Disadvantageous and *: Conditional.

Whole-Grain Breads

Under US Food and Drug Administration (US-FDA) regulations, 51% whole grain by weight must be incorporated in the food products to be able to claim as "whole grain". Meyer (2005) has listed his recommendations about preparation of whole-grain baked foods. There are: (1) besides whole-wheat flour, consider flakes, grits, cuts, whole kernel and soaked grains; (2) use special ingredients to increase dough strength, i.e. vital wheat gluten; (3) add mix-time adjustment agents; (4) use compatible sweeteners, i.e. brown sugar, honey, raisin juice; (5) be aware of differences between conventional dough and whole wheat dough development time; (6) be prepared for additional friction in the mixer, i.e. bowl refrigeration; (7) monitor the addition

water absorption caused by the fiber component of whole grains; and (8) do not over-work or over-sheet whole grain doughs.

Selection of grains also has some influence on the taste and texture of the end products (Moon 2006). Moon (2006) quoted that the whole-wheat hard white wheat flour has 'better' taste that could associated with consumer preferences in taste, flavor and appearance. Some offered a strategy to masked the bran flavor via adding natural flavors, such as honey, molasses, and raisin juice and organic acids for wholegrain sourdough (Beaven 2007; Moon 2006). Beaven (2007) recommended producing naturally emulsified system to support the heavy fiber content of whole-grain breads via adding some isolates proteins from soy, wheat, and dairy.

In US, most researchers in bread baking used AACCI Approved Method to run an experiment regarding bread-baking. The AACCI established two standard methods for bread baking namely Optimized Straight-Dough Bread-Baking Method (10-10.03) and Basic Straight-Dough Bread-Baking Method-Long Fermentation (10-09.01). These methods are based on straight dough methods. Currently, there is no official whole-wheat bread baking method published by official organization or association. Most researchers (Cai et al. 2014; Khalid and Simsek 2015; Li et al. 2012) used the published standard method (developed for refined flour) to conduct their whole-wheat bread experiments.

Impact of Genotype and Environment on Whole Wheat Flour and Dough Quality

Investigations on effects of genotype and environment on wheat flour and dough quality are conducted to determine the best genotype of wheat that meets consumers' needs (Williams et al. 2008). It is essential to note that most of the studies on genotype and environment effects for end product quality (wheat-based) were done with refined flour. Basically, different locations will have varying environmental conditions, such as soil variability, temperature differences and

available moisture. The environmental factors mentioned above have been reported to affect the quality parameters of harvested wheat grain. Different genotypes of wheat grains contain varying proportion of heritable genes that dictate the amount, features and type of quality traits (Gebruers et al. 2010a). Studies on the effects of environmental factors and genotype on the quality of whole wheat flour and dough have received less attention compared to that of refined white wheat flour. Details on impact of genotype performance and environmental influence on whole wheat flour/dough compositions and end quality parameters are briefly presented in the paragraphs that follow.

Study of effect of genotype and environment on wheat revealed that protein and water-soluble pentosans were affected significantly in hard and soft wheat. The result showed that effect due to genotype was 1.6 times greater than that of environment (Hong et al. 1989). Wheat genotypes affect the amount of water extractable arabinoxylan and total endoxylanase activity (Dornez et al. 2008; Li et al. 2009). Mendis et al. (2013) had earlier reported that arabinoxylan (AX) composition of wheat was not significantly ($P<0.05$) affected by genotype and/or location, but was by location-genotype interaction. The arabinose substitution pattern of AX (A/X ratio) was significantly affected by genotype and location-genotype interaction, but not location only. It was equally observed that genotype contributed about 72 % to the variability of xynalase inhibitor activity; thus, can be a stable parameter in segregating wheat genotypes with varying xyalanase activity (Mendis et al. 2013).

Wheat grain protein quality was reportedly influenced by exposure to high temperature and relative humidity. Long exposure of wheat to elevated temperature led to decline in protein quality (Graybosch et al. 1995). There is a correlation between temperature and polymeric protein content, where an increase in temperature resulted in increased in polymeric protein

content. However, this was not the case for the low molecular weight flour protein. A similar study by Uhlen et al. (1998) showed that different genotypes exhibited different protein quality parameters.

Effect of genotype differences on end-use quality of six hard red spring wheat genotypes was reportedly more than effect due to genotype × irrigation interactions (Guttieri et al. 2000). Wheat genotypes responded differently to moisture stress by increasing protein content during grain filling as a result of relocation nitrogen from vegetative part of plant to grains. Bread loaf volume and rheological properties of flour reacted similarly to protein content in all genotypes as a result of moisture stress (Guttieri et al. 2000).

Study on effect of type of fertilizers applied showed that there was greater increase in grain protein and gluten contents when complete mineral fertilizer was applied compared to application of only nitrogen; however, these disparities had no effect on bread loaf volumes (Rieux et al. 2013). The effect of genotype on quality of whole wheat flour, dough and bread was prominent. Whole wheat protein content (associated to water absorption and loaf volume) was reportedly affected by variation in genotype. The possible reason is due to effect of the quality of bran fraction from different genotype in whole-wheat flour (Bruckner et al. 2001). Differences in bread baking qualities have been associated with differences in bran characteristics resulting from different genotypes. Bran competes with gluten for water; thus, gluten are poorly hydrated. Poorly hydrated gluten resulted in lower loaf volume and altered dough properties (Lai et al. 1989a).

Phenolic acids and antioxidant properties of wheat were reportedly affected by genotype and environment. Phenolics are secondary metabolites synthesized by plants during normal development and also in response to stress condition such as infection, wounding and ultra-violet

(UV) radiation. Therefore, environment effects may contribute to the larger extent towards the phenolics content on whole-wheat bread. Environmental effects of wheat were considerably larger than genotype effects for vanillic acid, syringic acid and ferulic acid and their antioxidant properties as well (Mpofu et al. 2006). Based on previous findings that phenolic acids disrupt gluten network by preventing the disulfide crosslink (Han and Koh 2011b; Koh and Ng 2009), wheat that contain high amounts of phenolics content may produce whole-wheat bread with low loaf volume than moderate amount of phenolic content.

A study was carried out on stone-ground whole wheat flour and bread samples obtained from five wheat genotypes grown organically on eight farms in Quebec, Canada (Gélinas et al. 2009). Grain yield, grain protein and dough mixing stability of whole wheat flour were reportedly affected by the location. Equally, end product qualities, which is pan bread loaf volume, was significantly ($P<0.05$) affected by location. Gelinas and McKinnon (2011) extensively evaluated 25 wheat genotypes (21 spring wheat and 4 winter wheat) harvested at four different growing locations within 2 years for their performance in whole-wheat bread. The results showed that the effect of location impacted most on overall bread making qualities of whole wheat flour samples compared to the effect of genotype and crop year. Dough from whole wheat flour exhibited high variation in terms of farinograph water absorption due to effect of genotype. Also, the dry gluten content of whole grain exhibited large variations among different wheat genotypes (Gélinas and McKinnon 2011). Overall, whole wheat dough, flour, and bread qualities are affected by genotype, location and occasionally genotype-location interaction.

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PAPER 1. WHOLE-WHEAT FLOUR PRODUCTION USING ULTRA-CENTRIFUGAL MILL

Abstract

Interest has been growing in whole grain products. However information regarding the influence of ultra-centrifugal mill on whole grain flour was limited. An experiment was conducted to produce whole-wheat flour with Hard Red Spring (HRS) wheat using an ultra-centrifugal mill. This study determined the effect of centrifugal mill parameters as well as grain moisture (10-16%) on producing whole-wheat flour and its final products. Mill parameters studied were rotor speed (6,000 – 15,000 rpm) and feed-rate (12.5 – 44.5 g/min). Results showed that fine particle size was favored by low seed moisture content (10-12%) and high rotor speed (12,000 – 15,000 rpm). Flour moisture content was positively related to seed moisture content. Wheat grain with low seed moisture content (10 – 12%) milled using high rotor speeds (12,000 – 15,000 rpm) produced desirable whole wheat flour quality, with 70-90% of fine particle size portion and low starch damaged (less than 11%). This whole-wheat flour produced uniform and machinable dough that had low stickiness and formed bread with high loaf volume.

Introduction

There is increasing demand in the domestic and world markets for whole grain bread products. Research has shown that whole grain consumption has been associated with reduced the plasma total cholesterol and LDL-cholesterol concentration (Tong et al. 2014), reduced risk of cardiovascular disease (Mellen et al. 2008), heart disease (Jacobs et al. 1998), obesity (Pauline and Rimm 2003), diabetes (Slavin 2004), and certain types of cancer (Schatzkin et al. 2008). In 1999, American Association of Cereal Chemists International (AACCI) through its Board of Directors defined whole grain as "shall consist of the intact, ground, cracked or flaked caryopsis,

whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis"(AACCI 1999). However the whole grains council put out this definition in 2004 as "Whole grains or foods made from them contain all the essential parts and naturally-occurring nutrients of the entire grain seed in their original proportions. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver the same rich balance of nutrients that are found in the original grain seed. This definition means that 100% of the original kernel – all of the bran, germ, and endosperm – must be present to qualify as a whole grain" (Whole-Grains-Council 2004).

Whole wheat bread quality depends on ingredient formulation and the quality of wheat and milling techniques used to produce the flour (Kihlberg et al. 2004b). Stone and disc mills generate high temperatures during wheat grinding. High temperature can result in elevated protein degradation especially the high molecular weight glutenin proteins, and loss of total amino acids(Prabhasankar and Rao 2001). Whole wheat flour is produced via two methods: milling the whole kernel directly into flour, and recombining all milled fractions (endosperm, bran, and germ) at the end of roller milling (Doblado-Maldonado et al. 2012).

Tempering is a process where water is added to the grain. The wetted grain is allowed to rest for a period of time before milling. Water is applied to wheat kernels to toughen the bran and soften the endosperm (Delcour and Hoseney 2010), both of which aid in the removal of bran and germ from the endosperm during roller milling. Most tempering studies have focused on tempering mechanism and milling yield but not flour quality. Moisture content after tempering can affect the quality of flour. Flour had lower ash content and lower polyphenol oxidase activity when derived from grain that was tempered to 15% compared to 12% moisture (Kweon et al.

2009). Kweon et al (2009) explained that wheat kernel with 12% tempered moisture may have more starch granule fracture (causing increased damaged starch) and also have increased bran particles. Those bran particles would contain relatively greater mineral, PPO, and water-unextractable arabinoxylans.

Starch granules can be damaged during wheat milling/grinding. Damaged starch refers to small particles of starch broken away from the main starch granules. These small particles hydrate easily during dough preparation. The level of starch damage affects the water absorption and dough mixing properties (Bettge et al. 1995). Damaged starch has much greater water retention capacity; however, too much starch damage leads to sticky dough, strong proofing, undesirable red crust color (Bettge et al. 1995) and low specific volume (Barrera et al. 2007).

Centrifugal mill uses the impact and shearing forces for particle size reduction. Literature search failed to find any published articles concerned with whole grain milling for flour production using a centrifugal mill. However, a centrifugal mill has been used to reduce bran particle size before blending it back with white flour from roller mill stream (Seyer and Gélinas 2009; Villeneuve and Gélinas 2007). The centrifugal mill used in this research was configured with a grain feeder that controlled feed rate into the mill; a rotor with blades, mesh screen, and a vacuum air flow cooling system. Present study was undertaken with the objective to produce whole-wheat flour for bread-baking using a centrifugal mill. Rotor speed, feed-rate, and seed moisture content were evaluated for their effects on flour quality and subsequent baking quality.

Materials and Methods

Samples

Bulk hard red spring wheat (var. Barlow, Prosper, Glenn) was used. Moisture and protein contents were determined in triplicate using a Dickey-John Model GAC 2100b (Dickey-John

Corp., Auburn, IL, USA). Wheat kernel quality was measured via single-kernel characterization system (model 4100; Perten, Springfield, IL USA). Wheat was equilibrated to room temperature (25°C) and tempered to 10, 12, 14, and 16% moisture content 24 h before milling.

Wholegrain Flour Milling

Wheat (200 g) was ground using an ultra-centrifugal mill (Retsch ZM200, Haan, Germany) configured with a 250 µm screen. The mill was operated using a vibratory feeder (model DR100, Retsch GmbH, Haan, Germany) and a vacuum (Nilfisk GM 80, Hungary) attachment that air cooled the mill and mill product. The feed rate was varied by adjusting vibration settings to 30 and 40 to achieve 12.6 g/min and 44.5 g/min, respectively. Rotor speed was varied from 6,000 to 15,000 rpm. Milling was done in the Durum Wheat Quality Laboratory during winter season, with air temperature of 20°C and relative humidity 17%. Milled product was collected and sealed in a zip lock plastic bag and stored at -20°C.

Size reduction by the ultra-centrifugal mill occurs by impact and shearing effects caused by the rotor and the fixed ring sieve. Centrifugal acceleration throws the kernel outward with great energy. The kernel is crushed and sheared on impact with the ring sieve. The energy of impact is determined by the rotor speed.

Physical and Chemical Properties of Whole-Wheat Flour

Temperature of the whole-wheat flour and the rotor were measured immediately after milling using an infra-red digital thermometer (VWR International, Radnor, PA, USA). Particle size distribution was determined using vibratory sieve shaker (Retsch AS200, Haan, Germany) with a stack of six sieves (50 µm, 150 µm, 250 µm, 425 µm, 500 µm, and 600 µm). Each sieve contained five plastic sieving balls. Sample (100 g) was shaken for 5 min and the weight retained on each sieve and in pan was recorded as percent of the total.

Whole wheat flour was characterized by flour moisture content (AACCI Approved Method 44-15.02), ash content (AACCI Approved Method 08-01.01), protein content (AACCI Approved Method 46-30.01), and starch damage (AACCI Approved Method 76-30.02).

Dough and Baking Properties

Dough and baking properties were evaluated for the whole wheat flour samples. The mixogram was obtained using 10 g bowl mixograph according to the AACCI Approved Method 54-40.02. Flour protein content was used to determine optimum water absorption. Ten gram of flour (14% mb) was mixed with the optimum amount of water for 8 min or until mix time could be determined at 25°C.

Bread formulations were baked according to AACCI Approved Method 10-09.01, basic straight dough with modifications. Fungal α-amylase and instant dry yeast were used instead of malt powder and compressed yeast, respectively. Ammonium phosphate at 5 ppm was added to improve yeast function. The bread was prepared using 2 h fermentation schedule, with an extra 10 min time for proofing.

Baking qualities were characterized by baking absorption, dough handling properties, bread loaf volume, and bread crumb score. Baking absorption was determined as the amount of water required for optimum dough baking performance and was expressed as a percent of flour weight on a 14% mb. Dough handling properties was evaluated at panning on a scale of 1 to 10 with higher scores preferred. Loaf volume was determined by rapeseed displacement method (AACCI Approved Method 10-05.01). Subjective analysis of final loaf score was evaluated by the Guidelines for Scoring Experimental Bread (AACCI Approved Method 10-12.01) using a constant illumination source. The score ranged from 1 to 10, with the higher scores preferred.

Experimental Design and Statistical Analyses

The experimental design was a randomized complete block with a factorial arrangement of tempering moisture (10, 12, 14, and 16%), feed rate (12.6 and 44.5 g/min), and rotor speed (6,000, 9,000, 12,000, and 15,000 rpm). Individual treatments were milled three times each time on separate days, which were considered as replicates. Data were analyzed using SAS System for Windows (version 9.3, SAS Institute, Cary, NC). Analysis of variance was performed using the GLM procedure in SAS. Treatment means were separated by Fisher's protected Least Significant Difference test at P=0.05.

Results and Discussion

Wheat Kernel Quality Characteristic

The bulk grain sample of HRSW had large and medium kernel distributions of 61% and 39%, respectively. The test weight (79.8 kg/hL), 1,000-kernel weight (34.4 g), protein content (14.9%, 12% mb), and moisture content (13.2%) of the grain indicates that the starting material had good quality (Regional-Quality-Report 2012).

Physical and Chemical Properties of Whole-Wheat Flour

Flour and Mill Temperature. Feed rate by rotor speed interaction was significant for mill surface temperature. The low feed rate (12.6 g/min) generally resulted in lower mill surface temperatures than did the high feed rate (44.5 g/min) (Figure 10). The exception occurred with wheat milled using the low feed rate and 15,000 rpm rotor speed, which resulted in mill surface temperature similar to that of high feed rate. At the high feed rate, the mill surface temperature did not differ with rotor rpm. In general, mill surface temperature ranged between 25.9 – 28.2 ℃, which represents an increase of 5.9 – 8.2 C throughout this experiment (Figure 10). Mill surface temperature during milling was not affected by seed moisture content.

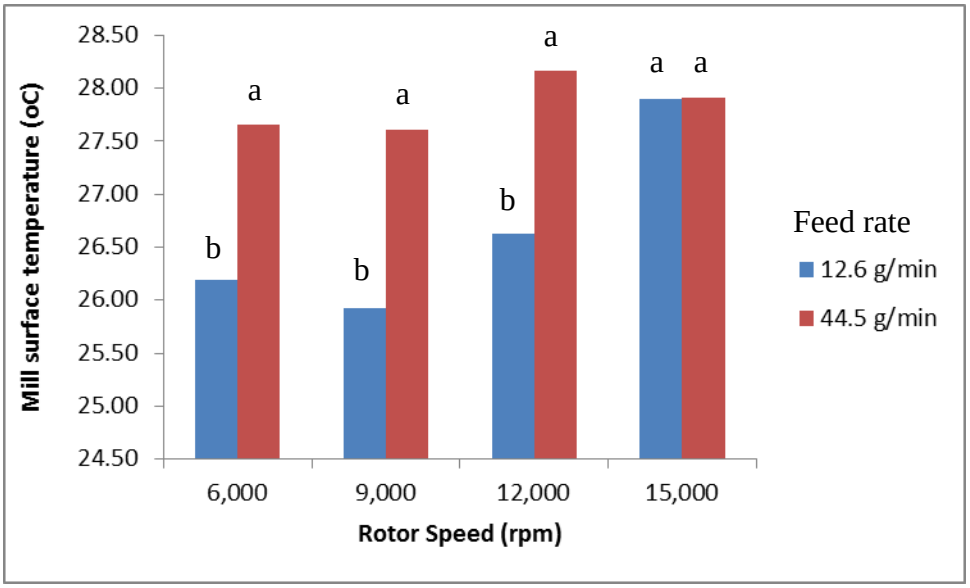

Figure 10. Mill surface temperature (^{o}C) as affected by feed-rate and rotor speed interaction for 250 µm screen mesh size.

Feed rate and seed moisture content main effects were significant for flour temperature. Flour temperature increased from 25.8 – 28.0 °C as moisture content of the seed increased from 10 to 16 % (Table 3). Flour temperature was also greater with high than low feed rate. Average air temperature during milling was 20°C. Thus, there was a 5-8 °C increase in temperature caused by friction generated during milling. These temperatures would not cause a detrimental effect on flour and bread quality since the temperature did not reach the denaturation temperature of wheat protein, gelatinization temperature of wheat starch (approximately 52-63°C) and did not alter the structure of the starch granules. Ngamnikom and Songsermpong (2011) agree with these findings as they reported temperature of 32°C for rice flour produced by milling rice on a hammer mill, roller mill, and pin mill did not affected the rice starch granules (rice starch gelatinization temperature was ranging between 65-78 °C).

Table 3. Flour temperature (^{o}C) of whole-wheat flours milled on an ultra-centrifugal mill as affected by main factors of seed moisture content and feed-rate

Factors	Flour Temperature (^{o}C)[a]
Moisture	
10%	25.8 ± 1.6 c
12%	26.1 ± 1.3 c
14%	27.0 ± 1.4 b
16%	28.0 ± 1.2 a
Feed-rate	
12.6 g/min	26.5 ± 1.7 b
44.5 g/min	27.0 ± 1.4 a

[a]Mean ± standard deviation; n = 24 for moisture; n = 48 for feed-rate; mean values followed by the same letters within factors are not significantly different.

Flour Moisture Content. Seed moisture content main effect was significant for flour moisture content. Other factors such as rotor speed and feed-rate factors did not significantly ($p>0.05$) impact whole-wheat flour moisture content. Whole-wheat flour moisture content ranged from 8 to 10%. Flour moisture content was directly related to seed moisture content, which ranged from 10 to 16%. The highest ($p<0.05$) flour moisture content was recorded at 10% when milling at 16% seed moisture content, while the lowest flour moisture content was 8% when milled at 10% seed moisture content. Moisture loss was greater at 16% moisture content (6 percentage units loss) compared to 10% seed moisture (2 percentage units loss). Moisture loss is attributed to increased exposed surface area of flour particles, to evaporation due to air cooling system and to the low relative humidity (17%) in the mill room.

Particle Size Distribution. Generally, 70% to 89% of whole-wheat flour was distributed at fine particle size category ($\leq$ 150 µm) (Table 4). Feed rate by seed moisture content interaction and rotor speed by seed moisture content interaction were significant for percent fine particle size portion. The fine particle size portion distributions of whole-wheat flours milled on the ultra-centrifugal mill is shown in Tables 4. Seed moisture content caused greater effect on fine particle size portion than did feed-rate. Fine particle size portion was greatest with high seed

moisture content between 14-16% for both feed-rates. Increasing moisture content greater than 14% did not significantly ($p>0.05$) produce more fine particle size portion with ultra-centrifugal mill. Eighty-two percent (82%) seems to be optimal for highest fine particle size portion production for HRS whole-wheat flour production using ultra-centrifugal mill with 12.6 or 44.5 g/min feed-rate and 14% seed moisture content.

Rotor speed by seed moisture content interaction was significant for fine particle size distribution (Figure 11). Changing seed moisture content did not increase fine particle size distribution when milling at 12,000 and 15,000 rpm rotor speed. At low rotor speed (6,000 and 9,000), higher seed moisture content did result in more fine particle size whole-wheat flour. However, at high rotor speed (12,000 and 15,000) little to no difference in percent fine particle size occurred with change in grain moisture content. Percent fine particle size was greatest (86.8-89.6%) with rotor speed of 15,000 rpm regardless of seed moisture content.

Table 4. Mean[a] fine particle size distribution as affected by feed-rate setting and seed moisture content interaction for 250 µm screen mesh size.

Feed-rate (g/min)	Seed moisture content (%)	Fine particle size (%)
12.6	10	79.6 ± 9.9 bc
	12	80.7 ± 8.1 ab
	14	81.9 ± 6.7 a
	16	82.2 ± 6.3 a
44.5	10	79.6 ± 9.1 bc
	12	78.6 ± 8.2 c
	14	81.4 ± 6.4 a
	16	82.1 ± 6.7 a

[a]Mean ± standard deviation; values followed by the same letters within column are not significantly different.

Referring to our experiment, the seed moisture content levels determined to be optimal (which is 16%), in as much as further moisture applied (up to 16%), did not significantly produce greater fine particle size portion (82% fine portion was the maximum). Tempering grains before

milling is to toughen the bran and soften the endosperm. Higher tempering moisture content resulted in mellower endosperm thus easier to mill (Posner and Hibbs 2005). In contrast, milling whole wheat flour on a roller mill (Buhler experimental mill) produced whole-wheat flour with coarse particle size and the amount of coarse particles increased with high seed moisture content (Doblado-Maldonado et al. 2013). Roller mill is configured to maximize shear action to remove bran in large particles (Posner and Hibbs 2005) while centrifugal mill involves impact and cutting action (Retsch 2015). Grinding of fibers is a machine-driven process. It is similar to grain size reduction of powders. However, due to extreme non-spheroid habit of fibers, the process is more complex and one can distinguish between length reduction (cutting) and a diameter reduction (fibrillation) (Bartl et al. 2004). Fibrous plant material from crops, such as wheat bran, is well ground under impact and cutting action (Kukla 1991). Usually fibers need to be sized (length reduction) in a cutting mill prior to the impact milling process (Hixon 1991). Hixon (1990) described generally on fibrous materials from crops, such as grass fibers.

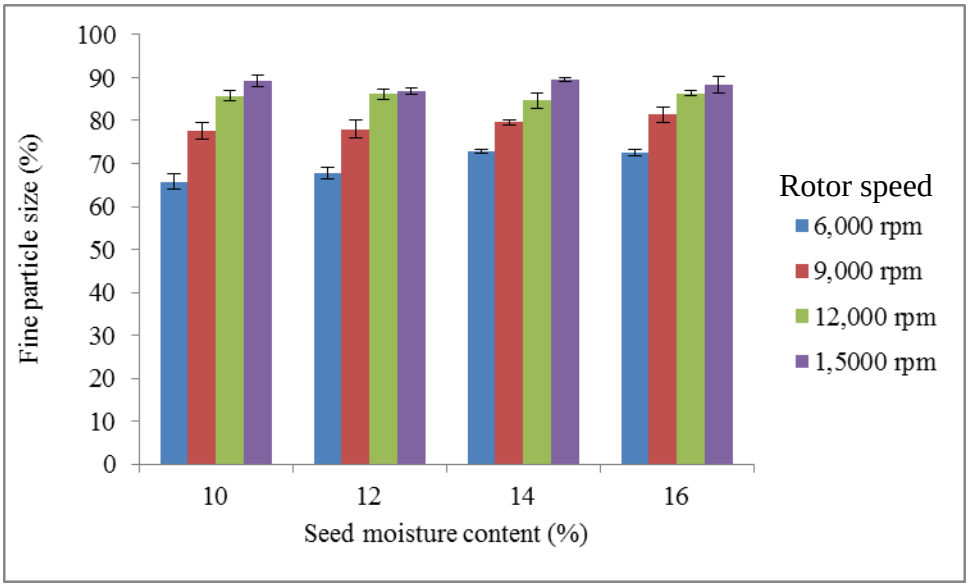

Figure 11. Fine particle size as affected by rotor speed and seed moisture content interaction for 250 µm screen mesh size

Higher rotor speed produced significantly ($p<0.05$) greater fine particle size portion. Higher rotor speed gives greater impact and shearing action towards the kernel thus produces finer flour. Faster feed-rate produced 1% less fine particle size. Feed-rate at 12.6 g/min produced 81% of fine particle size while feed-rate at 44.5 g/min produced 80% of fine particle size. One percentage difference may not be practical; however if taken into account the output from giant milling company, the 1% less in production may cause some loss in profit. High feed rate cause greater amount to be fed into the grinding chamber at one time; thus it will generate heat as there is an increase in collisions in the milling chamber compare to low feed rate.

Damaged Starch. Feed rate by rotor speed by seed moisture content interaction was significant for damaged starch. Aggressive impact and shearing action inside the milling chamber caused some damage to starch granules. In this experiment, damaged starch ranged between 6.2 to 11.2% for all 32 treatments (Table 5). High rotor speed produced less starch damaged for both feed-rates at each seed moisture content level. It might be due to air-cooling system in this centrifugal mill, high rotor speed may resulted in high air stream and the flour discharged more rapidly from the grinding chamber, spent less time inside the grinding chamber, thus less starch was damaged (we did not measure the air flow inside the grinding chamber). High seed moisture content caused greater starch damaged at each rotor speed level for both feed-rates. Low seed moisture resulted in less fine particles produced (Table 4 and Figure 11) therefore less impact and shearing forces towards the seed.

Table 5. Mean[a] damaged starch as affected by feed-rate x rotor speed x seed moisture content interaction for HRS whole-wheat milling

Feed-rate (g/min)	Rotor speed (rpm)	Damaged starch (14%mb)			
		Seed moisture content (%)			
		10	12	14	16
12.6	6,000	8.94hi	8.85i	9.30gh	9.90ef
	9,000	8.16k	9.62fg	10.72c	12.11a
	12,000	8.57ijk	8.65ij	10.05ef	10.32cd
	15,000	7.06mn	7.24lm	8.17k	9.33gh
44.5	6,000	9.35gh	8.14k	10.58cd	10.27de
	9,000	7.61l	8.15k	9.33gh	11.23b
	12,000	6.18o	7.08mn	8.67ij	9.00hi
	15,000	6.32o	6.78n	7.59l	8.29jk

Key: High; Medium; Low

[a]Mean values followed by the same letters are not significantly different. mb = moisture basis; rpm = rotation per minute.

The least damaged starch recorded as 6.2 and 6.3 % when milling whole-wheat flour at these combination 12,000 rpm with 10% seed moisture content at 44.5 g/min and 15,000 rpm with 10% seed moisture content at 44.5 g/min respectively. The highest damaged starch was recorded at 12.1% when milling at 16% seed moisture content with 9,000 rpm and 12.6 g/min. When the seed moisture and rotor speed were kept in constant, high feed-rates had higher starch damage. These results are in agreement with Larsen's study (Larsen et al. 1989a). Larsen milled the wheat at 16% moisture on Buhler and Brabender experimental laboratory mills with two different feed-rates namely high and low-feed-rates. They found that flours milled at high feed-rates had starch damaged levels averaging 7.5% and those milled at low feed-rates, 8.0%. Larsen et al (1989) did not offer any explanation on why low feed rate caused greater damaged starch than high feed rate.

Dough and Baking Properties

Mixograph. Main effects of rotor speed and seed moisture content were significant for mid line peak time (MPT). MPT indicates optimum mixing time with well-developed gluten in dough system and is expressed in minutes. Data in Table 6 shows the value for MPT as affected by the main effects of rotor speed and seed moisture content. Whole-wheat flour produced from high rotor speed and low seed moisture content using centrifugal mill needed longer mixing time. In baking industry, longer mixing time is an indication of strong gluten flour. MPT does not correlate with particle size portion. MPT correlate negatively with damaged starch and flour moisture content and positively with loaf volume (data not shown).

Table 6. Selected mixograph parameters of whole-wheat flour with different moisture, rotor speed, and feed-rate[a].

Factors	MPT (min.)	TA (%)	TW (%Tq*min)
Moisture			
10%	4.1 ± 0.4 a	294.1 ± 17.4 d	8.3 ± 1.5 a
12%	4.0 ± 0.5 a	306.4 ± 20.4 c	8.7 ± 1.6 a
14%	3.7 ± 0.4 b	319.0 ± 22.3 b	8.1 ± 1.4 a
16%	3.6 ± 0.3 b	332.0 ± 18.7 a	8.3 ± 1.8 a
Rotor speed			
6,000 rpm	3.7 ± 0.3 a	319.6 ± 23.9 a	7.4 ± 1.8 c
9,000 rpm	3.7 ± 0.4 b	321.5 ± 25.4 a	8.2 ± 1.5 b
12,000 rpm	3.8 ± 0.4 b	308.2 ± 21.0 b	9.0 ± 1.4 a
15,000 rpm	4.1 ± 0.6 b	302.3 ± 21.7 b	8.7 ± 1.1 ab
Feed-rate			
12.6 g/min	3.9 ± 0.5 a	307.5 ± 23.4 b	7.9 ± 1.4 b
44.5 g/min	3.8 ± 0.3 a	318.3 ± 23.8 a	8.9 ± 1.7 a

[a]Mean ± standard deviation; n = 24 for moisture; n = 24 for rotor speed; n = 48 for feed-rate; values followed by the same letters within factors are not significantly different; MPT = midline peak time; TA = total area under the midline curve, measured at the end of mixing process; TW = midline curve width measured after peak at 5min; Tq = Torque.

Dough strength and mixing tolerance were recorded as area under the midline curve measured after peak time (TA) and midline curve width after peak time respectively (Chung et al.

2001; Martinant et al. 1998; Miles et al. 2013). Feed rate by rotor speed interaction was significant for TA. Data in Table 7 shows the value for TA as affected by feed rate and rotor speed interaction. TA was expressed as percent while TW was expressed as percent torque by minute. Changing feed rate did not impact ($p>0.05$) dough strength when milling at low rotor speed (6,000 and 9,000 rpm). However, dough strength was less for whole wheat flour when milled at high rotor speed (12,000 and 15,000 rpm) with low feed rate (12.6 g/min).

Table 7. Total area under the midline curve for whole wheat flour as affected by feed-rate and rotor speed interaction.

Feed-rate (g/min)	Rotor speed (rpm)	TA (%)
12.6	6,000	321.6 ± 20.3 a
	9,000	320.2 ± 25.9 a
	12,000	295.3 ± 14.4 bc
	15,000	292.8 ± 16.2 c
44.5	6,000	317.5 ± 27.9 a
	9,000	322.7 ± 26.1 a
	12,000	321.2 ± 18.8 a
	15,000	311.8 ± 23.0 ab

[a]Mean ± standard deviation; values followed by the same letters within column are not significantly different; TA = total area under the midline curve, measured at the end of mixing process; rpm = rotation per minute.

Main effects of rotor speed and feed rate was significant for curve width after peak time. None of the main effects interactions were significant for curve width after peak time. Curve width after peak time was highly correlated with mixing tolerance scores (Chung et al. 2001; Miles et al. 2013). High feed rate as well as high rotor speed produced stronger whole wheat flour as shown in Table 4. Higher torque was needed for the mixer's pins to pull the dough while mixing. In general, high rotor speed and high feed rate produced whole wheat flour with less starch damaged (Table 5) and greater fine particle size portion (Table 4 and Figure 11). Since the flour spent less time inside the grinding chamber, less rupture towards the starch and protein

granules of the wheat during grinding, therefore stronger flour produced (more torque needed). Less fracture towards the starch granule produced less damaged to the starch. Therefore, the protein granules in flour might get sufficient water during mixing, and less competence for the water between damaged starch and protein granules.

Baking Performance. Rotor speed by seed moisture content interaction was significant for baking absorption. Baking absorption is based on flour weight. Data in Table 8 shows the value for baking absorption. Baking absorption was ranged between 77.5 to 79.4%. Generally, high seed moisture content and high rotor speed produced flour that needed less water for baking. Less starch damage was found in whole wheat flour milled with high rotor speed (Table 5); therefore less water was needed during mixing. Damaged starch caused high water absorption capacity and is more readily hydrolyzed by α-amylase (Bettge et al. 1995; Bushuk and Scanlon 1993). Damaged starch has been reported to cause increased initial water absorption and prevent optimum gluten formation during mixing (Barrera et al. 2007). This effect might be explained by competition for the water between damaged starch and protein that prevents optimum gluten formation during mixing.

Table 8. Mean[a] baking absorption as affected by rotor speed and seed moisture content interaction

Rotor speed (rpm)	Seed moisture content (%)	Baking absorption (%)
6,000	10	79.42
	12	78.18
	14	78.84
	16	78.30
9,000	10	77.45
	12	79.36
	14	78.74
	16	78.70
12,000	10	78.18
	12	78.69
	14	77.89
	16	76.88
15,000	10	78.35
	12	77.88
	14	77.91
	16	77.59
LSD		1.25

Key
High
Medium
Low

[a]Mean ± standard deviation; values followed by the same letters within column are not significantly different; rpm = rotation per minute.

Feed rate by seed moisture content interaction was significant for dough handling properties. Dough handling properties were subjectively scored by an expert baker. The dough handling properties score ranged from 1 to 10, where 1 indicated poor/difficult to handle dough while 10 indicated the best/easy to handle dough. Figure 12 shows the dough handling property score for dough made from whole wheat flour as affected by feed rate and seed moisture content interaction. Dough was easier to handle (high score) when it was made from whole wheat flour that was derived from wheat with low seed moisture content, regardless of feed rate. Generally, whole wheat flour produced from grain with low seed moisture content had low damaged starch

(6-9%, 14%mb) (Table 5), long midline peak time (Table 6), and averaged 78-81% fine particle size (Table 4). In yeast-leavened products, a little content of damaged starch is desirable in order to obtain fermentable sugars after starch hydrolysis by amylase, but excessive starch damage leads to sticky dough (Bettge et al. 1995; Drapron and Godon 1987) thus the dough will be unmanageable and less favorable.

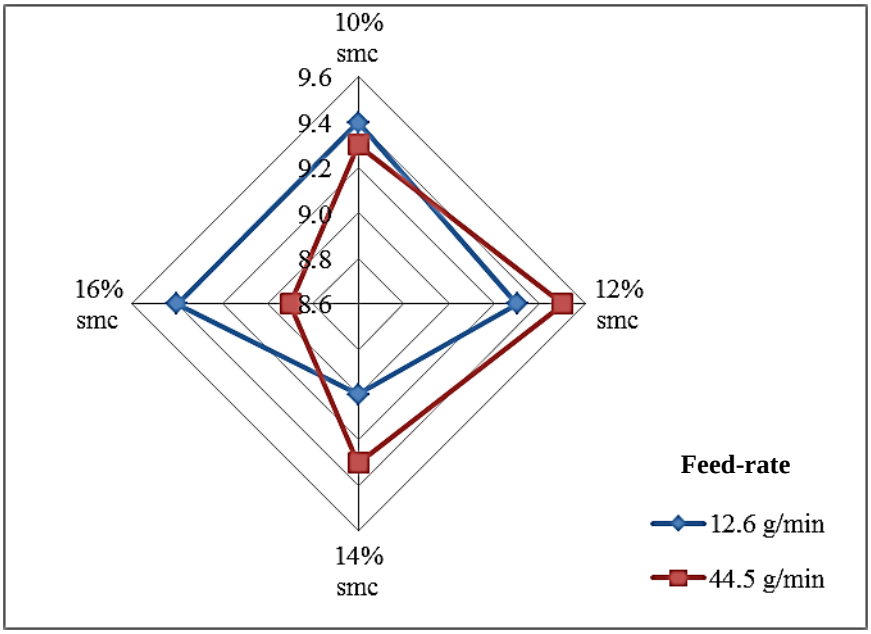

Figure 12. Dough handling properties score as affected by feed-rate and seed moisture content interaction.
smc = seed moisture content

Crumb score was significantly affected by feed rate and rotor speed interaction. Crumb texture was subjectively scored by a human expert. Crumb texture score ranged between 1 to 10 where 1 indicates extremely poor, coarse and large cells and many bad defects, while 10 indicates perfect crumb texture with tiny elongated cells with silky touch. Figure 13 shows the effect of feed rate and rotor speed on the crumb texture score of whole wheat bread. Crumb texture score was high for bread made from flour with low feed rate and high rotor speed

combination. However, at high feed rate, there was little effect on crumb score even though the rotor speed changed.

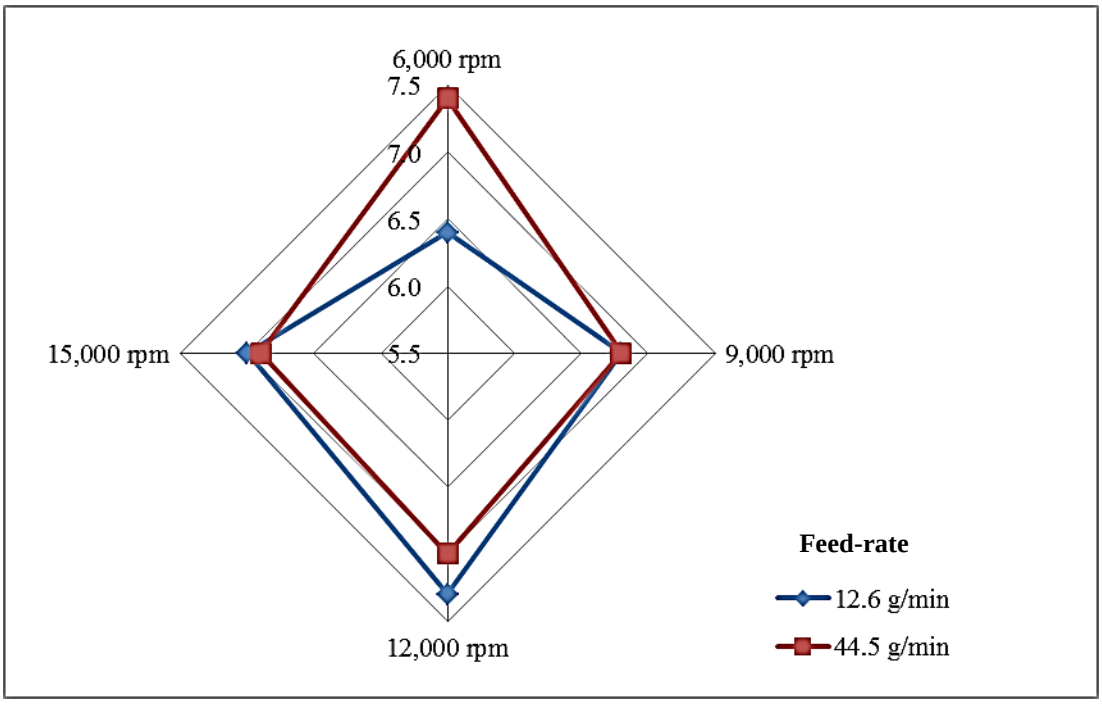

Figure 13. Crumb texture score as affected by feed-rate and rotor speed interaction.
rpm = rotation per minute

Rotor speed affected significantly the loaf volume and dough handling properties. Results show that grain milled at the high rotor speed produced whole wheat flour that had better dough handling properties and produced high loaf volume (Table 9). Milling with high rotor speed produced more fine particles of whole wheat flour (Figure 11), less damaged starch (Table 5) and longer peak time (Table 6); therefore the developed dough was easier to handle – more uniform and machinable, less sticky, and had good viscoelastic properties.

Table 9. Baking qualities of whole-wheat flour as affected by rotor speed[a]

Factors	Dough handling properties score	Corrected loaf volume (cc)
Rotor speed		
6,000 rpm	9.0 ± 0.5 b	145.8 ± 12.3 b
9,000 rpm	9.3 ± 0.5 a	154.5 ± 13.1 a
12,000 rpm	9.5 ± 0.5 a	154.0 ± 12.3 a
15,000 rpm	9.3 ± 0.6 a	153.3 ± 8.4 a

[a]Mean ± standard deviation; n = 24 for rotor speed; values followed by the same letters within column are not significantly different. rpm = rotation per minute; cc = cubic cube.

Conclusion

Whole-wheat flour was successfully produced using centrifugal mill. Milling resulted in low starch damaged (below 10%), low flour temperature (below 30^{o}C), low flour moisture content (less than 11%), and greater fine particle size portion (70 to 90%). Whole-wheat bread made from whole-wheat flour with centrifugal mill setting of high rotor speed (12,000 and 15,000 rpm) and low seed moisture content (10% and 12%) produced dough with good characteristics, easy to handle (score of 9.3-9.5), which resulted in high loaf volume (153 to 155 cc) and high score of crumb texture.

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PAPER 2. CENTRIFUGAL-MILLING OF WHEAT BRAN

Abstract

Bran and germ are considered byproducts from the milling process. This milling fraction generally consists of large flakes. Flour often adheres to the bran particles. Commercially, this flour is removed via bran finishers. Current study was aimed to investigate the effect of flour removing process on ground bran characteristics and to determine the milling parameters required to produce high yield of fine bran flour. Different tempering levels (10-16%) on bran were applied for size reduction using centrifugal mill. Mill parameters studied were rotor speed (6,000 – 15,000 rpm) and feed rate (6 – 12 g/min). Results showed that the bran and germ fraction contained 10% flour and the flour was 1.4 fold higher in protein content compare to the bran. Ground bran moisture content was positively correlated with moisture level during tempering. Ground bran particle size increased as tempering level increased. Bran without flour removing treatment impacted on final product temperature, protein content, and total starch. Ground bran milled from centrifugal mill at setting of high rotor speed (12,000 – 15,000 rpm) with low tempering level (10 – 12%) and low feed rate (6 g/min) produced ground bran with high yield (52 – 59%) of fine particle size (< 150 μm) regardless whether the flour being removed or not.

Introduction

Wheat bran and germ are valuable by-products of wheat milling and account for about 20-25% of the grain weight (Neves et al. 2006). Bran is a complex multi-layered material made up of several adhesive tissues: outer pericarp, testa, hyaline layer, aleurone layer, and some starchy endosperm residues (Hemery et al. 2011). Bran and germ contain phenolic compounds

(Kim et al. 2005), starches (Xie et al. 2008), soluble and insoluble dietary fiber (Cui et al. 1999), and proteins (Zhang et al. 2007).

In conventional wheat milling, a roller mill is used to remove the bran and germ from the endosperm and then to reduce the endosperm to flour (Posner and Hibbs 2005). The bran and germ are considered by-products. To aid in removing the bran and germ, wheat grain is tempered (Glenn and Johnston 1992). During tempering, water hydrates the bran and acts as a plasticizer, toughening the bran. The resulting bran is less likely to fracture during milling and remains in relatively large thin flakes which aids in the separation of the bran from the endosperm. Flour often adheres to the bran particles. Commercially, this flour is removed via bran finishers and added back to the flour stream. This process helps the millers with their flour extraction efficiency. On the other hand, research level small scale mills do not utilize the bran finishers to remove the flour.

Whole wheat flour is often composed of refined flour and reground bran and germ that are added back to the refined flour in proportions equivalent to that in the original seed. One of the problems associated with whole wheat flour is its bran's particle size. Various studies have been reported the effects of bran particle size on dough rheology and bread quality (Galliard and Gallagher 1988; Khalid and Simsek 2015; Zhang and Moore 1999). Some studies reported that fine bran particle size produced better baking performance (Khalid and Simsek 2015; Lorenz 1976; Moder et al. 1984b; Shetlar and Lyman 1944; Zhang and Moore 1997), while other studies claimed that fine bran particle size gave a detrimental effect on bread quality (Galliard and Gallagher 1988). The technique used to prepare the bran sample may contribute to the variances of the results. Most researchers prepare their different particle size fractions by sifting the whole bran. This may lead to major differences in chemical composition of bran fractions (Antoine et al.

2003; Hemery et al. 2009a; Hemery et al. 2007); such as large flakes fraction (pericarp-rich fraction) may ample in fiber (Antoine et al. 2003); and small particles fraction (aleurone-rich fraction) may abundant in vitamins, minerals and antioxidant compounds (Brouns et al. 2012).

Ball-milling and impact-milling have been used to decrease the particle size of the wheat bran (Antoine et al. 2004a; Rosa et al. 2013). Ball milling wheat bran was studied to investigate the antioxidant properties (Rosa et al. 2013) and biochemical markers (Antoine et al. 2004a) influenced by particle size. Wheat bran size reduction by hammer mill was investigated by Zhu et al. (2010) for bran's dietary fiber composition, hydration, and antioxidant properties. The centrifugal mill was used to reduce bran particle size before it was mixed with refined flour (Seyer and Gélinas 2009) and to investigate the wheat bran chemical composition after milling and sieving (Chen et al. 2013). Seyer and Gelinas (2009) used the centrifugal mill to grind bran with 1,000μm aperture screen size, and found that high loaf volume was correlated with low friability of the bran.

Prehydration or presoaking of bran was reported by Lai et al. (1989b) and Nelles et al. (1998) to improve its functional property in whole wheat flour breadmaking. Cai et al. (2015) studied the influences of different levels of bran hydration and physical treatments on bread-baking quality. They found that bran hydration and their physical treatments (autoclaving and freezing) were promising approaches to improving whole wheat bread loaf volume.

Particle size plays a significant role in flour functionality (Noort et al. 2010). The particle size impacts fiber's water absorption and retention, as well as end product quality (Al-Saqer et al. 2000; Sidhu et al. 1999). The aims of the present work were 1) to investigate the effect of flour removing process on ground bran characteristics; and 2) to determine the milling parameters

required to produce high yield of fine bran flour. These inputs are needed to produce whole wheat flour with similar particle size distribution as white endosperm flour.

Materials and Methods

Sample Procurement and Preparation

A composite sample of durum wheat (*Triticum turgidum* var. *durum*) harvested in North Dakota in 2013 was milled into semolina and bran/germ using an experimental mill (Buhler, model MLU 202) fitted with two laboratory-scale purifiers (Buhler-Miag, Minneapolis, MN, USA). A portion of the bran was passed through a bran finisher (Bühler, Uzwill, Switzerland), which removed flour particles adhering to the bran (labeled as cleaned bran). Original and cleaned bran samples were stored at -20°C until needed. Bran was equilibrated to room temperature (25°C) and tempered to 10, 12, 14, and 16% moisture content 24 h before milling.

Bran Milling

Tempered bran (150 g) was ground using an ultracentrifugal mill (Retsch ZM200, Haan, Germany) configured with a 250μm screen. The mill was operated using vibratory feeder (Model DR100, Retsch GmbH, Haan, Germany) and a vacuum (Nilfisk GM 80, Hungary) attachment that air cooled the mill and mill product. Feed rate and rotor speed were the mill parameters evaluated. The feed rate was varied by adjusting vibration setting 30 and 40 to achieve 6 g/min and 12 g/min, respectively. The rotor speed was varied from 6,000 to 15,000 rpm. Milling was done in the Durum Wheat Quality Laboratory during winter/spring season, with an average air temperature of 22°C and relative humidity of 17%. Milled product was collected and sealed in a zip lock plastic bag, stored at -20°C until flour analysis.

Physical and Chemical Properties of Ground Bran

Immediately after milling, the temperature of the ground bran and the rotor surface were measured using a digital infrared thermometer, (VWR International, Radnor, PA, USA). Particle size distribution was determined using a vibratory sieve shaker (Retsch AS200, Haan, Germany) configured with a stack of six sieves. Sieves used were 50, 150, 250, 425, 500, and 600 µm. Each sieve contained five plastic sieving balls. Sample (100g) was shaken via a vibratory amplitude displacement of 3 mm at 15 sec intervals for 5 min. Weight retained on each sieve and in pan was recorded as percent of the total.

Composite ground bran was characterized by moisture content (AACCI Approved Method 44-15.02), ash content (AACCI Approved Method 08-01.01), protein content (AACCI Approved Method 46-30.01), starch damage (AACCI Approved Method 76-30.02) and total starch (AACCI Approved Method 76-13.01).

Experimental Design and Statistical Analyses

The experimental layout was split-plot design with three replications. Bran was divided into two main plot treatment levels, original bran (no bran cleaning) and cleaned-bran (adhering flour removed using bran finisher). Subplot were factorial arrangement of tempering (10, 12, 14, 16%), feed rate (6.04 g/min and 12.01 g/min), and mill rotor speed (6000, 9000, 12000, 15000). Analysis of variance was performed using the ‘Mixed’ procedure in SAS software (SAS Institute, Cary, NC). Treatment means were compared with Least Significant Difference tests at 5% level. Pearson correlation coefficients were estimated between variables using CORR procedure in SAS. Stepwise regression was also performed using SAS software.

Results and Discussion

Bran Characteristics

The bulk bran samples of *Triticum turgidum* var. *durum* and its flour (from bran finisher) characteristics were listed in Table 10. Durum was tempered to 17.5% moisture before milling and bran were stored at -20°C right after milling before next treatment. There was 6% of moisture loss during low-temperature storage of the flour that adheres to bran. When unwrapped foods are frozen and/or stored in the frozen state or with a non-adhering packaging, weight losses take place due to sublimation of the surface ice (Campañone et al. 2001).

Flour constituted 10% of the weight of original bran (Figure 14). The flour from bran cleaning process (FBCP) had 1.4 times higher protein content than the bran (Table 10). The FBCP may contain hyaline and aleurone layers and also peripheral starchy endosperm cells. The aleurone layer is the innermost layer of the wheat bran (Brouns et al. 2012). It is relatively high in minerals, vitamins, and bioactive phytochemicals, such as antioxidant compounds and lignans (Antoine et al. 2003; Buri et al. 2004; Delcour and Hoseney 2010; Fardet 2010). Buri et al. (2004) found 20.8% protein in aleurone layer, and furthermore, the essential amino acids were well balanced.

The ash content of bran is much higher than that of FBCP, which contained 5.5 and 2.9 g of crude ash per 100 g of 14% moisture basis, respectively. A bran finisher is a beater machine that frees endosperm from the bran by impact and friction (Posner and Hibbs 2005). Using a bran finisher to remove any flour adheres to the bran is not an effective way to isolate aleurone and hyaline layer. Aleurone layer is tightly bound to the seed coats, and different fractionation methods have been developed to isolate each layer (Brouns et al. 2012).

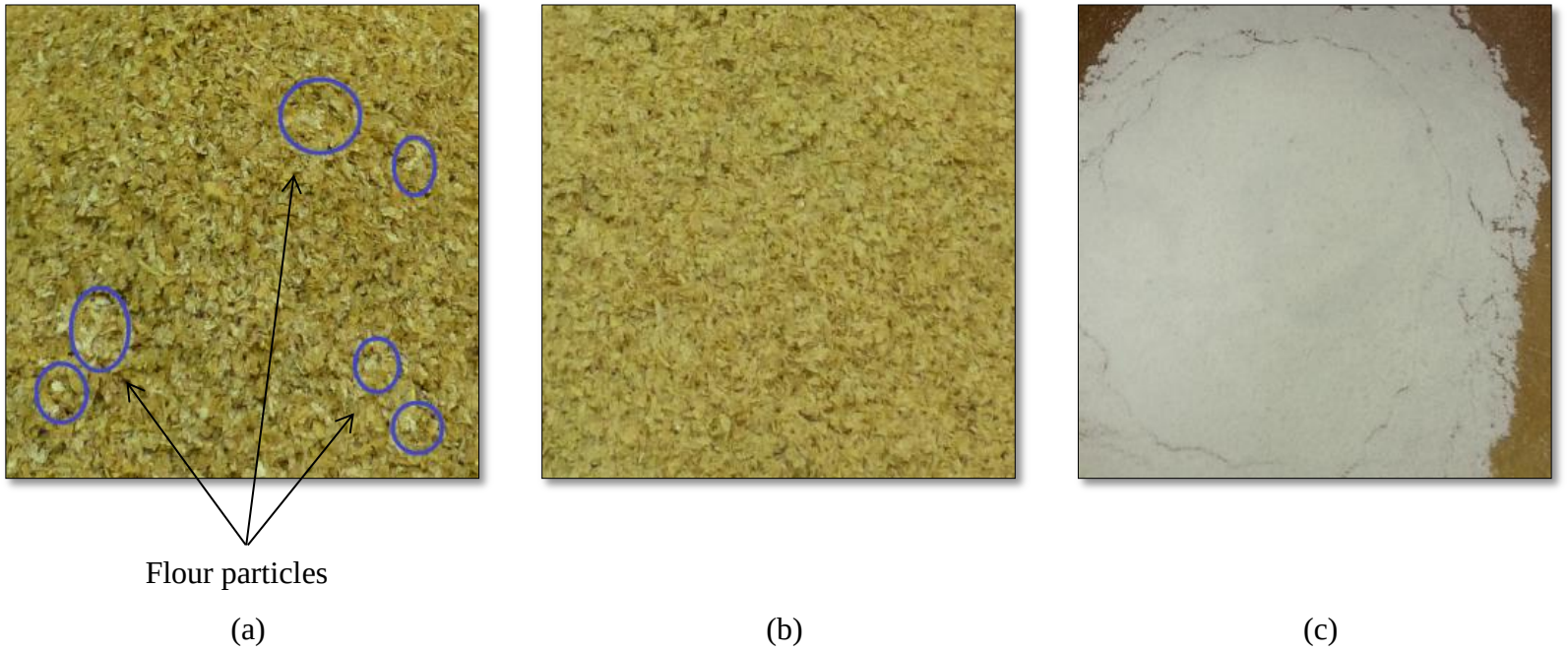

Figure 14. Fresh bran with flour particles (a); clean bran (b); flour particles adhered to bran (c)

Table 10. Proximate composition[a] of bran and flour from bran cleaning process (FBCP)

Sample	Moisture (%)	Protein (N x 5.7; 14%mb)	Ash (14%mb)	Damaged Starch (14%mb)	Total Starch (14%mb)
Bran	16.71	17.43	5.54	1.15	12.62
FBCP	11.23	23.97	2.92	3.56	40.37

[a] = average of three replications; mb = moisture basis.

Total starch and damaged starch was higher in FBCP than bran. The peripheral starchy endosperm cells are the first row of cells in the aleurone layer; they are small and are equal in diameter in all directions or slightly elongated (Delcour and Hoseney 2010). Bran particles are very light in weight. The vibratory feeder was causing the bran particles to stack against each other (agglomerate) and eventually the bran particles stopped moving. Therefore, while milling, the bran particles had to be stirred occasionally to facilitate movement (Figure 15a, c). The vibratory feeder was also causing some separation from bran and FBCP while milling was performed because of the vibration action (Figure 15b).

Physical Properties of Ground Bran

Ground Bran Temperature. Analysis of variance for ground bran temperature is shown in Appendix Table B1. Bran cleaning by feed rate by tempering level interaction was significant ($p<0.001$) for changes in ground bran temperature (Table 11). The high feed rate (12 g/min) generally resulted in higher changes in ground bran temperatures than did the low feed rate (6 g/min). Ground bran temperature was increased as bran tempering level increased for cleaned bran. An exception occurred with non-cleaned bran, where changes in ground bran temperature after milling seems to be fluctuating at each tempering moisture level (Table 11).

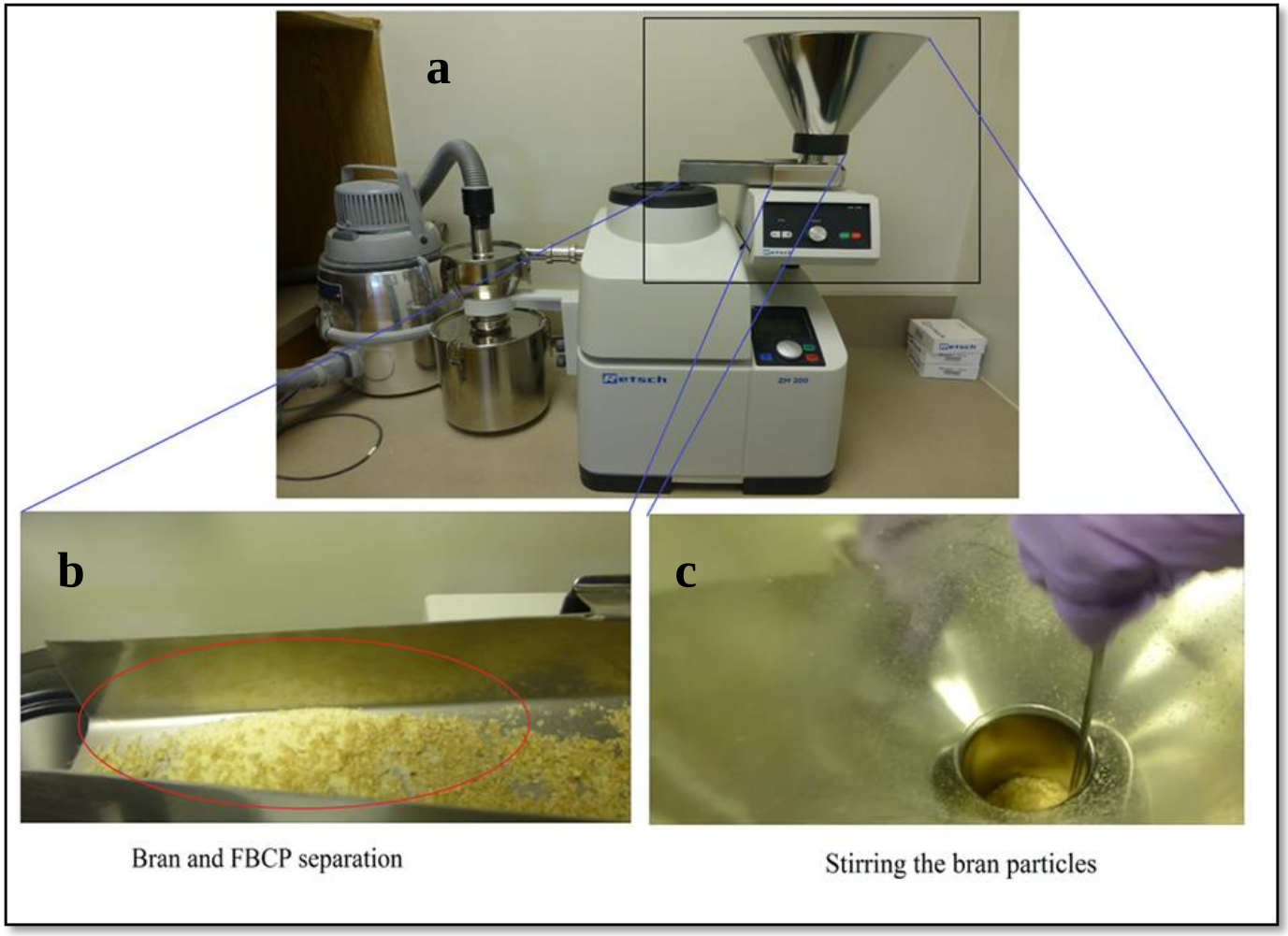

Figure 15. Vibratory feeder (a); bran and FBCP separation during milling (b); stirring action to facilitate the bran movement during milling (c).
FBCP = flour from bran cleaning process.

Bran cleaning by feed rate by rotor speed interaction was significant ($p<0.001$) for ground bran temperature changes after milling. Generally, high rotor speed (12,000-15,000 rpm) and high feed-rate (12 g/min) caused high changes in ground bran temperature for both cleaned and non-cleaned bran (Table 12). However, non-cleaned bran had lower changes in ground bran temperature than cleaned bran with exception for 15,000 rpm and high feed-rate. High feed rate (12 g/min) resulted in greater changes in ground bran temperature than low feed rate (6 g/min). In this case, the high feed rate was associated with increasing vibration of the feeder. This action resulted in more bran being fed into the grinding chamber at one time (6 g vs. 12 g per minute), and increase the collisions inside the milling chamber and cause the high final product temperature. Ground bran temperature was ranging between 27 and 32°C, and this is about 6 to 10°C changes in temperature. This temperature is not detrimental to starch and protein, as Olkku and Rha (1978) mentioned that wheat starch, in general will gelatinized in a range of 58-64°C; while Schofield et al. (1983) mentioned that gluten declined its functionality when temperature reach 75°C.

Table 11. Changes in temperatures (°C) for ground bran after milling as affected by two-way interaction of bran cleaning-tempering level.

Bran Cleaning	Tempering Level (%)	Feed-rate (g/min)	
		6	12
Non-cleaned Bran	10	6.23	7.38
	12	6.13	8.13
	14	7.18	8.36
	16	5.95	8.39
Cleaned Bran	10	6.85	8.58
	12	7.48	8.18
	14	7.89	8.98
	16	7.96	8.89

Key
High
Medium
Low

Table 12. Changes in temperatures (°C) for ground bran after milling as affected by two-way interaction of bran cleaning-rotor speed level.

Bran Cleaning	Rotor Speed (rpm)	Feed-rate (g/min)	
		6	12
Non-cleaned Bran	6,000	5.28	7.00
	9,000	5.41	7.19
	12,000	6.91	7.93
	15,000	7.88	10.14
Cleaned Bran	6,000	6.43	8.06
	9,000	6.33	8.01
	12,000	8.00	8.83
	15,000	9.43	9.71

Key
High
Medium
Low

Changes on Mill Surface Temperature. Analysis of variance for mill surface temperature shown in Appendix Table B1 indicated that four-way interaction (bran cleaning by tempering level by rotor speed by feed rate) was significant at $p<0.05$. The lowest mill surface temperature achieved after milling was 25°C for ground bran milled at 6,000 rpm-6 g/min feed rate-10% tempering level-non-clean bran. The highest mill surface temperature achieved was 33°C when milled at these three milling combinations parameters: 1) cleaned bran-10% tempering level-

15,000 rpm-12 g/min feed rate; 2) cleaned bran-14% tempering level-15,000 rpm-6 g/min; and 3) cleaned bran-16% tempering level-12,000 rpm-12 g/min feed rate (Table 14). These three milling combination parameters were associated with cleaned bran and high rotor speed level. Absence of FBCP may increase the chances of bran particles to absorb energy from the rotor without any competition. High rotor speed generates more energy to transfer to bran particles, and absence or less amount of FBCP makes the bran particles produced high temperature.

Main effects of feed rate, rotor speed and tempering level were significant ($p<0.0001$). Mill surface temperatures increased 1.3°C as temper moisture increased from 10 to 16%; increased 2.2°C as rotor speed increased from 6,000 to 15,000 rpm; and increased 0.7°C as feed rate increased from 6 to 12 g/min (data not shown).

Particle Size Distribution of Ground Bran

Overall, about 0.9 to 5.6% of total ground bran was classified as coarse particles and remained on top >425 µm sieve (Appendix Table B6). Since the bran was milled using a 250 µm sieve size aperture, a major portion of ground bran was distributed at medium ($425 > x > 150$ µm) and fine (<150µm) particle size portion, which were 51-64% and 28-46% respectively. Analysis of variance for medium and fine particle size of ground bran shown in Appendix Table B2 indicated that four-way interaction (bran cleaning by tempering level by rotor speed by feed rate) was significant ($p<0.0001$), as well as the main effect except bran cleaning main plot.

Table 13. Temperature changes on mill surface (°C) as affected by four-way interaction of bran cleaning-tempering level-rotor speed-feed rate

Bran Cleaning	Tempering Level (%)	Rotor Speed (rpm)	Feed rate (g/min)	
			6	12
Non-cleaned Bran	10	6,000	3.30	5.35
		9,000	5.75	5.95
		12,000	6.05	5.60
		15,000	7.40	5.05
	12	6,000	4.15	6.35
		9,000	6.60	7.00
		12,000	5.95	6.70
		15,000	5.80	8.60
	14	6,000	6.25	5.40
		9,000	6.75	6.30
		12,000	6.05	7.70
		15,000	8.05	9.90
	16	6,000	5.45	7.15
		9,000	5.75	5.75
		12,000	6.10	6.90
		15,000	6.15	8.00
Cleaned Bran	10	6,000	5.10	7.60
		9,000	5.40	8.45
		12,000	8.85	8.95
		15,000	9.95	10.60
	12	6,000	8.15	7.90
		9,000	6.75	7.30
		12,000	8.20	9.00
		15,000	9.55	9.40
	14	6,000	7.95	8.80
		9,000	7.80	8.70
		12,000	9.40	9.90
		15,000	10.55	10.35
	16	6,000	7.75	7.85
		9,000	9.00	8.70
		12,000	9.95	11.05
		15,000	9.80	10.45

Key

High
Medium
Low

Fine Particle Size Distribution. Fine particle size portion of ground bran decreased with increased tempering level (Appendix Table B6). Opposite trend occurred for rotor speed, where fine particle size fraction increased with increasing rotor speed (Appendix Table B6). Negative association seen with feed rate, fine particle size fraction was greater with low feed rate (6 g/min) than high (12 g/min) feed rate (Appendix Table B6). Four-way interaction (bran cleaning by tempering level by rotor speed by feed rate) for ground bran fine particle size portion was shown in Table 14. In general, a significant amount of fine particles size portion of ground bran were found when milled at high rotor speed (12,000 – 15,000 rpm) and low tempering level (10 – 14%). A large reduction in fine particles occurred when milled at 16% tempering level with both high rotor speeds (12,000 – 15,000 rpm). Decreasing amount of fine particle size portion of ground bran were found when milled at increasing tempering level (from 10% - 16%) with both low rotor speeds (6,000 – 9,000 rpm).

With regards to roller milling practices, tempering or conditioning the wheat prior to milling was done to toughen the bran, reduce the formation of bran powder, soften the endosperm, and to facilitate the separation of bran from endosperm (Shellenberger 1980; Sugden 2001; Yamazaki and Donelson 1983). Wheat bran becomes more compliant and resilient (plastic and elastic) with moisture content (Glenn and Johnston 1992). Conditioning treatments in wheat before milling facilitate the separation of outer grain layer and endosperm, thus improves millability (Shellenberger 1980; Ziegler and Greer 1971). The strength of the bran and its capacity to deform without breaking, especially under humid conditions, contrasts sharply with the mechanical properties of the starchy endosperm (Glenn and Johnston 1992; Glenn et al. 1991). The tensile strength of the bran in Glenn and Johnston study (1991) was five- to 10-fold greater than the tensile strength reported for the starchy endosperm.

Table 14. Fine particle size portion (%) of ground bran as affected by bran cleaning process-tempering level-rotor speed-feed-rate interaction

Bran Cleaning	Rotor Speed (rpm)	Tempering Level (%)	Feed rate (g/min)	
			6	12
Non-cleaned Bran	6,000	10	40.75	32.60
		12	31.45	28.80
		14	29.05	31.10
		16	25.30	21.45
	9,000	10	44.30	47.80
		12	41.15	35.55
		14	39.35	38.95
		16	38.45	38.25
	12,000	10	48.45	42.65
		12	51.20	53.40
		14	44.50	39.90
		16	48.20	27.50
	15,000	10	53.80	46.10
		12	52.35	45.75
		14	58.55	45.75
		16	48.80	37.20
Cleaned Bran	6,000	10	36.10	28.20
		12	36.43	25.40
		14	26.65	21.30
		16	26.10	13.25
	9,000	10	46.70	44.60
		12	39.50	34.75
		14	43.68	30.72
		16	37.02	23.48
	12,000	10	54.40	52.35
		12	44.30	44.30
		14	45.55	49.18
		16	33.00	36.05
	15,000	10	45.90	39.65
		12	52.25	42.05
		14	43.80	48.30
		16	50.70	27.25
LSD1 (0.05)			5.72	
LSD2 (0.05)			11.89	

Key: High, Medium, Low

LSD1=LSD between subplot means at the same bran treatment; LSD2=LSD between subplot means at different bran treatment.

Chemical Composition of Ground Bran

Ground Bran Moisture Content. Analysis of variance for ground bran moisture content shown in Appendix Table B3 indicated that main effects of feed rate, rotor speed and tempering level were significant ($p<0.0001$). Ground bran moisture content was increasing as tempering level and feed rate increase (Appendix Table B7). However opposite trend was seen with increasing rotor speeds (Appendix Table B7). Two-way interaction of rotor speed and tempering level was highly significant ($p<0.001$) for ground bran moisture content compare to other factors interactions (Appendix Table B3). Ground bran moisture content (6.4 to 8.9%) was directly related to tempering moisture level (10 to 16%). The negative association could be seen with ground bran moisture contents and rotor speeds (Figure 16). The highest ground bran moisture content was recorded at 8.9% when milling at 16% tempering level and 6,000 rpm rotor speed. The least moisture content of ground bran was recorded at 6.4% when milled at 10% tempering level and 15,000 rpm rotor speed.

Moisture loss was greater at 16% tempering level, ranging from 7.1 to 8.7 percentage unit losses (data not shown). Moisture loss is attributed to increased exposure of bran's particles surface area to evaporation due to air cooling system and to the low relative humidity in the mill room (17%), in agreement with the results showed in Paper 1. Ground bran moisture content was negatively correlated with fine particle size distribution and rotor speed and positively correlated with tempering level and medium particle size distribution (Appendix Table B7). Higher rotor speed produced greater fine particle size portion thus generated more energy and heat to pass on to ground bran. This heat transfer movement will cause great moisture loss on ground bran.

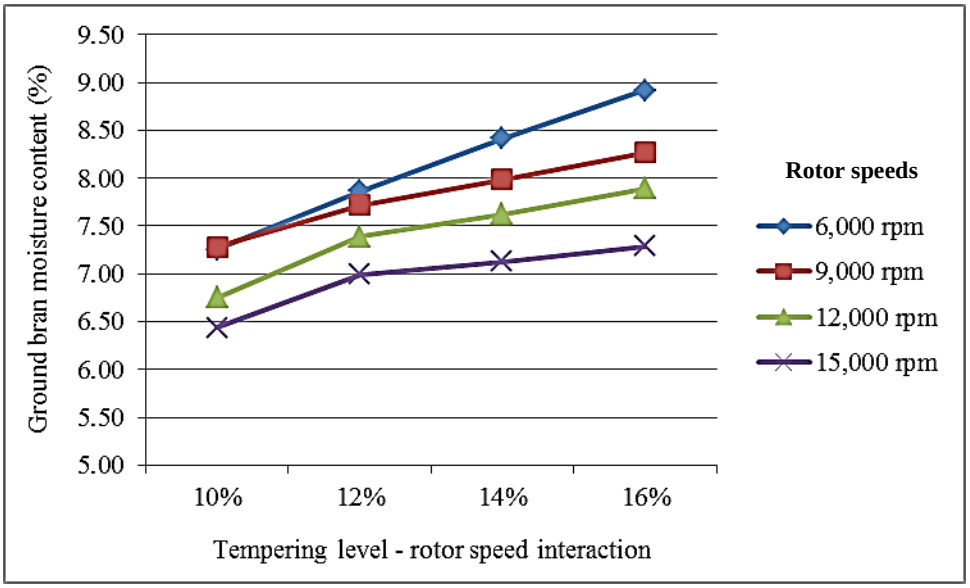

Figure 16. Ground bran moisture content (%) as affected by tempering level-rotor speed interaction.

Ground Bran Ash Content. Analysis of variance for ground bran ash content shown in Appendix Table B3 indicated that two-way interaction of bran cleaning and tempering level was highly significant ($p<0.0001$) for ground bran ash content compare to other factors interactions (Appendix Table B3). High ash content was recorded for cleaned bran with increased tempering level (Figure 17). While decreased ash content was found in ground bran when milled at increased tempering main effects of rotor speed were significant ($p<0.001$). Other main effects such as feed rate and tempering level were significant at $p<0.05$ for ground bran ash content. Results in Table 16 show the ground bran ash content as affected by the main effects. Even though it was statistically different , but practically it is meaningless since the difference among each main effects treatment was very small, which were 0.02, 0.05, 0.08, and 0.03 for bran cleaning, tempering level, rotor speed, and feed rate respectively (Appendix Table B7).

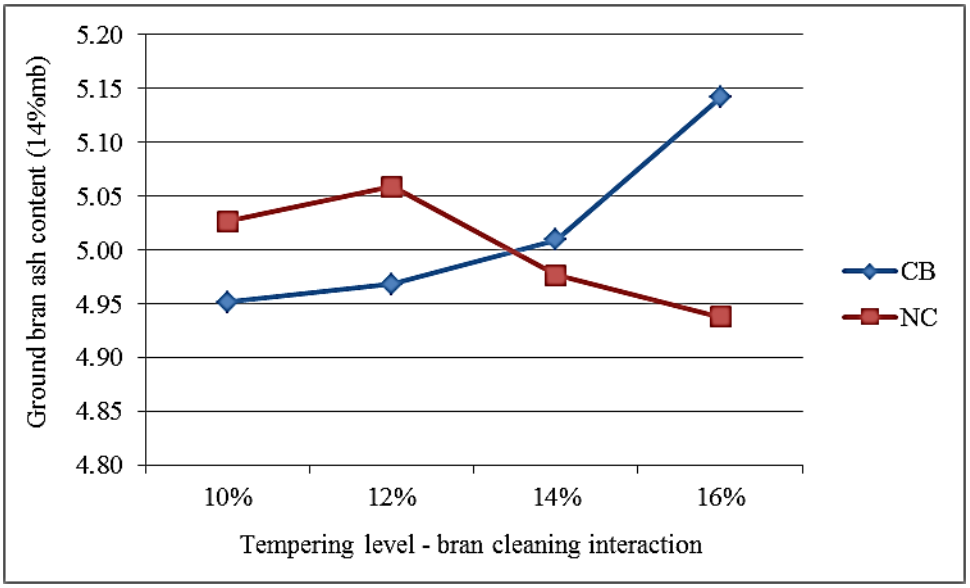

Figure 17. Ground bran ash content (14%mb) as affected by bran cleaning-tempering level interaction.
mb=moisture basis; CB=cleaned bran; NC=non-cleaned bran.

Hinton (1959) defined ash content as inorganic material left after incineration and that ash content increased from the center to the outer layers of the wheat kernel. Lower ash content in flour indicates less contamination with wheat bran and germ. Ash content is used to evaluate milling performance by constructing cumulative ash curves (Posner 1991; Shellenberger 1980). However, in bran milling experiment (this study), we found that drier non-cleaned bran (low tempering moisture level) tends to have high ash content, slightly equivalent to cleaned bran treated with high tempering level (Fig. 4). The ash content ranged from 4.95 to 5.14% (14%mb).

Ground Bran Protein Content. Analysis of variance for ground bran protein content shown in Appendix Table B4 indicated that all three-way interactions of bran cleaning-feed rate-tempering level, bran cleaning-rotor speed-tempering level, and feed rate-rotor speed-tempering level were significant ($p<0.0001$). Other three-way interaction of bran-cleaning-feed rate-rotor speed was significant at $p<0.01$. Table 15-18 shows the three-way interaction on ground bran protein content. Table 15,16, and 17 exhibited that higher protein content was found in non-

cleaned bran compared to cleaned bran. These results were relevant to data shown in Table 10, where FBCP's protein content was 1.4-fold higher than bran's protein content. The FBCP may contain hyaline and aleurone layers and also pheripheral starchy endosperm cells. The aleurone layer is the innermost layer of the wheat bran (Brouns et al. 2012), and it is relatively high in minerals, vitamins, and bioactive phytochemicals, such as antioxidant compounds and lignans (Antoine et al. 2003; Buri et al. 2004; Delcour and Hoseney 2010; Fardet 2010). No absolute trend could be seen for cleaned bran main plot with increasing tempering level (Table 15 and 16).

Different senario was revealed for non-cleaned main plot, where protein content was increasing with increasing tempering level (Table 15 and 16). The protein content ranged from 15.32 – 16.32% (Table 15) and 15.35-16.24% (Table 16) for non-cleaned main plot. The FBCP contains aleurone layer, as it is the innermost layer of the wheat bran (Brouns et al. 2012). The aleurone layers contain high activities of peptidases when germinating (Mikola and Kolehmainen 1972). However, the germs/kernels need 42-44% of moisture level to start the germination (Delcour and Hoseney 2010). In this case, the moisture content was not high enough to start the germination. However, there is still some possibility that it will occur. Another possible explanation would be concentrated nitrogen level in the ground sample. Protein content was measured using combustion method (AACCI Approved Method 46-30.01). Ground bran was dried at 135°C for 1 h (AACCI Approved Method 44-15.02) to determine the moisture content. Fructose, which found around 4.8% in wheat germs/aleurone layer (Dubois et al. 1960), will caramelize at 110°C and formed a volatile compound. Ultimately this will concentrate the nitrogen concentration.

Table 15. Protein content of ground bran as affected by three-way interaction of bran cleaning-tempering level-feed rate.

Bran Cleaning	Tempering Level (%)	Feed rate (g/min)	
		6	12
Non-cleaned Bran	10	15.3	15.5
	12	15.4	15.6
	14	16.1	15.8
	16	16.3	15.8
Cleaned Bran	10	15.2	15.2
	12	15.2	15.2
	14	15.1	15.1
	16	15.2	15.1
LSD1 (0.05)		0.1	
LSD2 (0.05)		0.1	

Key
High
Medium
Low

LSD=least significant different; LSD1=LSD between subplot means at the same bran treatment; LSD2=LSD between subplot means at different bran treatment.

Increasing rotor speed resulted in small changes in protein content of ground bran for cleaned bran (Table 17). However, changes in rotor speed affect negatively on the ground bran protein content for non-cleaned bran (Table 17). Protein content ranged from 15.5 – 16.0 % for non-cleaned bran (Table 17). When averaged the main plot treatments, data were presented in Table 18. In general, higher moisture content exhibited higher protein content. However, when we examined at individual tempering level and low (6,000 – 9,000 rpm) rotor speed, high feed rate (12 g/min) resulted in increasing the protein content at 10 to 14% tempering level and decreased at 16% tempering level, while the low feed rate (6 g/min) showed fluctuate trends with highest protein content recorded as 15.5% and 15.7% for combination of 9,000 rpm-12% tempering level and 12,000 rpm-14% tempering level respectively (Table 18).

Table 16. Protein content of ground bran as affected by three-way interaction of bran cleaning-rotor speed-tempering level.

Rotor Speed (rpm)	Tempering Level (%)	Bran Cleaning	
		Non-cleaned Bran	Cleaned Bran
6,000	10	15.7	15.2
	12	15.5	15.2
	14	16.2	15.1
	16	16.2	15.0
9,000	10	15.3	15.3
	12	15.4	15.4
	14	16.0	15.0
	16	16.1	15.0
12,000	10	15.3	15.1
	12	15.5	15.2
	14	15.8	15.4
	16	15.9	15.3
15,000	10	15.3	15.1
	12	15.5	15.0
	14	15.7	14.9
	16	16.0	15.4
LSD1 (0.05)		0.2	
LSD2 (0.05)		0.2	

Key
High
Medium
Low

LSD=least significant different; LSD1=LSD between subplot means at the same bran treatment; LSD2=LSD between subplot means at different bran treatment

At 10% tempering level, low feed rate (6 g/min) exhibited low protein content with increasing rotor speed, while high feed rate (12 g/min) seems to show no trend. Another interesting occurrence happened at 16% tempering level, where low feed rate (6 g/min) seems to offer high protein content with increasing rotor speed. The protein ranged 15.6-15.9%. However, the high feed rate (12 g/min) demonstrated opposite trend, where the protein content decreases with increasing rotor speed. The protein ranged 15.3-15.6%.

Table 17. Protein content of ground bran as affected by three-way interaction of bran cleaning-rotor speed-feed rate

Bran Cleaning	Rotor Speed (rpm)	Feed rate (g/min)	
		6	12
Non-cleaned Bran	6,000	15.8	16.0
	9,000	15.8	15.7
	12,000	15.7	15.6
	15,000	15.7	15.5
Cleaned Bran	6,000	15.1	15.1
	9,000	15.2	15.1
	12,000	15.3	15.2
	15,000	15.1	15.2
LSD1 (0.05)		0.1	
LSD2 (0.05)		0.1	

Key
High
Medium
Low

LSD=least significant different; LSD1=LSD between subplot means at the same bran treatment; LSD2=LSD between subplot means at different bran treatment.

Ground Bran Total Starch. Analysis of variance for ground bran total starch is shown in Appendix Table B5 indicated that all interaction between independent variables was significant. Table 19 shows the four-way interaction for total starch content of ground bran. Clearly could be seen that non-cleaned bran possesses higher total starch content compared to cleaned bran. This result was relevant to data shown in Table 10, where FBCP's total starch content was 3.2-fold higher than bran's total starch content. Millers use this flour to enhance their milling extraction yield. The total starch content ranged between 8.6-19.8% for the entire experiment, where the total starch content for cleaned and non-cleaned ground bran was fall between 8.6-13.2% and 14.0-19.8% respectively. Largest differences between low (6 g/min) and high (12 g/min) feed rate could be seen when milled at 10% tempering level for both cleaned and non-cleaned bran (Table 19).

Table 18. Protein content of ground bran as affected by three-way interaction of rotor speed-tempering level-feed rate.

Rotor Speed (rpm)	Tempering Level (%)	Feed rate (g/min)	
		6	12
6,000	10	15.5	15.4
	12	15.2	15.5
	14	15.6	15.7
	16	15.6	15.6
9,000	10	15.3	15.3
	12	15.5	15.4
	14	15.5	15.5
	16	15.8	15.3
12,000	10	15.2	15.3
	12	15.2	15.5
	14	15.7	15.4
	16	15.7	15.5
15,000	10	15.1	15.4
	12	15.2	15.3
	14	15.4	15.2
	16	15.9	15.5
LSD1 (0.01)		0.2	

Key
High
Medium
Low

LSD=least significant different; LSD1=LSD between subplot means at the same bran treatment; LSD2=LSD between subplot means at different bran treatment.

Three-way interactions for total starch content of ground bran were significant. Similar pattern were exhibited for interaction of bran cleaning-rotor speed-feed rate, bran cleaning-tempering level-feed rate, and bran cleaning-rotor speed-tempering level, where non-cleaned bran showed higher total starch than cleaned bran (data not shown). Total starch content difference was greatest (2.8%) between high and low feed rates when milled at 15,000 rpm for cleaned bran treatment. Total starch content was increasing with increasing tempering level for non-cleaned bran (both high and low feed rate).

The greatest differences between low (6 g/min) and high (12 g/min) feed rate was 1.9% for non-cleaned bran when milled at 10% tempering level. As seen in Table 20, the low (6 g/min) feed rate showed declining in total starch content (14.6-13.6%) with increasing rotor speed level when milled at 10% tempering level. However, opposite phenomena seen when milled at 16% tempering level, where increasing in total starch (14.4-15.4%) was observed with increasing rotor speed (Table 20). For the high (12 g/min) feed rate, greatest total starch (16.5%) was recorded when milled at 14% tempering level-9,000 rpm, while lowest total starch (13.5%) was recorded when milled at 16% tempering level-9,000 rpm (Table 20).

Ground Bran Starch Damaged. Bran clean by feed rate interaction and rotor speed main effect were significant for starch damage (Appendix Table B4). Figure 19 illustrated the interaction between feed rate and bran cleaning for ground bran starch damaged. Cleaned and non-cleaned bran were performed about equally starch damaged content (1.72-1.97%) in high feed rate (12 g/min). However, non-cleaned bran possesses considerably higher starch damaged than cleaned bran at low feed rate (6 g/min).

Table 19. Total starch content of ground bran as affected by four-way interaction of bran cleaning-tempering level-rotor speed-feed rate

Bran Cleaning	Tempering Level (%)	Rotor Speed (rpm)	Feed rate (g/min)	
			6	12
Non-cleaned Bran	10	6,000	17.78	17.67
		9,000	16.23	17.39
		12,000	16.57	18.88
		15,000	14.04	18.26
	12	6,000	16.62	17.23
		9,000	17.69	16.87
		12,000	17.37	18.65
		15,000	17.36	17.73
	14	6,000	18.77	19.06
		9,000	18.19	19.52
		12,000	18.82	17.57
		15,000	18.41	18.55
	16	6,000	18.19	18.07
		9,000	19.32	18.26
		12,000	19.42	17.39
		15,000	19.85	19.23
Cleaned Barn	10	6,000	11.39	10.99
		9,000	12.45	9.22
		12,000	11.65	10.58
		15,000	13.20	10.80
	12	6,000	10.49	9.80
		9,000	11.61	10.95
		12,000	9.72	11.57
		15,000	11.80	10.52
	14	6,000	10.78	9.43
		9,000	10.88	13.41
		12,000	9.75	10.91
		15,000	10.32	11.23
	16	6,000	10.57	9.55
		9,000	11.08	8.65
		12,000	11.32	11.13
		15,000	10.99	11.63
LSD1 (0.05)			0.97	
LSD2 (0.05)			0.99	

Key
High
Medium
Low

LSD=least significant different; LSD1=LSD between subplot means at the same bran treatment; LSD2=LSD between subplot means at different bran treatment.

Table 20. Total starch of ground bran as affected by three-way interaction of tempering level-rotor speed-feed rate.

Tempering Level (%)	Rotor Speed (rpm)	Feed rate (g/min)	
		6	12
10	6,000	14.59	14.33
	9,000	14.34	13.30
	12,000	14.11	14.73
	15,000	13.62	14.53
12	6,000	13.56	13.51
	9,000	14.65	13.91
	12,000	13.54	15.11
	15,000	14.58	14.13
14	6,000	14.78	14.24
	9,000	14.53	16.46
	12,000	14.28	14.24
	15,000	14.97	14.89
16	6,000	14.38	16.31
	9,000	15.20	13.46
	12,000	15.37	14.26
	15,000	15.42	15.43
LSD1 (0.05)		0.68	

Key
High
Medium
Low

LSD=least significant different; LSD1=LSD between subplot means at the same bran treatment; LSD2=LSD between subplot means at different bran treatment.

Main effects of rotor speed shows higher starch damaged content in ground bran milled at high rotor speed (12,000 to 15,000 rpm) compared to low rotor speed (6,000 to 9,000 rpm). Higher total starch content was found in ground bran milled at high rotor speed (Appendix Table B7). Therefore, greater amount of damaged starch was found in ground bran milled at those rotor speeds range.

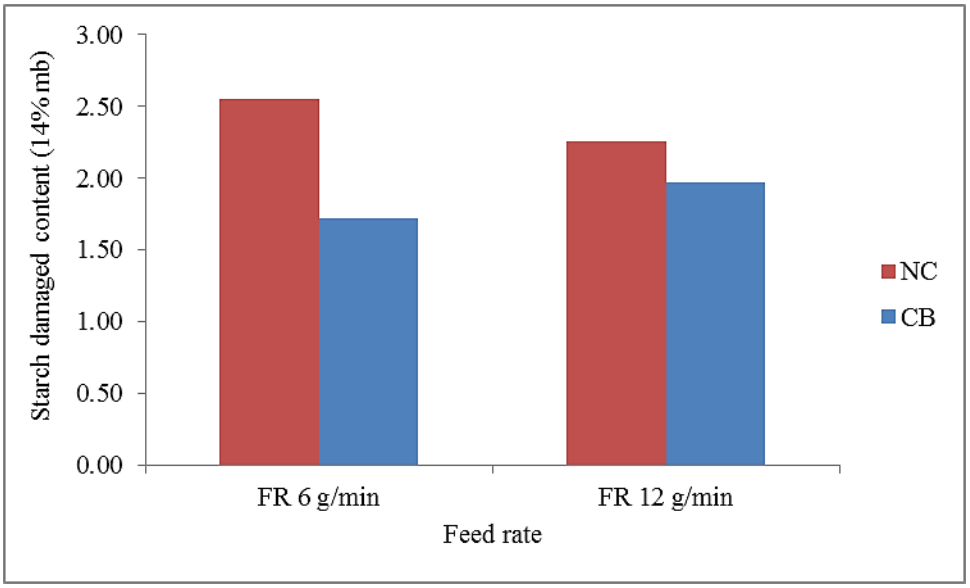

Figure 18. Ground bran total starch content (14%mb) as affected by bran cleaning-feed rate interaction.
mb=moisture basis; CB = cleaned bran; NC = non-cleaned bran; FR = feed rate.

Relationships among Milling Parameters and Ground Bran Characteristics

Correlation. Simple linear correlation coefficients among milling parameters and selected ground bran characteristics (cleaned and non-cleaned) were summarized in Table 21. Cleaned ground bran temperature was positively correlated with feed rate, rotor speed, tempering level, mill surface temperature, moisture content and starch damaged. However, for the non-cleaned ground bran temperature, only four factors of feed rate, rotor speed, mill surface temperature and starch damaged were correlated.

Table 21. Correlation coefficients (n=96) between milling parameters and (a) cleaned and (b) non-cleaned ground bran characteristics.

	GBT		MST		MED		FINE		MC		PC		SD	
(a)														
FR	0.40	**	0.23	*	0.27	**	-0.31	**	0.20	NS	-0.04	NS	0.31	**
RS	0.67	**	0.68	**	-0.55	**	0.63	**	-0.67	**	0.01	NS	0.44	**
TM	0.22	**	0.34	NS	0.49	**	-0.42	**	0.53	**	-0.11	NS	-0.03	NS
GBT			0.91	**	-0.13	NS	0.13	NS	-0.27	**	-0.09	NS	0.47	**
MST	0.91	**			-0.14	NS	0.18	NS	-0.28	**	-0.04	NS	0.34	**
MED	-0.13	NS	-0.14	NS			-0.96	**	0.68	**	-0.08	NS	-0.20	NS
FINE	0.13	NS	0.18	NS	-0.96	**			-0.70	**	0.09	NS	0.21	*
MC	-0.27	**	-0.28	**	0.68	**	-0.70	**			-0.16	NS	-0.27	**
PC	-0.09	NS	-0.04	NS	-0.08	NS	0.09	NS	-0.16	NS			0.04	NS
SD	0.47	**	0.34	**	-0.20	NS	0.21	*	-0.27	**	0.04	NS		
(b)														
FR	0.46	****	0.16	NS	0.24	*	-0.24	*	0.12	NS	-0.12	NS	-0.32	**
RS	0.59	****	0.29	**	-0.53	****	0.62	****	-0.53	****	-0.28	**	0.49	****
TM	0.11	NS	0.15	NS	0.27	**	-0.30	**	0.65	****	0.69	****	0.10	NS
GBT			0.74	****	0.06	NS	-0.01	NS	-0.01	NS	-0.10	NS	0.22	*
MST	0.74	****			0.28	**	-0.24	*	0.27	**	0.06	NS	0.22	*
MED	0.06	NS	0.28	**			-0.97	****	0.66	****	0.26	*	-0.22	*
FINE	-0.01	NS	-0.24	*	-0.97	****			-0.72	****	-0.35	***	0.27	**
MC	-0.01	NS	0.27	**	0.66	****	-0.72	****			0.64	****	-0.19	NS
PC	-0.10	NS	0.06	NS	0.26	*	-0.35	***	0.64	****			0.03	NS
SD	0.22	*	0.22	*	-0.22	*	0.27	**	-0.19	NS	0.03	NS		

*Significant at P<0.05; ** significant at P<0.01; ***significant at P<0.001; ****significant at P<0.0001; NS=non-significant; FR=feed rate; RS=rotor speed; TM=tempering level; GBT=ground bran temperature; MST=mill surface temperature; MED=medium particle size distribution; FINE=fine particle size distribution; MC=ground bran moisture content; SD=ground bran starch damaged; TS=ground bran total starch.

The fine particle size portion of cleaned ground bran was positively correlated with rotor speed levels (r=0.63) and starch damaged (r=0.21), and negatively correlated with feed rate (r=-0.21), tempering level (r=-0.42), medium particle size portion (r=-0.96) and moisture content (r=-0.70). Non-cleaned ground bran fine particle fraction was correlated (positive and negative) with all eight factors except the ground bran temperature. Multiple studies have investigated on bran particle size effects on digestion, noting that a reduced particles size usually coincides with a decrease in total stool water (Brownlee 2011). Various studies also reported that ultra-fine grinding of wheat bran increases the antioxidant capacity (Rosa et al. 2013; Zhou et al. 2004). Hemery et al. (2010) showed that the reduction in particle size was correlated with an increase in the bioaccessibility of phenolic acids. Investigation on whole wheat dough found that dough containing fine particle size bran exhibited more strength than dough containing coarse bran after an 180 min rest period as measured by the extensigraph (Zhang and Moore 1999). However, the same study also concluded that bran particle size had no significant effect on the farinograph water absorption, and dough containing coarse bran resulted in greater mixing stability.

The correlation between protein content of cleaned ground bran and other factors were not significant. While different situations occur with non-cleaned ground bran protein content; which was rotor speed, tempering, fine particle size portion, and moisture content had a correlation with non-cleaned ground bran protein content. Rotor speed had a positive (ground bran temperature, mill surface temperature, fine particle size portion, and starch damaged) and negative (medium particle size portion, ground bran moisture content) correlation with almost all ground bran characteristics except no association with ground bran protein content (with the cleaned bran treatment). Tempering level gave a strong association with ground bran moisture content and fine and medium particle size portion (for both cleaned and non-cleaned bran).

Regression. Stepwise multiple regression was used to generate regression equations for the prediction of ground bran characteristics from dependent milling variables (Table 22). The regression equations for both cleaned and non-cleaned ground bran temperature, medium particle size portion, fine particle size portion, ground bran moisture content, and ground bran starch damaged were significant (1% level). Only two variables were required for non-cleaned ground bran temperature, both cleaned and non-cleaned ground bran starch damaged. Tempering level was not associated with ground bran temperature and starch damaged. Values of $R2$ ranged from 0.49 to 0.81; in the latter case, 81% of the variability of non-cleaned ground bran could be explained by two milling parameters. Variables associated with rotor speed and feed rate predominate in this regression equation.

Table 22. Regression Coefficients, Intercept, R^2, F, and Probability of F of the Prediction Equations for Ground Bran Characteristics

Parameter		Regression Coefficients	Intercept	R^2	F	Prob>F
Ground bran temperature	NC	0.169 (FR)** +0.00032 (RS)**	19.88	0.81	61.47	<0.0001
	CB	0.111 (FR)** +0.0003 (RS)** +0.1372 (TM)*	21.56	0.76	28.84	<0.0001
Medium particle size portion	NC	0.478 (FR)** -0.0016 (RS)** +1.165 (TM)**	39.56	0.66	17.72	<0.0001
	CB	0.4701(FR)* -0.0014(RS)** +1.893 (TM)**	32.31	0.65	17.22	<0.0001
Fine particle size portion	NC	-0.518 (FR)* +0.00198 (RS)** -1.415 (TM)**	56.618	0.75	27.94	<0.0001
	CB	-0.633(FR)** +0.002(RS)** -1.956 (TM)**	65.32	0.69	21.18	<0.0001
Ground bran moisture content	NC	0.0168 (FR)* -0.0001 (RS)** +0.2099 (TM)**	5.64	0.72	78.39	<0.0001
	CB	0.028 (FR)** -0.0001 (RS)** +0.168 (TM)**	5.72	0.77	103.5	<0.0001
Ground bran starch damaged	NC	-0.029 (FR)** +0.000067 (RS)**	2.72	0.78	52.81	<0.0001
	CB	0.0253 (FR)** +0.00005 (RS)**	0.39	0.49	13.74	<0.0001

*Significant at P<0.05; ** significant at P<0.01; NC=non-cleaned bran; CB=cleaned bran; FR=feed rate; RS=rotor speed; TM=tempering level.

Conclusion

Flour removing process from bran (collected from roller milling facilities) may be useful to enhance the flour extraction rate from milling (FBCP recovery was 10%). The FBCP contains high protein levels and may contribute to the nutritional quality of the final products. The feed rate affects ground bran temperature the most compared to other ground bran characteristics. The rotor speed gave the most influence on the ground bran characteristics. The higher the rotor speed used, the higher the ground bran temperature, mill surface temperature, and greater fine particle size portion. The tempering level impacted the coarse particle size and ground moisture content. The higher the tempering level used, resulted in coarser particle size and high moisture content of ground bran. Fifty-two to fifty-nine percent of fine particle size portions for ground bran milled with ultracentrifugal mill was obtained with these milling parameters: 6 g/min; 12,000 to 15,000 rpm rotor speed level; 10-12% tempering level. Whether it was cleaned or non-cleaned treated bran, the yields of fine particle size portion were fall in those ranges. However, the non-removing flour treatment may impact on final product temperature, protein content, and total starch. Further study may be needed on how the differences of bran particle size (with and without flour removal) act in dough rheology and bread baking system.

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PAPER 3. IMPACT OF BRAN COMPONENTS ON THE QUALITY OF WHOLE WHEAT BREAD

Abstract

Consumption of whole-wheat based products is encouraged due to its important nutritional elements that beneficial to human health. However, processing of whole-wheat based products, such as whole-wheat bread, results in poor end-product quality (i.e. low loaf volume and dense crumb texture). Bran was postulated as the major problem. Four major bran components including lipids (oil), extractable phenolics (EP), hydrolysable phenolics (HP), and fiber (FB) were evaluated for their specific functionality in flour, dough and bread baking. The experiment was done by reconstitution approach using the 2^4 factorial experimental layout. Among all four main factors, FB was identified to have highly significant ($P<0.05$) and negative influence on wet gluten, gluten index, farinograph stability, oven spring, and loaf volume while increasing water absorption. HP was another main factor that impacted negatively ($P<0.05$) on bread loaf volume. Consequently, reconstituted breads prepared without FB or HP had higher loaf volume than white bread. HP was also found to have positive effect on farinograph stability. Especially when HP was reconstituted with oil, farinograph stability was significantly ($P<0.05$) higher than other samples. The protein solubility was also investigated for bread crumb flour sample. The residual protein fraction in bread crumb that was not solubilized by sonication in SDS buffer had significant and positive correlations with farinograph stability and loaf volume. The solubility of polymeric proteins in crumb samples increased significantly for the sample reconstituted with FB and HP. In general, oil, EP, HP, and FB in bran appeared to have complex influence on whole-wheat flour and bread-making characteristics showing significant ($P<0.05$) interaction of the four main factors for dough and baking characteristics.

Introduction

The wheat kernel consists of three main parts, embryo or germ, the outer seed coats, and endosperm. Each anatomically and chemically differentiated from the others. During milling, much care has been taken to separate the endosperm from germ and seed coats, or better known as bran. Bran and germ consists of important nutritional elements including dietary fiber, starch, fat, antioxidant nutrients, minerals, vitamin, lignans, and phenolic compounds, which are beneficial to human health (Chinma et al. 2015; Slavin 2004). Therefore, the consumption of whole grain or whole-wheat is encouraged. Although bran and whole-wheat products offer important nutritional elements to human health, bran tends to negatively impact the dough viscoelastic properties (Zhang and Moore 1999), and bread characteristics such as low loaf volume and dense crumb structure (De Kock et al. 1999; Gan et al. 1992), grainy, nutty and bitter flavors (Chang and Chambers 1992) and poor end product quality in general (Zhang and Moore 1997; Zhang and Moore 1999).

Few researches have been published that finds the major factor that causes the detrimental effect on whole wheat bread quality. Different levels and particle sizes of wheat bran has been investigated by Noort et al. (2010) on pan bread. Fine bran particle size enhanced the adverse effects of pan bread loaf volume while Khalid et al. (2015) reported conflicting results. Possible explanation would be the bran layer used in the respective experiments. Noort et al (2010) used sieving techniques to obtain correspondent particle size of bran layer while Khalid et al. (2015) ground the whole kernel to obtain whole wheat flour. Different bran layers contain different physical and chemical characteristics (Hemery et al. 2010). In research conducted by Zhang and Moore (1999), panelists preferred the color of the samples containing coarse bran, while, they admitted that the samples containing fine bran were of a more uniform color.

Phenolic compounds were found abundantly in the aleurone layer (Brouns et al. 2012). Free phenolic acids, especially ferulic acid, has been postulated as the major component that alters gluten matrix during bread making (Han and Koh 2011b). Also, the free ferulic acid interacts with gluten fraction during mixing to cause dough breakdown (Jackson and Hoseney 1986b). Sidhu et al. (1980b) obtained evidence for the formation of a covalently-linked adduct between cysteine and fumaric acid during mixing. These phenomena reduce the functionality of gluten and ultimately results in low loaf volume of bread. However, the experiment by Sidhu et al. (1980b) and Han and Koh (2011b) were conducted using free phenolic acids supplemented from outsourced.

Lipids has have an important role in bread making, in particular in the areas of gas cell stabilization (Sroan and MacRitchie 2009) and the emulsifier properties (Selmair and Koehler 2010). In the germ and aleurone tissue, the non-polar lipids are predominant, and consist of FFA, MAG, and DAG (Chung et al. 2009). A study by McCann et al. (2009) proposed galactolipids interact with glutenin via hydrophobic and hydrogen interaction, while phospholipids interact with the gliadin or lipid binding proteins of gluten. Pareyt (2011) concluded that "the binding of free lipids with gluten proteins may provide them with the ability to align at the interface of gas cells during the initial phases of dough mixing and increase gas cell stability throughout the bread making process".

Despite excellent findings cited above, there is still a lack of knowledge about the impact of bran components on flour, dough and bread quality in hard red spring wheat. The bran components (lipids, phenolics, fiber) may interact with the protein or starch in flour, dough and bread in ways that will change the quality. It is necessary to determine the effect of the individual

bran components, as well as the effect of the combined components on flour, dough and bread characteristics in hard red spring wheat.

Materials and Methods

Materials and Sample Preparation

Bran and germs and flour sample was purchased from North Dakota Mill (Grand Forks, ND). Bran was passed through the bran finisher to remove any flour adheres to the bran. The size reduction was done using an ultracentrifugal mill (Retsch ZM200, Haan, Germany) configured with a 250µm aperture screen. The milling parameters are described in Paper 2. Ground bran was sealed in a zip lock plastic bag, stored at -20°C prior to next treatment. Heat stable α-amylase from *Bacillus licheniformis* (Termamyl ® 120, 1186 units/mg protein; 19.8 mg protein/mL; A-3403-1MU) and protease from *Bacillus amyloliquefaciens* (P-1236-50 ML) were purchased from Sigma-Aldrich Inc. (Saint Louis, MO). All other chemicals were of analytical grade.

Extraction of Bran Component

Lipid Extraction. Lipid was extracted from 500 g of ground bran using hexane (2 L). Extraction was done for two hours on an Orbit shaker (Lab-Line instruments Inc. Melrose Park, IL, USA). The material was then filtered through Whatman No 1 filter paper and dried under the hood for two days until no hexane smell was detected. The resulting material was called defatted bran (DFB) and was stored at 4°C until further extraction. The filtrate was evaporated to dryness, weighed, labelled as 'oil' and stored at -10°C until needed.

Extractable Phenolics. Extractable phenolics was using aqueous-organic solvents (Saura-Calixto and Goñi 2006) with some modifications. DFB (50 g) was mixed with 1 L of acidic methanol/water (50:50, v/v; pH 2) and vigorously stirring for 1 h at room temperature (23°C). The solution was then centrifuge at 3,000 Relative Centrifugal Force (RCF) or G-force for 15

min and the supernatant was recovered. One liter of acetone/water (70:30, v/v) is added to the residue, and vigorously stirred for 1 h at room temperature (23°C). The solution was then centrifuge (3,000 RCF for 15 min) and the supernatant is recovered. Methanolic and acetonic extracts were combined and concentrated via solvents evaporation using rotary evaporator (Model: RE400, Yamato Scientific Co., Ltd., Japan) equipped with water bath (Model: BM200, Yamato Scientific Co. Ltd., Japan) and water chillers (Type: 97058, VWR International, PA, USA). The concentration of extracted phenolics then was freeze dried (VIRTIS Co., Inc., Gardiner, NY, USA). The resulting lyophilized material was called extractable phenolics (EP). The residue of these extractions was called extractable phenolics-residue (EP-residue).

Hydrolysable Phenolics. Hydrolyzable phenolics were extracted using acidic hydrolysis (Hartzfeld et al. 2002). EP-residue was mixed with 900 mL of methanol and 100 mL of concentrated sulphuric acid. Samples were then placed in a water bath (Type: 89032, VWR International, PA, USA) with constant shaking at 85°C for 20 h. The hydrolysis solution was then centrifuged (3,000 RFC for 10 min) and supernatants recovered. After two washings with 450 mL of methanol and 50 mL of sulphuric acid, the samples were then centrifuged (3,000 RCF for 10 min) and supernatants recovered. The combined supernatant was then diluted with 10-fold of deionized distilled water and the pH was adjusted to pH 4.0 using 15M NaOH for phenolics precipitation. The mixture was then centrifuged (3,000 RFC, 10 min) and the phenolics precipitate was freeze dried (VIRTIS Co., Inc., Gardiner, NY, USA). The resulting lyophilized material was called hydrolysable phenolics (HP).

Fiber. Destarched and deproteinized of DFB was carried out according to Mendis (Mendis 2015) with some modifications. DFB (250 g) was mixed with deionized water (2 L) and pH was adjusted to 7.0 using 1M NaOH. The solution was then boiled for 20 min to inactivate

the endogenous enzymes. Then 250 µL of heat stable α-amylase from *Bacillus licheniformis* was added. Starch was hydrolyzed at 90-95 °C for 2 h, and then cooled in an ice bath to 50 °C. The pH was adjusted to 6.0 using 1 M HCl, and 10 mL of protease was added and protein was hydrolyzed at 50 °C for 4 h with shaking (200 strokes/min) in a water bath (Type: 89032, VWR International, PA, USA). Next, the enzymes were inactivated by boiling the mixture for 30 min and were cooled in an ice bath to room temperature and pH was adjusted to 7.0. The slurry was separated out from the glucose by dialysis for 5 days (dialysis bag cut off 12,000 – 14,000 Da) against triple distilled water, and lyophilized to obtain high-fiber bran (FB).

Proximate Analyses of Ground Bran and Extracted Samples

Ground bran was analyzed for moisture content (AACCI Approved Method 44-19.01), protein content (AACCI Approved Method 46-30.01) with a LECO FP 528 nitrogen/protein analyzer (LECO, MI, U.S.A.), ash content (AACCI Approved Method 08-01.01), crude fat content by ether extraction [AOAC Official Method 920.39 (A)], fatty acid and mineral content [AOAC Official Method 985.01(A, B, D)]. The fatty acid profile was according to AOAC official methods 996.06 (Analysis of methyl esters by Capillary GLC), Ce 2-66 (Preparation of Methyl Esters of Fatty Acids), 965.49 (Preparation of Methyl Esters of Fatty Acids) and 969.33 (oils and Fat, Boron Trifluoride method) (AOAC International, 2006). The soluble, insoluble and total dietary fiber were analyzed according to AACCI Approved Method 32-07.01 with procedures modified for the ANKOM^{TDF} Dietary Fiber Analyzer (Ankom Technology Corp., NY, USA).

All extracted components were characterized by selected proximate analysis as above. The phenolic compound contents were determined by the Folin-Ciocalteau procedure, using a

ferulic and gallic acid as standards (Singleton et al. 1999). The results were expressed as ferulic and gallic acid equivalents (FAE and GAE, respectively).

Flour, Dough and Baking Test

All four bran's components (oil, EP, HP and FB) were added into the refined flour as original amount as whole-wheat flour blending (26% bran: 74% refined flour). Refined flour and whole-wheat flour (26% bran: 74% refined flour) were analyzed simultaneously as control. Dough rheology properties were determined using computerized Farinograph® according to AACCI Approved Method 54-21.02 (C.W. Brabender Instruments Inc., NJ, USA) with a 10 g mixing bowl. The wet gluten content and gluten index were determined with a Glutomatic 2200 S system (Perten Instruments, Springfield, IL, U.S.A.) according to AACCI Approved Method 38-12.02. Gassing power of each reconstitution and control dough were measured according to AACCI Approved Method 89-01.01 with procedures modified for the ANKOM^{RF} Gas Production System (Ankom Technology Corp., NY, USA). Dough was prepared according to AACCI Approved Method 10-09.01 (will be described in the following paragraph). Rounded dough (50 g) was placed in a 500 mL plastic coated glass bottle and allowed to ferment for 90 min at 30°C. Pressure (psi) during the entire 90 min fermentation was recorded with 1 min interval.

Samples (reconstituted and control flour) were baked according to AACCI Approved Method 10-09.01 with the following modifications: fungal α-amylase (15 SKB) instead of malt dry powder, instant yeast (1.0%) instead of compressed yeast, and the addition of 10 ppm of ammonium phosphate. After baking, bread loaf volume was measured according to AACCI Approved Method 10-05.01. Subjective analysis of final loaf score was evaluated by the Guidelines for Scoring Experimental Bread (AACCI Approved Method 10-12.01) using a

constant illumination source. The score ranged from 1 to 10, with the higher scores preferred. The results were evaluated to determine the relationship between the extracted bran components dough and flour and baking quality.

Protein Extraction and Size-Exclusion High Performance Liquid Chromatography (SE-HPLC)

Bread crumbs were air dried for 48 h at room temperature (temperature range 18-20°C, RH range 15-18%) prior to grind. Dried bread crumbs proteins were extracted as described by Gupta et al. (Gupta et al. 1993) with minor modifications (Ohm et al. 2009). Bread crumbs powder (10 mg) was suspended in 1 mL of 0.5% SDS and 0.05 M sodium phosphate buffer (pH 6.9) and stirred for 5 min at 2,000 rpm using pulsing vortex mixer (Fisher Scientific). No defatting was done for dried bread crumbs flour. The supernatant was separated after centrifuging the mixture for 15 min at 17,000 g (Eppendorf Centrifuge 5424). The residue was sonicated in the 1 mL of extraction buffer for 30 sec at 10 W output to solubilize SDS unextractable proteins using a Sonic Dismembrator 100 (Fisher Scientific) (Gupta et al.,1993; Ohm et al., 2009) and sonicated mixture was also centrifuged as described for the extractable fraction. The supernatants form extractable and sonication extractable fractions were individually filtered by a membrane (0.45 mm PVDF, Sun Sri, Rockwood, TN) and heated in a water bath at 80°C for 2 min (Larroque et al. 2000) to remove any enzyme activity. Protein SE-HPLC was performed on a narrow-bore size exclusion column (BioSep SEC S4000, 300 x 4.5 mm, Phenomenex, Torrance, CA) with a guard cartridge (BioSep SEC S4000) using an Agilent 1100 Series chromatograph (Agilent Technologies, Santa Clara, CA) (Batey et al. 1991; Ohm et al. 2009). The SE-HPLC settings were as follows: injection volume,10 μL; eluting solution, 50 %

acetonitrile in aqueous 0.1 % trifluroacetic acid solution; flow rate of 0.5 mL/min; and detection, UV 214 nm absorbance (Photodiode array detector, 1200, Agilent Technologies).

MATLAB (2015, The MathWorks) functions were used to process SE-HPLC absorbance data (Ohm et al. 2009). UV absorbance values were interpolated at retention time interval of 0.002 min, and absorbance area and area percentage were calculated at 0.01 min interval using the interpolated data. Chromatogram was separated into five main fractions: F1 (3.5–5.8 min), F2 (5.8-6.9 min), F3 (6.9–7.3 min), F4 (7.3-8.0 min), and F5 (8.0-9.9 min) for both extractable and sonication extractable fractions (EXF and SEF, respectively). Primary components are known to be polymeric protein for F1; gliadins for F2; albumin and globulin for F3 (Baasandorj et al. 2015; Larroque et al. 1997; Ohm et al. 2009). The F4 and F5 that were not shown prominently for flour samples seem to be low molecular weight protein/peptide released during bread-making process (de la Pena et al. 2015). Negative absorbance values that appeared around retention time of 8 min for some samples were not included in absorbance area calculation. The residual protein content was obtained by subtracting quantity of non-residual protein obtained by vortex and sonication from total protein in crumb. Non-residual protein quantity was determined by converting total absorbance area of SE-HPLC to protein quantity using a calibration equation, which showed coefficient of determination of 0.901 (n=18).

Statistical Analysis

Statistical analysis was performed with SAS statistical software (version 9.3, SAS Institute, Cary, NC, U.S.A.). An analysis of variance (ANOVA) was performed to assess the effect of the four extracted components, lipid, extractable phenolics, hydrolysable phenolics, and fiber, on bread-making quality characteristics. The experimental design for the quality characteristics impacted by reconstitution of the bran components was a completely randomized

design (CRD) with the 2^4 factorial experimental layout and three replications. Actual independent variables were coded as 0 and +1, where 0 represented as the independent variable was not present in the system, and +1 represented as the independent variables was present in the system. The 'Mixed' procedure in SAS was used for ANOVA and 'LSMEAN' function was used to estimate least square (LS) mean and least significance difference (LSD) values. LSD with a 5% significance level was used to declare differences between treatments. The factorial model derived from the coded equation was employed to visualize and identify the trend that individual bran components impacted the quality traits using the Design Expert program (9.0 Stat-Ease, Inc. Minneapolis MN). The simple linear correlation coefficients were estimated between quality parameters and individual SE-HPLC absorbance area values at 0.01 min retention time interval using MATLAB (2015, The MathWorks) and shown as continuous spectrum over retention time (Ohm et al. 2009).

Results and Discussion

Bran Characteristics

The bran compositions used in this experiment were given in Table 23. Dietary fiber and bioactive compounds such as phenolic acids are concentrated in the bran fraction of cereals. The main part of dietary fiber in bran is insoluble, which influences the digestibility and bioavailability of nutrients and phytochemicals (Kamal-Eldin et al. 2009; Liukkonen et al. 2003). Generally, wheat bran comprises approximately 7.7% moisture, 17% protein and 5% fat. In contrast, Apprich (2014) reported that wheat bran contains 3.5% fat. The difference might be due to difference in the method used for fat extraction. We found that five percent of fat was extracted with soxhlet apparatus, while 3% of fat was extracted by orbital shaker (data not shown).

Bran and germ are abundant in phenolic acids (a type of antioxidant) but they are removed during milling (Adom et al. 2005; Zhou et al. 2004). Ferulic acid is one of the most common phenolic compound found in whole grains (Liu 2007), especially in the aleurone layer of grain (Antoine et al. 2004a; Brouns et al. 2012). Total phenolic contents (TPCs) of wheat bran in our study showed approximately at 31.9 mg of ferulic acid equivalents per gram (FAE/g) of bran (Table 23), with bound polyphenols 6.8-fold higher than free polyphenols. The oil quality of wheat bran showed that palmitic, oleic, and linoleic acids were the major fatty acids (Table 23).

Table 24 shows the composition of extracted bran component. No major difference was found between fatty acids profile of extracted bran's oil (Table 24) and bran sample (Table 23), indicating that the extracted oil was in good condition for further experiment. Lyophilized EP and HP showed high ferulic acid concentration with 24 and 70 mg FAE/g respectively (Table 24), which is 7- and 2.5-fold higher than the raw material (Table 23). The aleurone layer is rich in phenolic compounds. The most abundant compound belongs to the chemical class of hydroxycinnamic acids. The major component in hydroxycinnamic acids class is ferulic acid (FA) followed by diferulic acids, sinapic acid and p-coumaric acid (Brouns et al. 2012). FB shows high in total dietary fiber (78%) with 72% was insoluble dietary fiber. Lyophilized extracted bran components (oil, EP, HP, FB) were shown in Figure 19.

Table 23. Bran composition

Bran composition	Amount
Proximate Composition	(%)
Moisture	7.7
Crude Protein (N=5.27)	16.9
Ash	5.4
Crude Fat	5.3
Beta glucan content	1.7
Dietary Fiber:	
Total Dietary Fiber	54.0
Soluble Dietary Fiber	7.4
Insoluble Dietary Fiber	46.6
Sugar Composition:	(%)
Mannose	0.6
Galactose	0.9
Glucose	12.5
Arabinoxylan	18.1
Ratio A/X	0.7
	(mg FAE/g, db)
Total Phenolic	31.9
Extractable Phenolic	4.0
Hydrolysable Phenolic	27.9
Minerals:	(%)
Calcium	0.1
Phosphorus	1.3
Magnesium	0.6
Potassium	1.2
Zinc	0.0
Sulfur	0.2
Fatty Acid Profile	(%)
Palmitic (16:0)	0.8
Stearic (18:0)	0.1
Oleic (9c-18:1)	0.9
Linoleic (18:2n6)	3.0
Linolenic (18:3n3)	0.2

A/X = Arabinose and Xylose ratio; FAE = Ferulic Acid Equivalent.

Table 24. Composition of lyophilized extracted bran component

Component / composition	Amount
Oil:	(%)
Crude fat	100.0
Palmitic (16:0)	15.8
Stearic (18:0)	1.1
Oleic (9c-18:1)	17.7
Linoleic (18:2n6)	57.9
Linolenic (18:3n3)	4.3
Fiber:	(%)
Moisture	3.5
Crude Protein	13.0
Total Dietary Fiber	77.8
Soluble Dietary Fiber	5.4
Insoluble Dietary Fiber	72.4
Lyophilized Phenolic	(mg FAE/g)
Extractable Phenolic	24.2
Hydrolysable Phenolic	70.5

FAE=Ferulic Acid Equivalent.

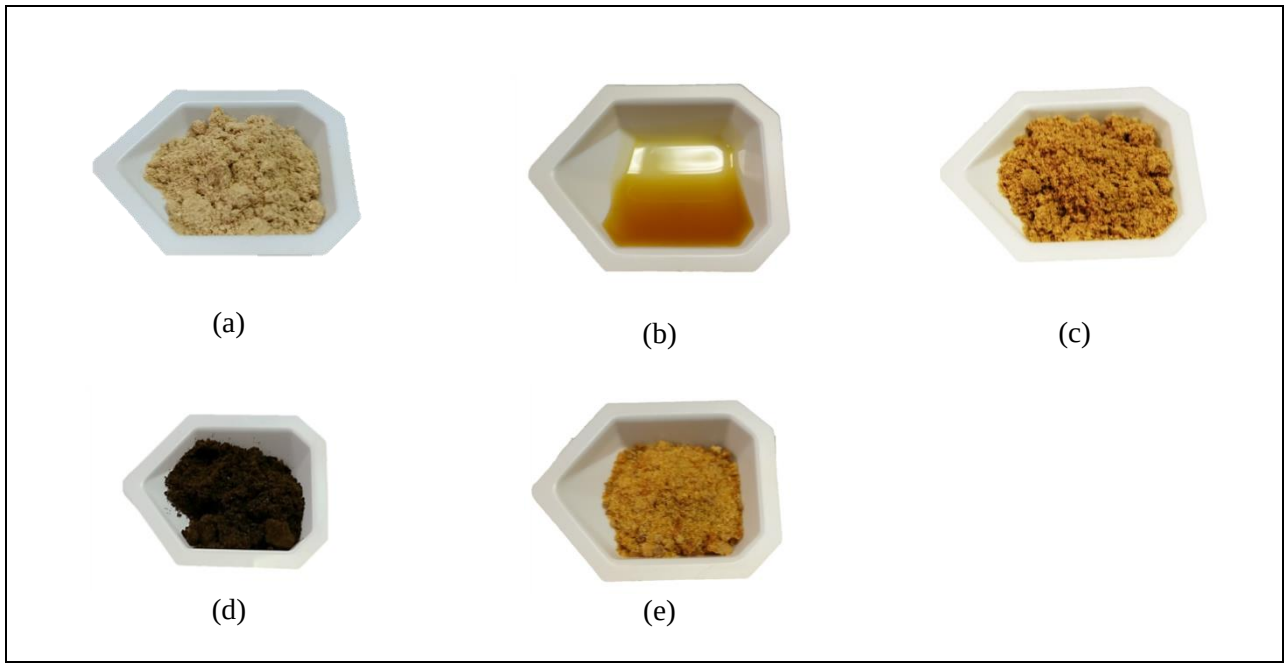


Figure 19. Ground bran and extracted bran component: (a) ground bran; (b) oil; (c) fiber; (d) lyophilized hydrolysable phenolics; and (e) lyophilized extractable phenolics.

Impact of Bran Components on Farinograph Parameters

Dough quality and baking quality parameters of control flour (commercial refined flour and commercial whole wheat flour) were given in Table 25 and Table 26, respectively.

Table 25. Dough rheology quality for refined flour (RF) and whole wheat flour (WWF) used in this experiment.

Sample	Farinograph Parameters				Gluten Index (%)	Gassing Power at 90 min. (psi)
	Water abs. (14% mb)	Dev. Time (min.)	Stability (min.)	Tolerance Index (BU)		
RF	64.0	2.7	8.9	24.3	95.5	6.0
WWF	71.5	5.8	8.2	26.7	81.6	5.6

mb=moisture basis; abs=absorption; Dev.=Development; BU=Brabender Unit.

Table 26. Baking parameters for refined flour (RF) and whole wheat flour (WWF).

Sample	Baking Parameters					
	Absorption (%)	Baked weight (g)	Loaf Volume (cc)	Crumb score (1-10)	Proof height (inch)	Oven spring (inch)
RF	65.0b	32.5b	185.3a	6.3a	6.3a	1.2a
WWF	75.7a	36.3a	134.0b	6.7a	5.7a	0.1b

Means with different letters within same column differ significantly ($p<0.05$).

Water Absorption. Analysis of variance for farinograph parameters were given in Appendix Table C1-C3. Water absorption was greatly affected by four-way interaction of independent variables (oil, EP, HP, FB) at $p<0.05$, demonstrated in Figure 20. Generally, absent of FB in the system caused low water absorption (60%). It was lower than refined flour, which was reported at 64% (Table 25). When FB is absent in the system, the water absorption was recorded between 54.7 – 63.3% (Fig.30a-b). Water absorption reduced 4.7% from 64.0% to 59.3% when HP and FB absent in the system (but EP and oil components were present in the system) (Fig. 20b). Water absorption was reduced as much as 8.6% when HP was present and FB absent in the system (Fig.20a); the water absorption ranged between 63.3% - 54.7%. When comparing

both Fig.20a and Fig.20b, the water absorption is reduced when the dough system has both EP and oil component (absent of FB).

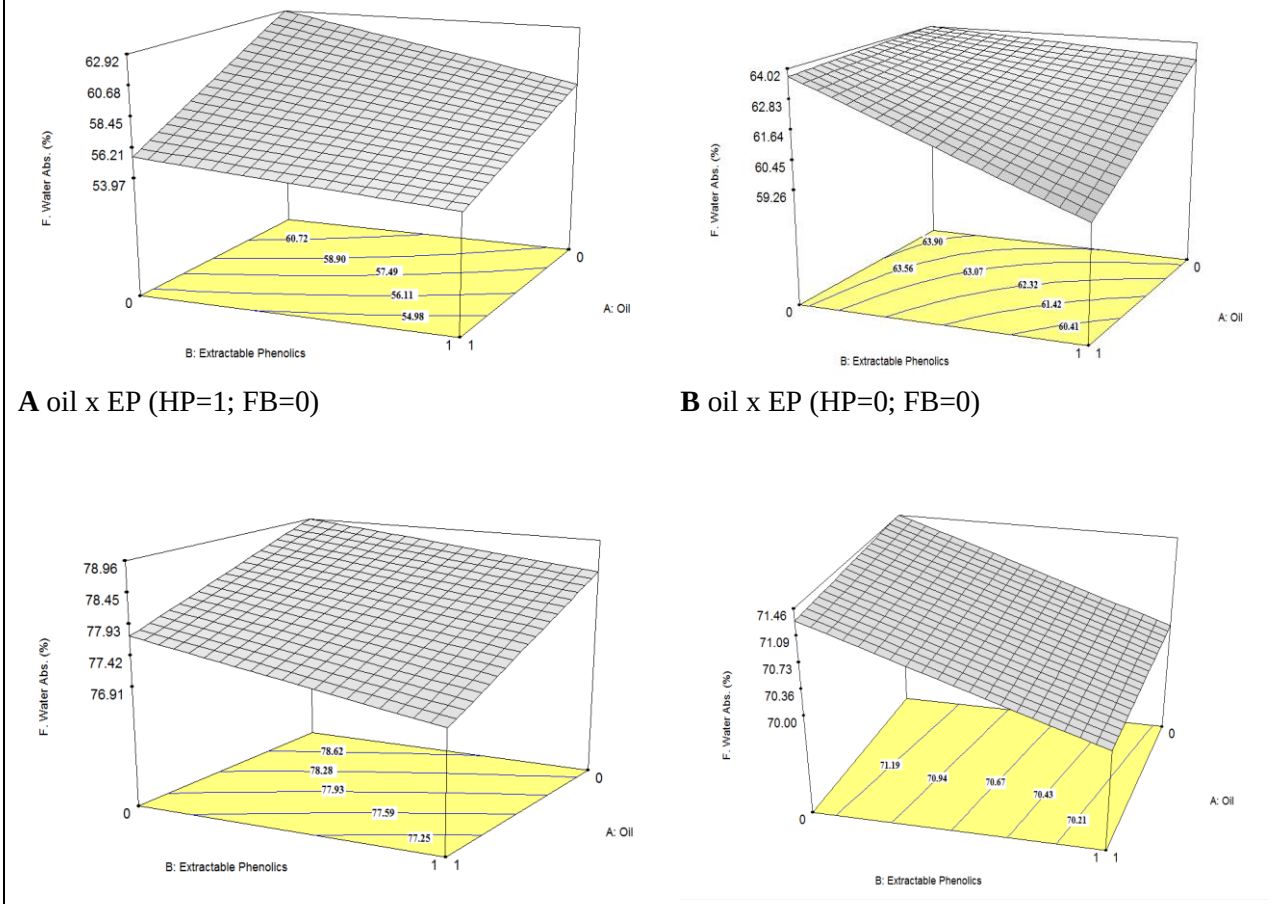
However, high water absorption (70 to 79%) was recorded when FB presents in the system (Fig.20c-d). The highest absorption was 79% with only FB in the system (Fig.20c), while the lowest water absorption was 70% with all four components were present in the system (Fig.20d). When comparing both two figures (Fig.20c and Fig.20d), the trend is as follow: "when FB present in the system, absent of HP associated with high water absorption (ranged from 77.0 to 79.1%) compared to when HP is present in the system (ranged from 70.0 to 71.5%). Present of HP (with FB) in the system, makes the EP component shows positive association with water absorption compare to oil component (Fig.20d)".

The high water absorption of wheat bran is explained by greater number of hydroxyl groups in the fiber structure, which allow more water interaction through hydrogen bonding (Anil 2012; Rosell et al. 2001). The observed effect agrees with Rosell et al. (2010), where the water absorption increased when different commercial dietary fibers were added into the wheat dough. Several studies (Biliaderis et al. 1995; Michniewicz et al. 1991; Vanhamel et al. 1993) showed significant increases in the farinograph water absorption when purified arabinoxylans were included. Rosell et al. (2010) explained that fiber might compete for water with dough main polymers, gluten and starch. The water soluble and insoluble portions of arabinoxylan have very high in water holding capacity (Izydorczyk and Biliaderis 1995; Jelaca and Hlynka 1972).

There is only about 1.0-1.5% of total arabinoxylan in refined bread flour (Izydorczyk and Biliaderis 1992; Ragaee et al. 2011) and 18% of arabinoxylan found in bran samples used in this study (Table 30). Arabinoxylan has been reported to affect flour and dough properties such as water absorption, viscosity, and gelling properties (Meuser and Suckow 1986). Phenolic

compounds such as ferulic acid can be bound with arabinoxylan via ester bond. Izydorczyk and Biliaderis (1995) showed an evidence where cross-linked arabinoxylans could hold up to 100 g of water per gram of polymer. On the other hand, phenolic compounds, especially ferulic acids are partly responsible for the insolubility of cell wall structures of cereal kernels, because ferulic acid can form cross-links between arabinoxylan polysaccharides and lignin (Faulds and Williamson 1999). The acidic condition during fermentation was favorable condition to release ferulic acids (Katina 2012).

Dough Development and Stability. Dough development and stability values are indicators of flour strength, with higher values indicating stronger doughs. Four-way interaction was significant at $p<0.001$ for dough stability (Appendix Table C2), as demonstrated in Figure 21a-d. Dough stability pattern was clearly affected by oil and EP component, when both HP and FB were absent in the system (Fig.21a), showing greater stability value especially when only oil was present (Fig.21c-d). FB decreased dough stability the most with values ranging between 2.8 to 4.4 min (Fig.21d). The wheat bran and aleurone layer have very high levels of arabinoxylan (70%) and structure of arabinoxylan in dough impacts its functionality. The decline in mixing stability might be due to the dilution effect caused by the presence of higher amount of fiber in the system, which may reduce the formation of the intermolecular disulfide bridges that is responsible for longer stability of dough during mixing (Autio et al. 2001).



A oil x EP (HP=1; FB=0)

B oil x EP (HP=0; FB=0)

C oil x EP (HP=0; FB=1)

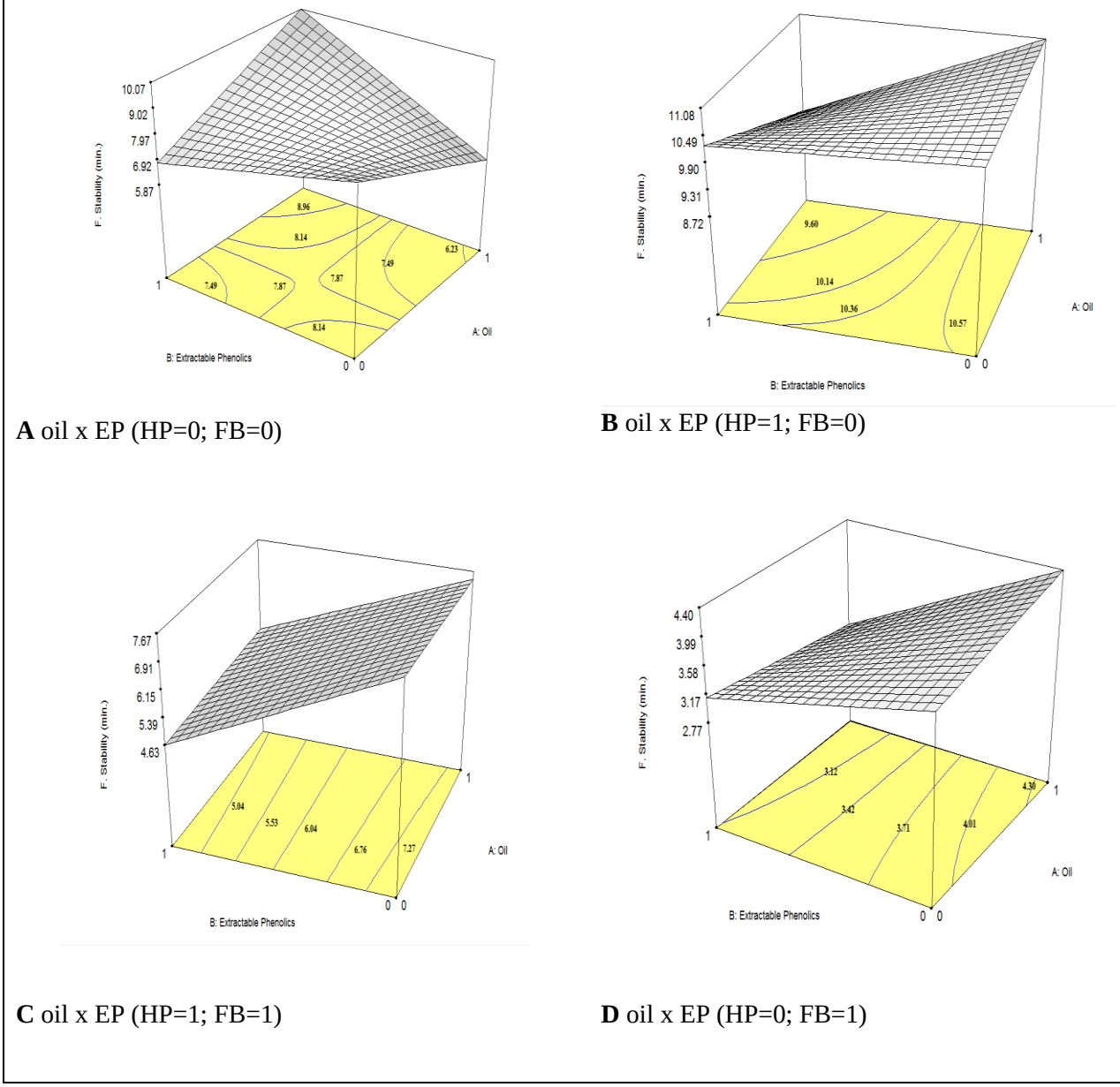
D oil x EP (HP=1; FB=1)

Figure 20. Factorial model plot for farinograph water absorption showing the effects of oil by EP interaction at different levels of HP and FB in the system.
EP=Extractable Phenolics; HP=Hydrolysable Phenolics; FB=Fiber; 0=not present in the system; and +1=present in the system.

Two-way interaction of FB and EP was significant ($p<0.0001$) for dough development time (Appendix Table C1). The difference between absent and present of FB was greater for dough without EP than dough with EP (Fig.22). EP component minimize the development time needed in dough system when FB was present. Non-starch polysaccharides (NSPs) have a high water-binding capacity (Hamed et al. 2014; Rieder et al. 2012). NSPs would compete with other dough components especially gluten for available moisture and affect water distribution in the dough system (Hamed et al. 2014). The NSPs may reduce the amount of free water in dough and therefore increase the amount of water required to reach a fully developed gluten network in the dough (Hamed et al. 2014; Rieder et al. 2012).

Impact of Bran Components on Gluten Index and Gassing Power

Gluten Index. All four independent variables interactions were significant (Appendix Table C3) at $p<0.0001$ for gluten index and is shown in Figure 23a-d. Gluten index (GI), which is indicative of gluten quality, shows declining pattern when FB present in the dough system. The lowest gluten index was recorded at 75.5% when the dough system has three extracted bran component (FB, EP and oil) (Fig.23d). Obviously, when all four components (oil, EP, HP, and FB) were not present in the dough system, the gluten index raised to 94% (Fig.23a). This observation is in agreement with many previous studies (Gularte et al. 2012; Jelaca and Hlynka 1972; Wang et al. 2002b; Wang et al. 2003; Wang et al. 2005).



A oil x EP (HP=0; FB=0)

B oil x EP (HP=1; FB=0)

C oil x EP (HP=1; FB=1)

D oil x EP (HP=0; FB=1)

Figure 21. Factorial model plot for dough stability showing the effects of oil by EP interaction at different levels of HP and FB in the system.
EP=Extractable Phenolics; HP=Hydrolysable Phenolics; FB=Fiber; 0=not present in the system; and +1=present in the system.

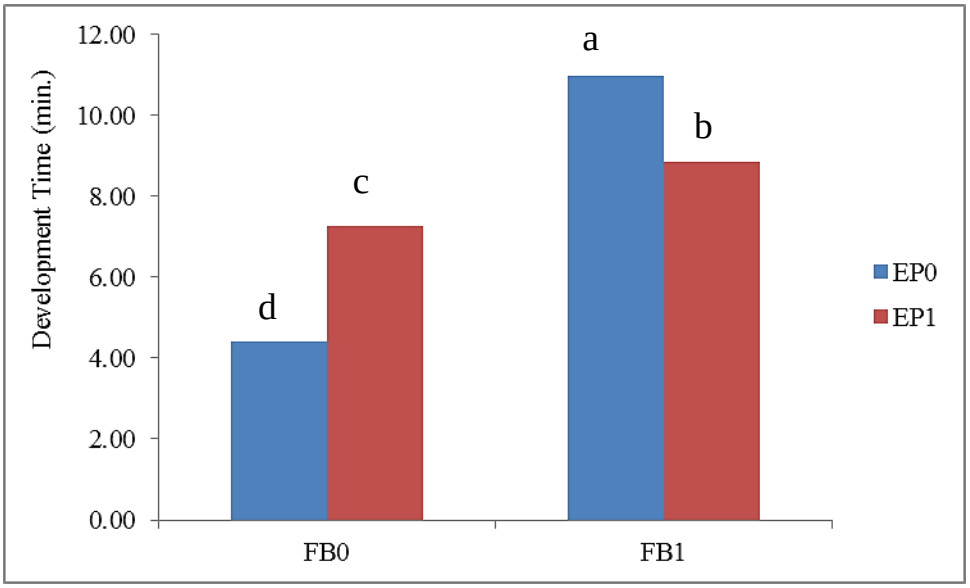


Figure 22. Development time (min.) in dough system as affected by Fiber (FB) and Extractable Phenolics (EP) component.
Means with the same letters are not significantly different ($p<0.05$).

With regards to Fig.23a, EP component had greater negative influence on the gluten index (from 95 to 83%) compared to oil component (95 to 92%). When HP was introduced in the dough system (absent of FB), gluten index was declined to 81%. Higher negative impact on gluten index was shown when HP was introduced with EP components (from 93 to 81%) than when introduced with oil component (93 to 87%) (Fig. 23b). Large variation in gluten index (ranged between 76 to 93%) was seen when FB was present in the dough system (Fig.23c-d). The gluten index ranged between 81 to 86% when FB and HP component present in the dough system (Fig.23c), where oil component gave higher negative impact than EP component. However, when HP component was removed in the dough system (present of FB), there was a greater difference on gluten index between"oil0" and "oil+1" at present of EP in the dough system (Fig.23d).

It has been reported that during high-speed mixing the disulfide bonds will weaken and creates thiol free radicals among gluten polymers (MacRitchie et al. 1991). The free radicals react with reducing compounds in flour, and eventually will inhibit disulfide crosslinking (Dahle and Murthy 1970). In an effort to prove this theory, many articles were published showing that ferulic acid, fumaric acid and free radical scavengers accelerate the breakdown of dough during mixing (Jackson and Hoseney 1986a; b; Koh and Ng 2008; Labat et al. 2000a; Labat et al. 2000b; Okada et al. 1987). However, Wang et al. (2003) suggested that free ferulic acid (addition of ferulic acid in dough) may be useful to overcome the negative effects of water unextractable arabinoxylan (WU-AX) on gluten yield. According to Wang et al. (2003), "free FA can either interfere with the important disulfide interchange reaction of gluten or prevent arabinoxylans from cross-linking through ferulic acid".

Three-way interaction of oil-EP-FB was significant at $p<0.0001$ for gluten index. Table 27 shows the value of gluten index as affected by three-way interaction. The highest gluten index (94%) was recorded with dough system not-containing oil, EP and FB components. While the lowest gluten index (78%) was recorded with dough system containing these entire three components, oil, EP and FB. HP component alone did not give significant negative impact ($p>0.05$) on gluten index (93.1%) when compared to gluten index of refined flour (95.5%) (data not shown).

Wet Gluten. Three-way interaction of oil-EP-FB was significant at $p<0.0001$ for wet gluten (Appendix Table C4), and the values were shown in Table 27. In general, low wet gluten (25.7 – 28.8%) was observed when FB was present in the dough system. It was noted that combination of oil0xEP0xFB0 exhibited considerably low wet gluten (Table 27) compared to other combinations in the same column. This combination was calculated as an averaged of

present and absent of HP component in the system, which were recorded as 28.7% and 31.2% respectively.

Table 27. Gluten quality on composite flour as affected by oil-EP-FB component.

OIL	EP	Gluten Index (1-100)		Wet Gluten (%)	
		FB0	FB1	FB0	FB1
oil 0	EP 0	94.26a	84.76c	29.95b	28.81bc
	EP 1	82.30d	89.65b	31.20a	25.91d
oil 1	EP 0	88.42b	83.83cd	32.44a	25.71d
	EP 1	85.68c	78.16e	31.54a	28.62c
		LSD-GI (0.05)	2.20	LSD-WG (0.05)	1.25

Means with different letters within same parameter differ significantly ($p<0.05$). EP=extractable phenolics; FB=fiber; GI=gluten index; WG=wet gluten; 0=not present in the system; +1=present in the system.

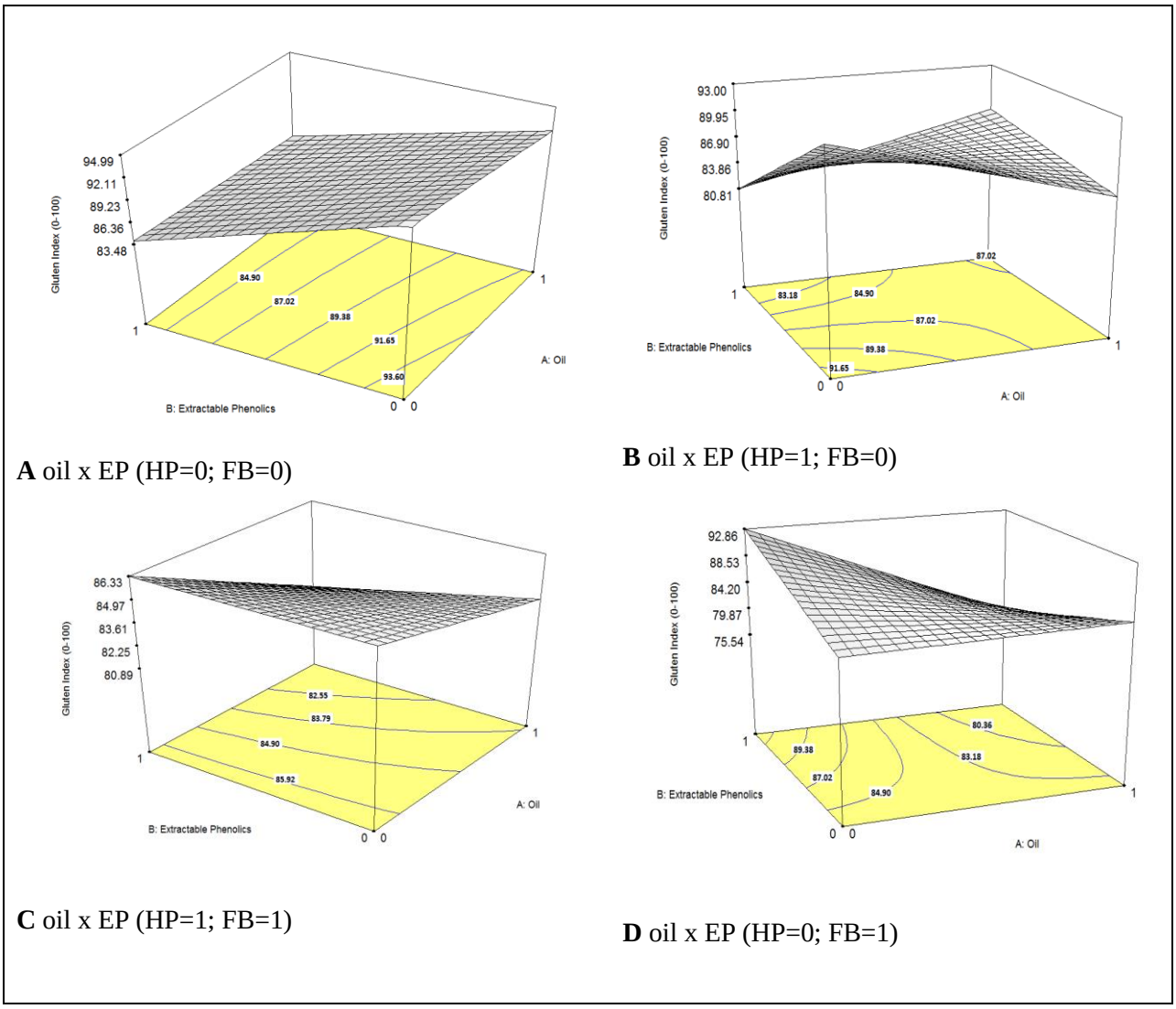
The non-starch polysaccharides of wheat flour comprise mainly of arabinoxylans and water-extractable arabinogalactan-peptides (WE-AGPs) (Loosveld and Delcour 2000). Arabinoxylans have been shown to decrease the amount of water available to gluten (Biliaderis et al. 1995). The lower water content of the gluten phase affects greatly the properties of the dough (Biliaderis et al. 1995). The low molecular weight of WE-AGPs have been postulated to interact with gluten proteins (Loosveld and Delcour 2000). Loosveld and Delcour (2000) studied WE-AGPs on bread-making properties and found that, purified wheat WE-AGPs exhibited a significant decrease in farinograph water absorption, an increase in maximum resistance and a decrease in extensibility. With regards to these effects (water absorption and dough rheological properties), Autio (2006) suggested WE-AGPs interfere with gluten formation by binding more water and thus changing the conditions for gluten formation.

Gassing Power. Gassing power was calculated at 90 min of fermentation. The gassing power is extensively used to investigate yeast strains that have high freeze tolerance in frozen dough (Hosomi et al. 1992; Shima et al. 1999; Van Dijck et al. 1995). Higher number, which associated with high pressure, indicated that more carbon dioxide was produced in the dough

system; thus resembled the high yeast activity during fermentation. In general, high yeast activity was exhibited when there was no HP and EP component in the system (FB and oil were present) (Fig.24a, 24d), showing gassing power range 5.4 – 6.0 psi. However when EP was introduced in the system, the gassing power declined to 4.8 – 5.1 psi (Fig.24a, 24d).

When HP component was introduced in the system, present of FB gave higher gassing power, value ranged between 5.0 – 5.7 psi (Fig.24c). Removal of FB component resulted in lower yeast activity (4.3 – 5.0 psi) (Fig.24b), indicating that FB enhance yeast activity. The decreased gassing power in doughs may have affected the rheological properties of final-proofed doughs, as suggested by Kilborn and Preston (1982). Wheat bran contains significantly higher amounts of calcium, magnesium, potassium, and sulfur (Juliano 2003; Kadan and Phillippy 2007; Khalid and Simsek 2015) (Table 23). These minerals are necessary for yeast health and nutrition (Spencer et al. 1997). In whole-wheat dough system, yeast may be actively propagated and produce more carbon dioxide as it was supplemented with essential nutrient for growth.

In general, FB component resulted in high gassing power, indicating yeast was actively propagated. As explained above, bran contains mineral (Table 23) needed for yeast growth (Spencer et al. 1997), thus yeast may actively producing carbon dioxide during fermentation in whole wheat dough system. Despite being a good mineral source for yeast fermentation in dough system, FB component exhibited a detrimental effect on gluten property (Fig.23). Even though yeast was actively propagated and produced high carbon dioxide, the defected gluten matrix could not trap the gas inside the dough during fermentation.



A oil x EP (HP=0; FB=0)

B oil x EP (HP=1; FB=0)

C oil x EP (HP=1; FB=1)

D oil x EP (HP=0; FB=1)

Figure 23. Factorial model plot for gluten index showing the effects of oil x EP with different levels of HP and FB in the system.
EP=Extractable Phenolics; HP=Hydrolysable Phenolics; FB=Fiber; 0=not present in the system; +1=present in the system.

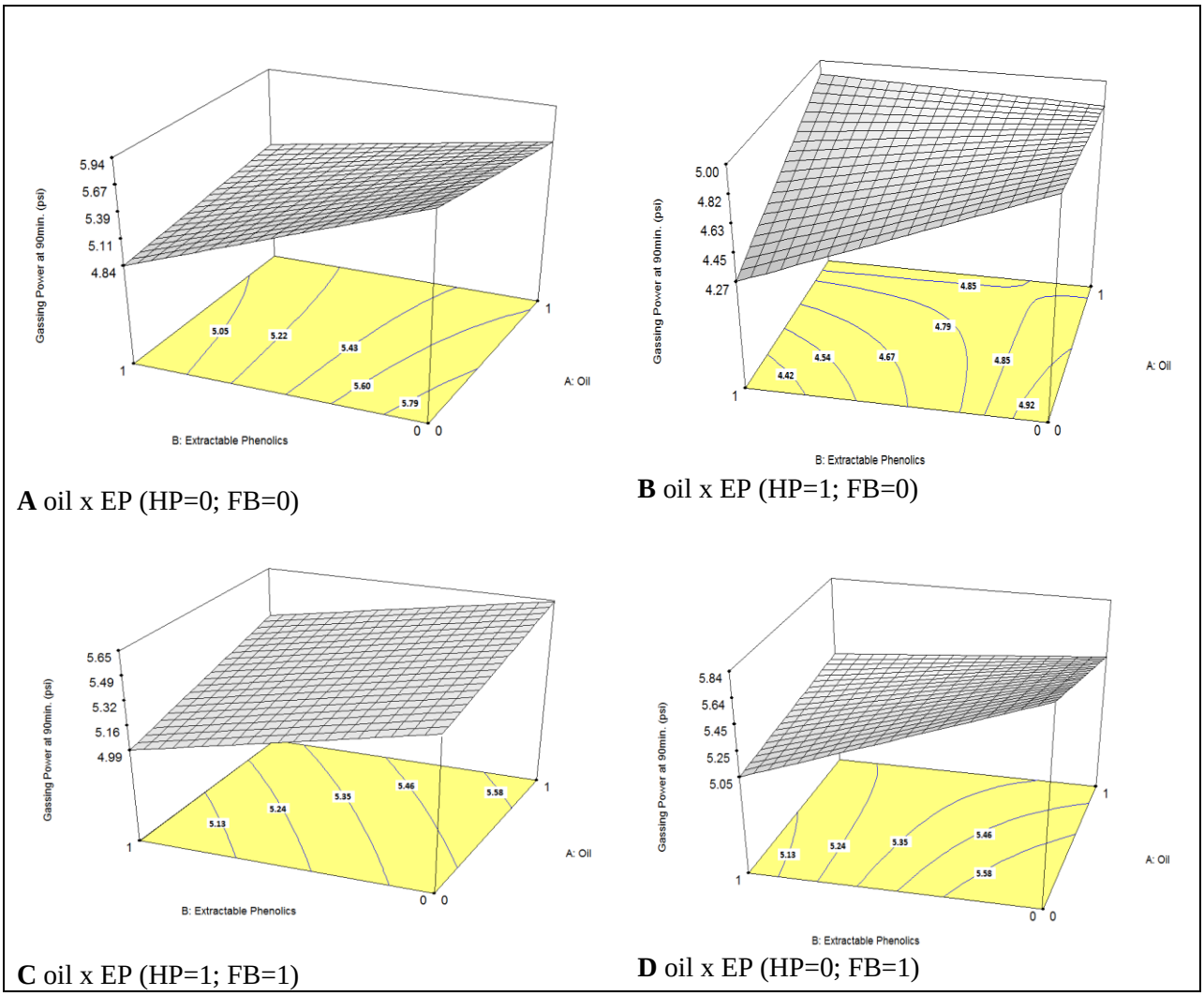
While FB component support the yeast activity through carbon dioxide production, the EP and HP component suppressed their activity. Phenols has been shown to form complexes with proteins (Loomis and Battaile 1966), interact with protein to form haze in beer, wine and fruit juices (Siebert 1999) and developed an off-flavor compound in wine making industry (Shinohara et al. 2000). In bread making, phenols could bind with water-extractable pentosans (Jackson and Hoseney 1986a). Another property of phenols is their antimicrobial and antioxidant

activities, especially ferulic acid (Ou and Kwok 2004). Ferulic acid inhibits the growth of bacteria, fungi and yeasts (Lattanzio et al. 1994). Stead (1995) found that ferulic acid can also appreciably inhibit growth of yeast such as *Pichia anomala, Debaryomyces hansenii* and *Saccharomyces cerevisiae*; however it is less effective than potassium sorbate.

Impact of Bran Components on Baking Qualities

Baking Absorption. Baking absorption was determined from farinograph data but was adjusted based on the feel and appearance of the dough by human expert. This was necessary because the baking formula included other ingredients in addition to flour and water. Analysis of variance for baking absorption was given in Appendix Table C4. Two-way interaction of HP-FB and oil-FB were significant at $p<0.01$ and $p<0.05$ respectively. Their interaction could summarize as, having FB in the system always resulted in high baking absorption regardless with or without HP or oil component (Fig.25). Also, having HP or oil component in the system decreased the baking absorption regardless with or without FB component (Fig.25a-b).

Oil extracted from bran decreased the baking absorption. Non-polar lipids retained in the gluten network through hydrophobic forces, while glycolipids interacts with glutenins through hydrophobic interactions and hydrogen bonding (McCann et al. 2009). Wheat lipids stabilize gas cells in dough system, and thereby affect the volume and crumb grain (MacRitchie and Gras 1973; Sroan and MacRitchie 2009). Collar et al. (1998) studied the lipid binding in dough and found that when lipid-starch complex has been formed, water penetration will be postponed. The lipids coat the continuous network of gluten and starch, therefore make the water absorption became harder (Collar et al. 1998; Krog 1981).



A oil x EP (HP=0; FB=0)

B oil x EP (HP=1; FB=0)

C oil x EP (HP=1; FB=1)

D oil x EP (HP=0; FB=1)

Figure 24. Factorial model plot for gassing power at 90min showing the effects of oil x EP with different levels of HP and FB in the system.
EP=Extractable Phenolics; HP=Hydrolysable Phenolics; FB=Fiber; 0=not present in the system; +1=present in the system.

Oven Spring and Loaf Volume. The expansion of dough in the oven, or so-called oven spring, results from continued yeast action. During heating, carbon dioxide diffuses and vaporization of ethanol and water in the cells expand dough. Oven spring was calculated as difference of dough height before and after baking. Three-way interaction of HP, EP and oil was significant for oven spring at $p<0.05$. Presence of HP in the system resulted in little to no oven spring (Fig.26a). With HP present, no oven spring was recorded especially when EP and oil components were present in the system (Fig.26a). The presence of oil and HP components resulted in a considerable oven spring (0.18 – 0.23 cm) when there was no EP in the system (Fig.26a). When EP was introduced in the system, there was a decline in oven spring regardless oil component was absent or present in the system (Fig.26a).

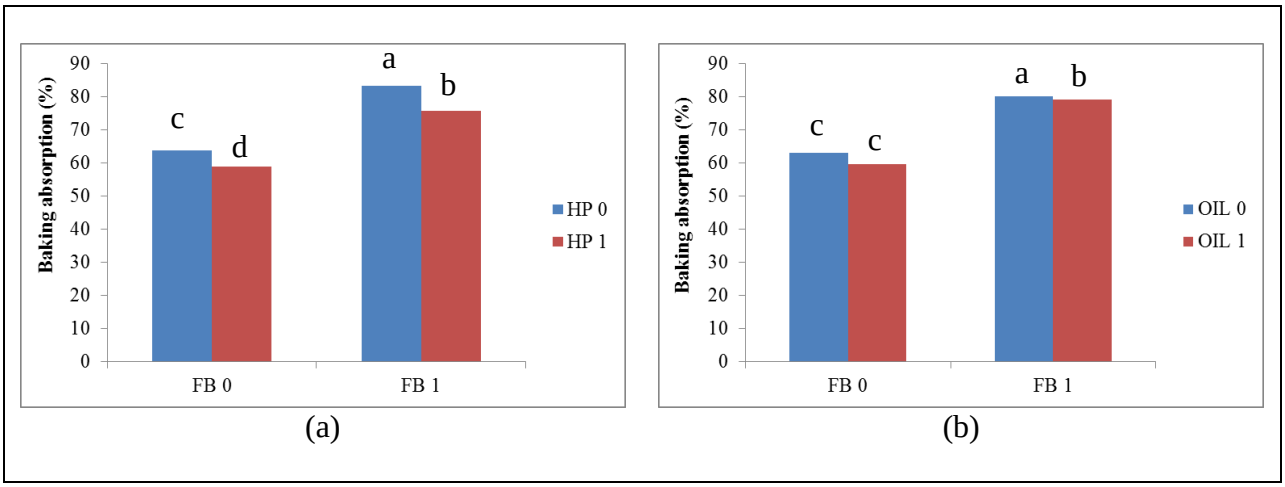
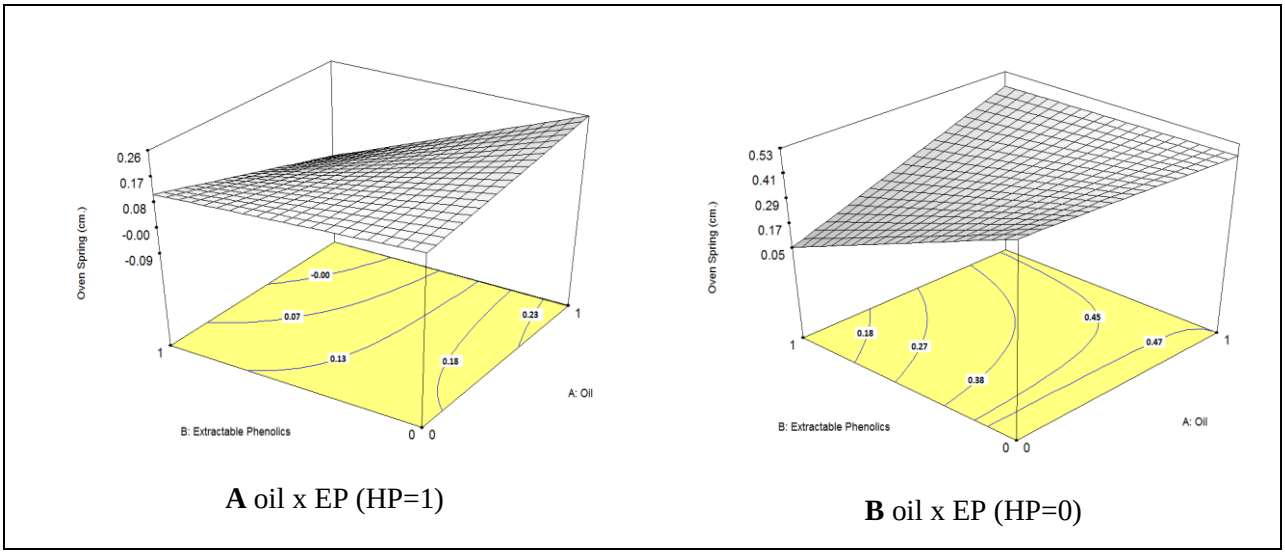


Figure 25. Baking absorption (%) as affected by two-way interaction: (a) FB-HP interaction; (b) FB-oil interaction.
FB=fiber; HP=hydrolysable phenolics; 0=not present in the system; +1=present in the system; means with the same letters are not significantly different ($P<0.05$).

On the other hand, absent of HP in the system exhibited considerably little to high oven spring (0.1 – 0.5 cm) (Fig.26b). Oil component did not impact the oven spring when EP component was removed in the system (Fig.26b). However, when EP was introduced in the system, declining of oven spring (from 0.5 to 0.2 cm) was observed with greater declining when oil component was absent (Fig.26b).

Two-way interaction of HP-FB was significant for oven spring at $p<0.01$. Fig.27 shows the value of oven spring as affected by HP-FB interaction. There was negative value recorded, or better known as no oven spring when FB and HP present. Overall, FB alone was more detrimental to bread oven spring that HP (Fig.27).

High oven spring was associated with high gluten index and wet gluten. Presence of FB and HP in the system has been proved with low gluten index and wet gluten (Fig.23 and Table 27). With low value of gluten index and wet gluten in the system, dough could not retain gas that has been produced by yeast during fermentation. Therefore, no oven spring could be seen for both treatments. Rogers and Hoseney (1982) noted that whole wheat dough which contains mostly insoluble fiber, has a normal proof height but gave only slight oven-spring. They attributed it to early solidifying of the loaf structure during baking because of premature starch gelatinization caused by the high level of water in the dough.



A oil x EP (HP=1)

B oil x EP (HP=0)

Figure 26. Factorial model plot for oven spring showing the effects of oil x EP with different levels of HP in the system.
EP=Extractable Phenolics; HP=Hydrolysable Phenolics; FB=Fiber; 0=not present in the system; +1=present in the system.

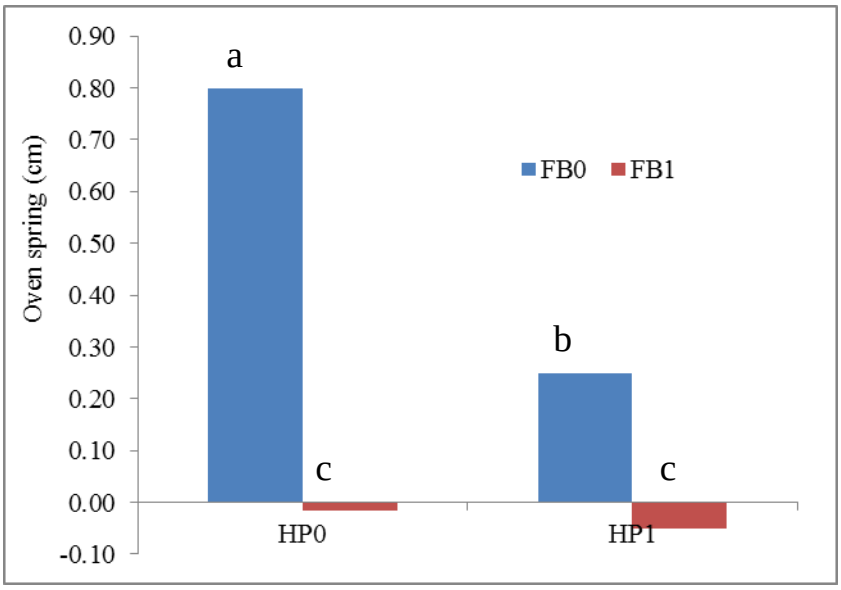


Figure 27. Oven spring as affected by HP-FB interaction.
EP=Extractable Phenolics; HP=Hydrolysable Phenolics; FB=Fiber; 0=not present in the system; +1=present in the system. Means with the same letters are not significantly different ($p<0.05$).

The individual effects of HP and FB were significant on loaf volume at $p<0.001$ and $p<0.0001$ respectively, while the effects of EP and oil were not significant. Figure 28 shows the value of loaf volume as affected by single effect of FB and HP. In general, HP and FB component negatively impact the bread loaf volume, with value of 127.9 and 102.8 cc respectively. Addition of fiber has been proved to have a detrimental effect on bread loaf volume (Lai et al. 1989b; Pomeranz et al. 1977; Sidhu et al. 1999; Wang et al. 2002a). Pomeranz et al. (1977) explained that low loaf volume was due to 'gluten dilution' by insoluble fiber, and poor gas retention. Many researchers concluded that these detrimental results could be explained by the interactions between fibers and gluten (Chen et al. 1988; Chen et al. 2011; Zhang and Moore 1997), however they did not offer any further explanation.

Phenolic compounds such as ferulic acid (found in bran) could be the major reason for dough breakdown. Ferulic acid and other phenolic acids have been found in wheat flour (Gallus and Jennings 1971), and they can bound to the water-soluble pentosans by ester bonds (Fausch et al. 1963; Yeh et al. 1980). Jackson and Hoseney (1986a) conducted an experiment on overmixed dough and found that ferulic acid in water-soluble fraction interacts with gluten/starch fraction during mixing to cause changes (lack of resilience, i.e. dough breakdown) in the gluten proteins. Sidhu et al. (1980a) and Jackson and Hoseney (1986b) obtained evidence for the formation of a covalently-linked complex between cysteine and fumaric acid during mixing and overmixed dough.

As suggested by Nowrocka et al. (2016) presence of fiber caused decreased of α-helix band, and induced the conformation of two α-helix protein complexes to form antiparallel-β-sheet structures. This might change the protein functionality. During bread making process, disulphide bonds acts as "chain extender" – connecting high and low molecular weight glutenin

subunits to form gluten network (Wieser 2007) and contribute to the gluten network elasticity (Shewry and Lucas 1997). Based on our findings, we speculate that in whole wheat dough system, where phenolic and fiber were found abundantly, the disulphide bonds were attracted to phenolics compounds, thus disrupt the gluten network, and ultimately exhibit poor gas retention capacity as well as low loaf volume. Also, fiber might change the protein functionality via changes the α-helix to β-sheet structures.

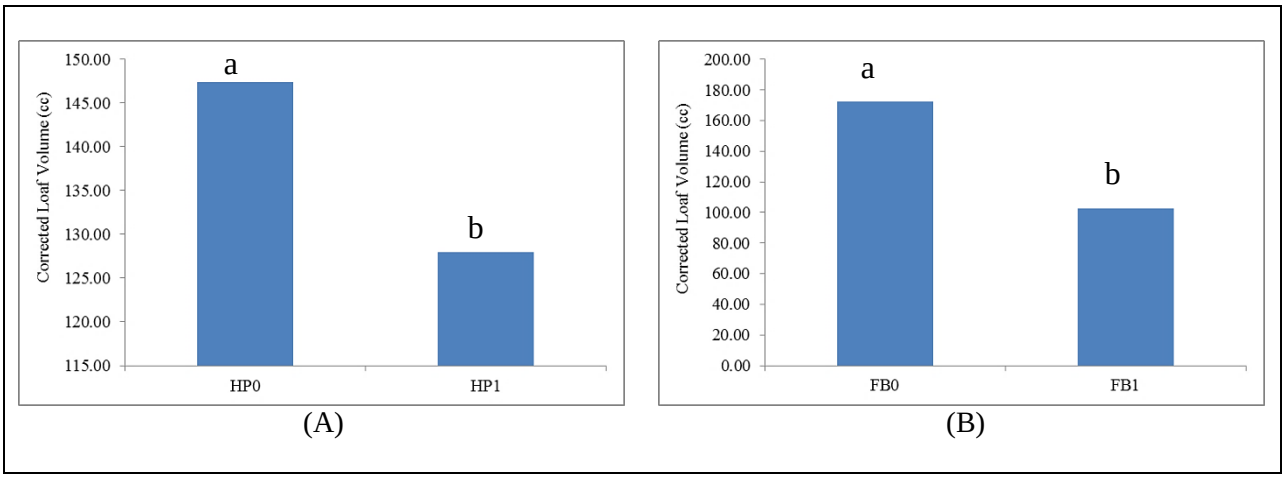


Figure 28. Loaf volume as affected by: (A) hydolisable phenolics; and (B) fiber (FB). Means with different letters within same histogram differ significantly ($p<0.05$); 0=not present in the system; +1=present in the system.

Influence of Bran Components on Solubility of Proteins in Bread Crumb

In the current research, we investigated the influence of individual bran components on solubility of proteins in bread crumb. The result exhibited that the protein amount left in the FLR after extraction by vortex and sonication in SDS buffer was 0.8 % (Table 28). That percentage increased to 7% after the flour was baked into bread after mixing and fermentation, indicating that protein solubility significantly decreased by bread-making. The decrease of protein solubility for bread crumb could be mainly due to the heating during bread-making process. Singh (2005) reported that protein solubility decreased due to aggregation and/or cross-linking of protein molecules with time of baking. Other studies reported that changes in the protein

solubility was observed when gluten protein were heated at 70°C (Schofield et al. 1983; Singh and MacRitchie 2004). De La Pena et al. (2015) also found that residual protein content for spaghetti was higher than semolina. The solubility of proteins in bread crumb was influenced by bran components. The addition of bran components significantly ($P<0.05$) increased residual protein percentage based on sample weight. Especially, ANOVA indicate highly significant ($P<0.001$) interaction effects of EP by FB and HP by FB for residual protein percentage values based on protein content. When all the bran components were added, percent of residual protein (% protein) was much lower than that for the crumb sample of whole wheat flour. In this experiment, major portion of protein component in bran was not included in reconstitution as indicated by lower protein content of reconstituted samples than whole wheat flour sample. Therefore, the high percentage of residual protein for whole wheat bread crumb was possibly caused by the proteins in bran. However, further research is necessary to clarify the influence of bran proteins on solubility of proteins in bread crumb.

Many studies have been reported using SE-HPLC to evaluate the molecular weight distribution of wheat proteins (Ohm et al. 2010; Simsek et al. 2010; Tsilo et al. 2010; Zhang et al. 2011). The solubilized protein fractions were also analyzed for protein molecular weight distribution in this experiment. Typical SE-HPLC profile of total proteins extracted from bread crumb samples of refined flour and whole wheat flour were given in Figure 29A. The “WWF” clearly show lower SE-HPLC peak heights for F1 and F2 and higher heights for F4 and F5 when compared the “RF”. Main proteins are polymeric proteins for F1, gliadins for F2 and low molecular weight monomeric proteins and peptides for F4 and F5. The chromatogram indicates that proteins obtained from crumb sample of RF contains greater quantity of gluten proteins while lower amount of albumin, globulin, and peptides. The difference in chromatogram was

ascribed to the changes in the structure and extractability of some protein fractions that occur during bread making (Lagrain et al. 2007; Singh and MacRitchie 2004; Weegels et al. 1996).

Table 28. Protein percentage of bread crumb, solubilized fraction, and residue.

Sample	Crumb Protein (%, 12% mb)	Solubilized Protein (% flour, 12% mb)	Residual Protein	
			Percent flour (12% mb)	Percent Protein (%)
FLR	12.5	11.4	0.8	6.8
WWF	14.0	4.5	9.5	68.1
RF	12.6	5.6	7.0	55.3
OIL	12.3	5.5	6.8	55.1
EP	13.0	5.8	7.3	55.8
HP	12.2	5.8	6.4	52.6
FB	12.8	5.9	6.9	53.7
OIL*EP	12.9	5.9	7.0	54.5
OIL*HP	12.2	6.0	6.1	50.4
OIL*FB	12.6	6.0	6.6	52.5
EP*HP	12.7	6.4	6.3	49.8
EP*FB	12.9	6.6	6.3	48.5
HP*FB	12.2	6.7	5.5	44.8
OIL*EP*HP	12.6	6.5	6.1	48.2
OIL*EP*FB	12.9	6.7	6.2	48.2
EP*HP*FB	13.0	8.2	4.8	36.8
OIL*HP*FB	12.2	7.1	5.1	41.9
OIL*EP*HP*FB	12.7	8.5	4.2	33.4
LSD (0.05)	0.4	0.4	0.6	3.9
LSD (0.01)	0.5	0.5	0.8	5.3

db=dry basis; FLR=flour; WWF=whole wheat flour; RF=refined flour; EP=Extractable Phenolics; HP=Hydrolysable Phenolics; FB=Fiber; LSD=least significant difference.

The chromatograms in Figure 29B revealed that as each bran components gave different impact on the area under the curve of SE-HPLC of solubilized protein profile. Bran's oil component exhibited less total area under the curve than RF, as indicated by lower solubilized protein content in Table 28. The oil component in bran had smaller impact on protein solubility in the bread system when compared to other bran components. Wheat bran oil was characterized by a yellowish color and a light odor. The major fatty acids of wheat bran oil were linoleic

(18:2n6), palmitic (16:0), oleic (9c-18:1) and linolenic (18:3n3) (Table 24). The only rich saturated fatty acid is palmitic, and the rest exhibited unsaturated fatty acid. Fats and oils in baking are added for lubrication purposes. Fats help ease expansion. Early studies showed that free nonpolar lipids (NL) generally depressed loaf volume while polar lipids (PoL) had an improving affect (Daftary et al. 1968; Gan et al. 1990; Larsen et al. 1989b). Flour lipids could stabilize foam structure of dough via enveloping the expanding gas cell (MacRitchie and Gras 1973). However, these studies were done on flour lipids, which correspond to endosperms portion. Tait et al (1988) reported on lipid composition during whole-meal storage on baking quality. He found that greater double bonds of fatty acid structure gave detrimental to loaf volume and texture scores (Tait and Galliard 1988). As explained by McCann et al. (2009) lipids interacts with flour protein during bread making, the complexes formed may attributed to extractability of the baked bread. Gluten proteins polymerize (Lagrain et al. 2007; Weegels et al. 1996) and the levels of SDS-extractable gliadin and glutenin decreased during baking (Lagrain et al. 2007).

EP and HP exhibited different behavior with regards on SE-HPLC protein profile (Fig. 29B). F1 of EP chromatogram decreased compared to of RF chromatogram. However, F2, F4 and F5 of EP exhibited the higher peaks than those of RF. The chromatogram for HP showed prominently higher peak for F4 while showing lower peak for F1 and F2 when compared those of RF. This indicated that solubilized proteins from crumb samples that reconstituted with HP contained lower gluten proteins while having much more quantity of proteins with similar level of molecular weight to albumin/globulins. HP component is rich in ferulic acid (Table 24). Ferulic acid is known to form a complex with cysteine fraction in gluten protein (Jackson and Hoseney 1986a; b; Sidhu et al. 1980a) during mixing and inhibit disulfide crosslinking (Han and

Koh 2011a), which ultimately causes the breakdown of dough. The inhibition of polymerization of proteins in dough system when HP was introduced might also increase extraction of proteins similar to albumin and globulin.

Crumb sample reconstituted with FB component exhibited greater peak areas for F1, F2, F3, and F4, indicating more gluten proteins were extracted from the bread crumb. The higher peak height for F1 were also seen with SE-HPLC profile for bread crumb samples made from flour added with FB and other components such as HP, and EP (Fig. 30). This indicates that that HP and EP might have synergistic effect with FB for increasing solubility of polymeric proteins in crumb samples. The addition of bran was observed to cause partial dehydration of gluten and collapse of β-spirals into β-sheet structures in dough (Bock and Damodaran 2013). Nowrocka et al (2016) reported that present of fiber caused decreasing of α-helix band, and induced the conformation of two α-helix protein complexes to form antiparallel-β-sheet structures. These polymerization changes may alter the molecular weight distribution of protein. Furthermore, the addition of these purified compounds may also cause some depolymerization of protein molecule during breadmaking and resulted in increase of solubility of polymeric proteins.

Since the bran components had significant effect on solubility of proteins in bread crumb, we investigated the associations between the protein solubility and quality parameters such as farinograph stability, wet gluten, and bread loaf volume. The residual protein content (% flour) showed significant and positive correlations with wet gluten (r=0.535, $P<0.05$) and loaf volume (r=0.699, $P<0.01$) while showing a non-significant correlation (r=0.185, $P\geq0.05$) with farinograph stability. This result indicates that reconstituted samples which had greater quantity of gluten and loaf volume showed lower solubility of proteins in bread crumb. Specifically, sample reconstituted with EP showed high values for wet gluten, loaf volume, and crumb

residual protein content while samples reconstituted with FB showed low values for those parameters.

Correlation coefficients were also estimated between the quality traits and SE-HPLC parameters of SDS-buffer extractable (EXF) and sonication extractable fractions (SEF). Correlation coefficients were shown specifically between farinograph stability, wet gluten, and loaf volume and SE-HPLC absorbance area values as a spectrum over profiles of EXF and SEF (Fig.31). While no significant correlation ($P < 0.05$) appeared between farinograph stability and SE-HPLC fractions of EXF (Fig.31a-1), significant and negative correlations were found between farinograph stability and SEF fractions including F1 ($r = -0.574$, $P<0.05$), F2 ($r=-0.770$, $P<0.001$), and F3 ($r=-0.742$, $P<0.001$) (Fig.31a-2). For the flour samples, polymeric proteins in SEF are found to have positive correlations with farinograph stability (Ohm et al. 2009). However, the results in the current research indicate that high quantity of polymeric proteins in SEF is associated with weak dough stability. And it is also indicates that high level of gliadins in SEF could be also associated with weak stability.

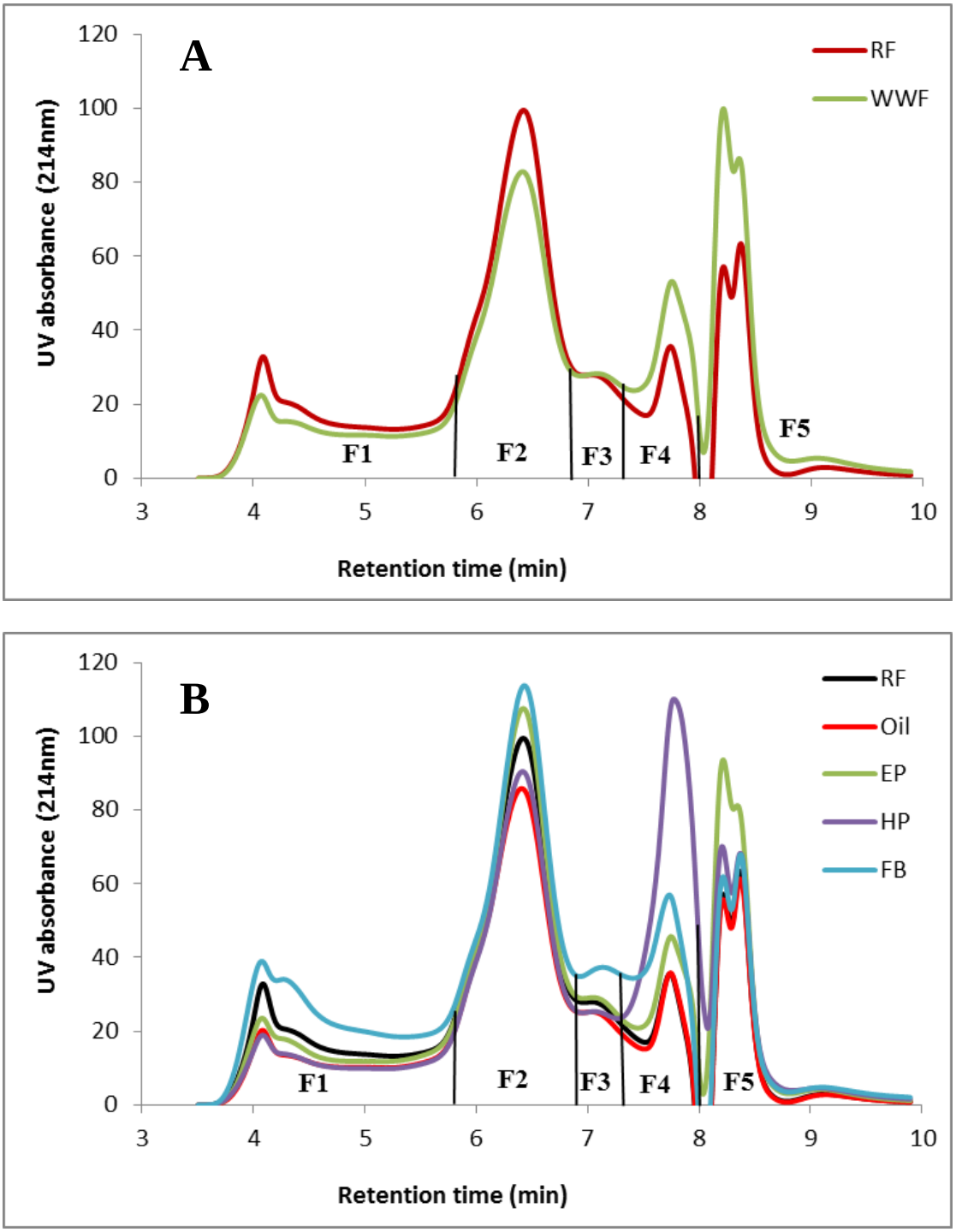


Figure 29. Size-exclusion HPLC profiles of protein extracts of (A) a flour and bread crumbs made from whole wheat flour, and refined flour and (B) its blend with extracted bran components.
FLR=flour; RF=refined flour; WWF=whole wheat flour; EP=extractable phenolics; HP=hydrolysable phenolics; FB=fiber.

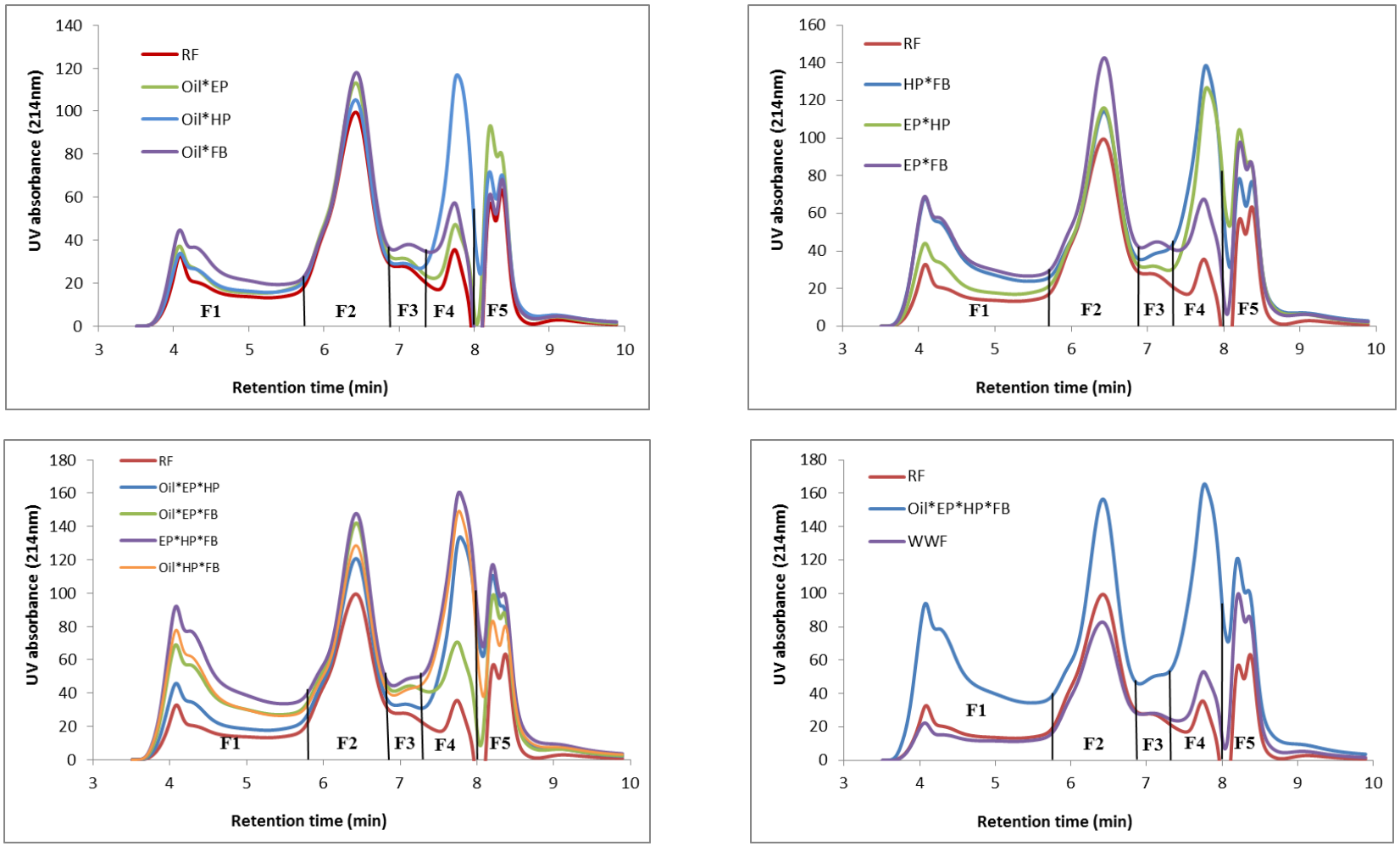


Figure 30. Size-exclusion HPLC profiles of protein extracts of a flour and bread crumbs made from composite flours. FLR=flour; RF=refined flour; EP=extractable phenolics; HP=hydrolysable phenolics; FB=fiber

Wet gluten and bread loaf volume showed similar trends for correlation profiles for both EXF and SEF (Fig 31b and c). Wet gluten had significant ($P < 0.05$) negative correlations with F1 (r = -0.515, $P<0.05$) and fraction eluted around 7.3 min (r = ~-0.65, $P<0.01$) of EXF (Fig.31b-1). When compared to wet gluten, bread loaf volume showed stronger associations showing r values of -0.626 ($P<0.01$) with F1, -0.651 ($P<0.01$) with F3 and -0.575 ($P<0.05$) with F4. For SEF, both wet gluten and loaf volume had significant ($P < 0.05$) and negative correlations with all the SE-HPLC fractions (Fig.31b-2 and 31c-2). The negative correlation of F1 of EXF and SEF occurred primarily due to the influence of FB that acted to increase solubility of polymeric proteins. The FB could be associated with correlations found for F2 and F3 as it increased solubility of those proteins in crumb. The significant correlations seem to be associative with interaction of FB with other components such as HP and EP that acted in synergistic way to increase solubility of proteins, especially polymeric proteins in crumb while having negative effect on the quality parameters. The interaction could be associated with correlations identified for F4 and F5 with quality parameters such as wet gluten and loaf volume. The addition of bran was observed to cause conformation change of proteins (Bock and Damodaran 2013; Nawrocka et al. 2016). Phenolic compounds such as ferulic acid (found in bran) cause depolymerization in the gluten proteins by interacting with gluten during mixing (Jackson and Hoseney 1986a; b). The results in this research indicates that FB mainly influence protein conformation interacting with other bran components such as HP and EP, which also resulted in decrease of breadmaking quality, with increasing protein solubility specially polymeric proteins. These findings could not be the definitive conclusions regarding distribution of protein molecular weight in bread crumbs as there were 30-70% of bread crumb proteins residue still bound in the bread crumb and could not extracted.

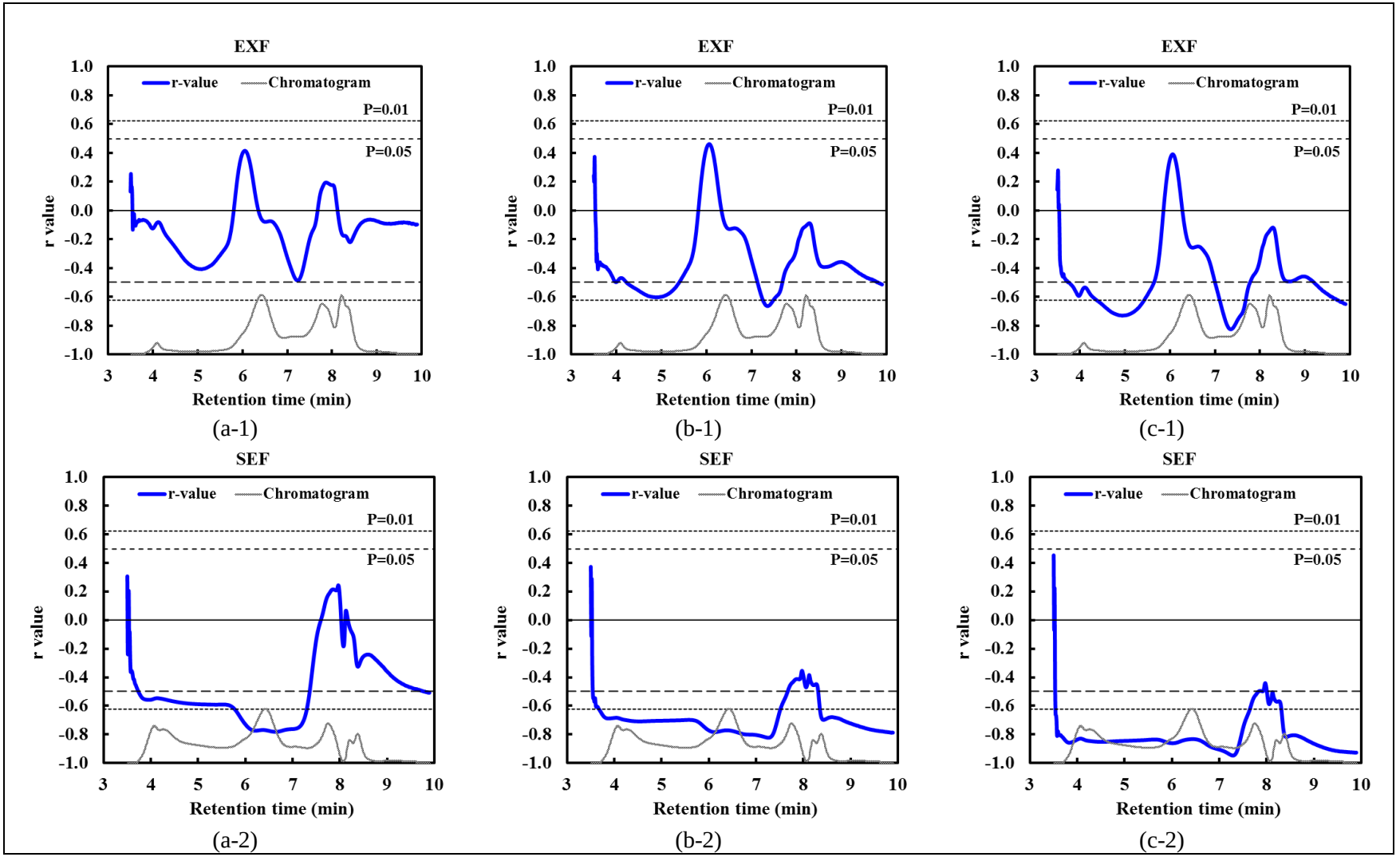


Figure 31. Spectrum of simple linear correlation coefficients (*r*) between farinograph stability (a), wet gluten (b), and corrected loaf volume (c) and size-exclusion HPLC absorbance area values of the SDS-buffer extractable (EXF) (1) and sonication extractable (SEF) (2) protein fractions for the 16 formulations.

Conclusion

Despite the health benefits of bran and whole-wheat products, bran tends to negatively impact dough viscoelastic properties, loaf volume and end product quality in general. However, limited information is available concerning the influence of individual bran components and their interactions on whole wheat breadmaking in hard red spring wheat. Therefore, this research aimed to investigate the association between bran components and breadmaking quality. For this, effects of different major bran components including lipids, phenolics (extractable and hydrolysable), and fiber fractions on the whole wheat bread-making quality were investigated by following up a reconstitution approach using the 2^4 factorial experimental layout. All four components exhibited pronounced effect on quality parameters. Interestingly, bran fiber was identified as a single main factor that had highly significant impact on all flour, dough, and baking parameters measured in this experiment. Specifically, presence of fiber in dough system increased water absorption and gassing power. However, fiber had strong negative influence on dough and baking quality characteristics including wet gluten, gluten index, farinograph stability and bread loaf volume. Other components appeared to have negative influence on breadmaking quality but it was not as pronounced as FB. The reconstitution of hydrolysable phenolics was found to impact positively on farinograph stability. However, the interaction of fiber with other components decreased bread loaf volume further more. Fiber and hydrolysable phenolics were the main factors that significantly impacted bread loaf volume. Reconstituted breads prepared without fiber or hydrolysable phenolics had higher loaf volume than white bread. The influence of FB was also associated with solubility of proteins in bread crumb. The individual bran components showed difference in proteins solubilized from bread crumb when analyzed by SE-HPLC. Especially, FB was found to increase solubility of polymeric proteins in bread crumbs

while other components decreased it. When FB was reconstituted with EP and HP, polymeric protein solubility increased furthermore, resulting in decrease of farinograph stability and loaf volume. This indicates that FB interacted with other components to change protein characteristics. The influence of FB on proteins might be mainly related to the change of protein conformation, which might sequentially cause increased protein solubility and decreased dough stability and loaf volume. Overall, influence of bran components on bread-making quality seemed very complex since analysis of variance showed that interaction of all four bran components (lipid, extractable and hydrolysable phenolics, and fiber) was highly significant ($P<0.05$). This study shows how each of these components effects on bread quality and might lead to further investigation about pre-treatments that could be performed to bran in an effort to improve whole wheat bread quality.

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PAPER 4. WHOLE-WHEAT BREAD-MAKING METHOD AND THE EFFECTS OF VARIETY AND LOCATIONS ON WHOLE-WHEAT BREAD QUALITY

Abstract

Whole-wheat bread quality, such as loaf volume and crumb texture, depends on whole-wheat flour characteristics, ingredient, and bread-making methods used. Four different types of commercial whole-wheat flours (CWWF) were used to produce whole-wheat bread using three different types of bread-baking methods, which were sponge-and-dough (SpD), straight dough (StD), and no-time dough (NoD). CWWF possess different physical and chemical characteristics of whole-wheat flour. StD and NoD method required higher water absorption than SpD method. Loaf volumes of whole-wheat bread made with SpD method were relatively higher than whole-wheat bread made with other baking method. Whole-wheat bread made using NoD method had the heaviest loaf weight, as ascribed by no fermentation in the bread-baking step. StD method recorded highest variation in baking mix time, baked weight, crumb grain score, and symmetry score. Higher variation is needed to identify differences among flour types as well as cultivars used. StD is the best method for whole-wheat bread in order to see the differences between flour used especially different cultivars. This is important to help breeders on evaluation of whole-wheat bread quality. Twenty-one cultivars from six locations were used in second experiment to evaluate the effects of cultivars and locations on whole-wheat bread quality. There were relatively high variability among cultivars for the whole-wheat bread loaf volume and symmetry, showing 47% and 41% contribution, respectively. Locations contributed to high variability for the whole-wheat baking absorption, showing 89% contribution.

Introduction

There is increase in consumption and demand for whole-wheat flour product due to improved nutritional and health related claims compared to refine flour products. Important nutritional compounds have been reported found in whole wheat grains including fiber, vitamins and minerals, was well as phytochemicals, such as phenolic compounds (Arvola et al. 2007; Slavin 2004). Whole wheat bread has been recognized as an acceptable and staple food for consumers that have great concerns of health implications of their food intakes (Slavin et al. 2001). Bread making process has been stated to have effect on nutritional properties and qualities parameters for whole meal wheat and rye breads (Dewettinck et al. 2008; Rosell et al. 2009).

Evaluation of different baking methods is necessary to achieve process efficiency and most importantly, to meet consumer quality requirements (Rosell et al. 2009). No-time dough refers to baking method that does not involve bulk fermentation, unlike straight dough and sponge-and-dough methods. Although, no-time dough method offer process advantages of less space requirement and short processing and operation time, flavor development is poor and product formulation is stringent (Baker et al. 1988). Sponge-and-dough method is widely used especially for the majority of mass-production of bread in the United States (Kulp and Ponte 2000). Straight dough method offers an intermediate fermentation time and is the most widely used method for experimental baking in breeding programs (Graybosch et al. 2013). Dough mixing and proofing have been reported to affect ferulic acid content in dough during bread making. The sourdough method has been reported to cause increasing in phenolic compounds (Katina et al. 2007; Lopez et al. 2003). Also, long fermentation during bread making process of whole wheat enhanced up to 30% enrichment of riboflavin and maintain vitamin B at high amount (5.5 μg/g) (Batifoulier et al. 2005). Recently, bread making methods were found to

impact phytonutrient in wholegrain bread differently. Straight dough method was adjudge as the best towards retention of total flavonoid in wholegrain bread, compared to sponge dough and sourdough methods while sourdough method enhances total carotenoid most. Therefore, baking process could be used to manipulate the phytonutrients in wholegrain bread (Sahli 2015).

It is quite important to know that there are still some technological bottle necks, requiring urgent intervention by cereal scientist, hindering the acceptance of whole-wheat bread. Among some of the setbacks are lower loaf volume, faster staling and coarser texture (Rosell et al. 2009). According to Bruckner et al. (2001), data obtained for white flour experiments could be used to estimate whole-wheat flour performance. However, Seyer and Gélinas (2009) reported that data for white bread would not suitable for whole-wheat bread because of disparities in the impacts associated with other constituents (wheat bran and short). Further studies have been demanded in order to fully explore the effects of bread baking methods on whole-wheat bread quality evaluated from whole-wheat flour samples (Seyer and Gélinas 2009).

Materials and Methods

Experiment 1: Whole-Wheat Bread-Making Method

Four types of commercial whole-wheat flour (CWWF) were purchased in North Dakota. Three types of bread-baking methods namely sponge-and-dough (SpD), straight dough (StD), and no-time dough (NoD) were used to prepare whole-wheat breads. StD loaves were prepared according to the AACCI Approved Method 10-09.01, basic straight dough with modifications. Fungal α-amylase and instant dry yeast were used instead of malt powder and compressed yeast, respectively. Ammonium phosphate at 5 ppm was added to improve yeast function. The bread was prepared using 2 h fermentation schedule, with an extra 10 min time for proofing (preliminary study, data not shown). SpD loaves were according to AACCI Approved Method

10.11.01 with modifications (using fungal α-amylase and instant dry yeast instead of malt powder and compressed yeast, respectively). NoD loaves were prepared according to lab procedure. Ingredients for these baking methods are summarized in Table 29; for each case, the dough was made from 100 g of flour. Figure 32 summarized the whole-wheat bread-making methods used.

Table 29. Ingredients (% baker's) of breadmaking for different baking methods

Bread-baking methods / Ingredients	Sponge-and-dough		Straight dough	No-time dough
	Sponge	Dough		
Flour	60	40	100	100
Water	60 water absorption	40 water absorption	Water absorption	Water absorption
Instant dry yeast	1	0	1	1
Sodium chloride	0	1	1	1
Sugar	0	5	5	5
Vegetable shortening	0	2	2	2
Fungal a-amylase	0	15-17SKB	15-17SKB	15-17SKB

CWWFs were characterized by protein content (AACCI Approved Method 46-30.01) with a LECO FP 528 nitrogen/protein analyzer (LECO, St. Joseph, MI, U.S.A.), ash content (AACCI Approved Method 08-01.01), moisture content (AACCI Approved Method 44-15.02), starch damage (Megazyme starch damage assay procedure according to AACCI Approved Method 76-30.02), and wet gluten content and gluten index were determined (AACCI Approved Method 38-12.02) with a Glutomatic 2200 S system (Perten Instruments, Springfield, IL, U.S.A.). Farinograph parameters of CWWFs were conducted using a 50 g mixing bowl by following AACCI Approved Method 54-21. CWWFs particle size distribution was determined using vibratory sieve shaker (Retsch AS200, Haan, Germany) with a stack of six sieves (50 μm, 150

μm, 250 μm, 425 μm, 500 μm, and 600 μm). Each sieve contained five plastic sieving balls. Sample (100 g) was shaken for 5 min and the weight retained on each sieve and in pan was recorded as percent of the total. Yeast activity measured as gas production (gassing power) was determined according to AACCI Approved Method 89-01.01 with modification using ANKOM^{RF} System (Figure 33).

Baking qualities were characterized by baking absorption, dough handling properties, bread loaf volume, and bread crumb score. Baking absorption was determined as the amount of water required for optimum dough baking performance and was expressed as a percent of flour weight on a 14% mb. Loaf volume was determined by rapeseed displacement method (AACCI Approved Method 10-05.01). Subjective analysis of final loaf score was evaluated according to the Guidelines for Scoring Experimental Bread (AACCI Approved Method 10-12.01) using a constant illumination source. The score ranged from 1 to 10, with the higher scores preferred. Firmness of bread was measured using texture analyzer (TA-XT2i, Texture Technologies Corp, NY) according to AACCI Approved Method 74-09.01.

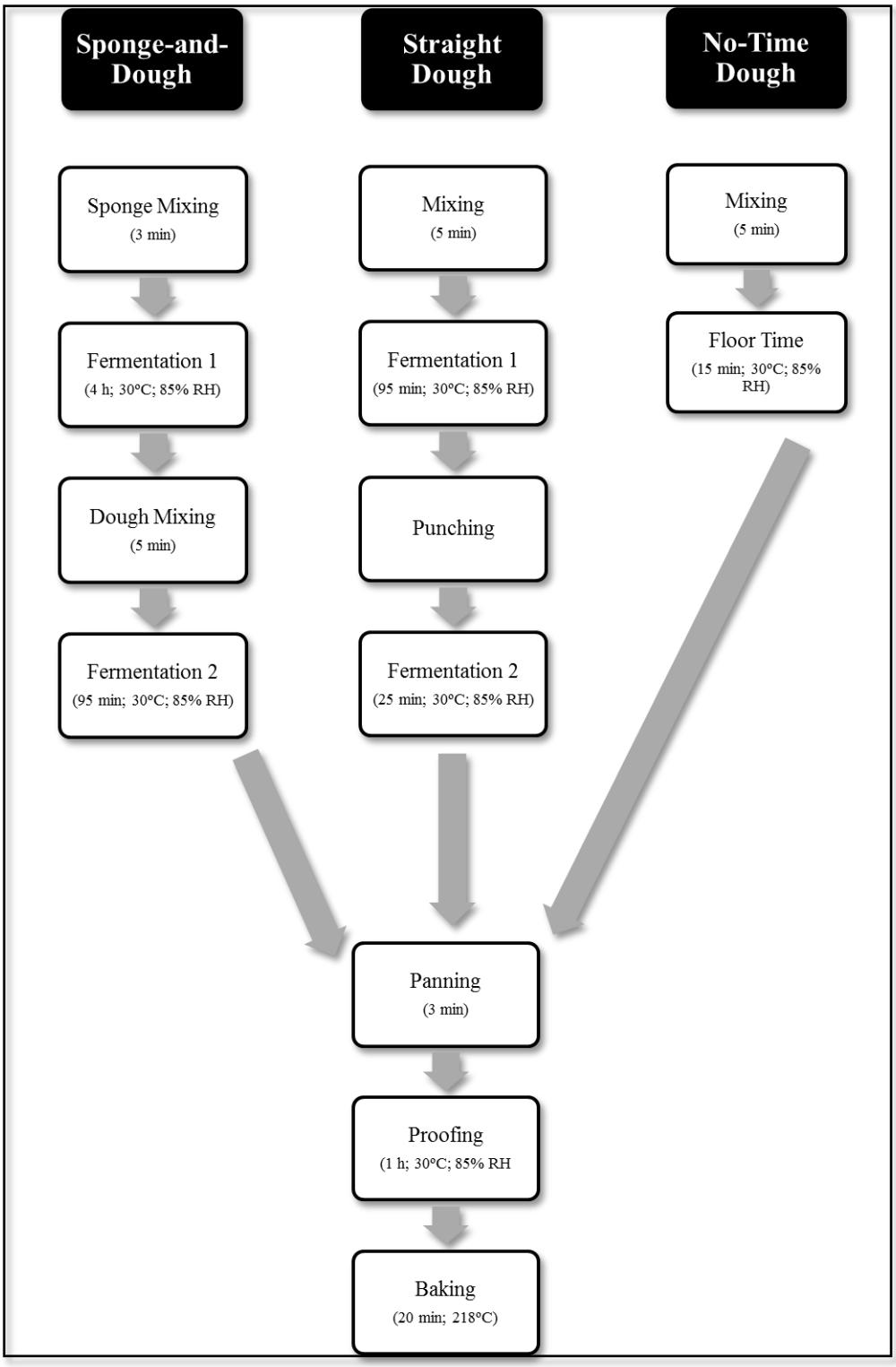

Figure 32. Flow diagram of different baking methods used in this experiment.

Figure 33. Gassing power measurement using ANKOM^{RF} System.

Experiment 2: Effect of Location and Cultivar on Whole-Wheat Bread-Making Quality

All 21 wheat samples were kindly provided by Dr. Mergoum in the Department of Plant Sciences, North Dakota State University. Samples of 21 wheat cultivars were grown at six locations (Carrington, Casselton, Dickinson, Hettinger, Langdon, and Minot) in 2012 and 2013 growing season. However, due to poor storage conditions (breakdown of freezer room) samples from 2013 growing seasons were excluded. Twenty-one hard red spring wheat samples were adapted to the U.S. Spring Wheat region (Table 30).

Wheat grains were cleaned by passing through on a Carter Day XT5 seed cleaner (Simon-Carter Co., Minneapolis, MN). A Bühler MLU-202 Mill (Bühler Industries Inc., Uzwil, Switzerland) was used to mill the wheat samples according to AACC Approved Method 26-21.02. Both flour and bran fractions produced from the Bühler mill were collected and stored at -20°C until needed. The bran portions were ground in cyclone sample mill with a 0.5mm screen (UDY Corp, Fort Collins, CO) and mixed with flour portion in its original percentage to produce whole-wheat flour.

Table 30. Genotype, class, origin and pedigree of hard spring wheat samples.

Genotype	Class	Origin	Pedigree
Alsen	HRS	NDSU	ND-674/ND-2710//ND-688
Barlow	HRS	NDSU	ND-744/ND-721
Breaker	HRS	Westbred LLC	KNUDSON/ALSEN,USA
Brennan	HRS	Syngenta Seeds, Inc.	REEDER//(N-98-0439)CHINA-SCAB-140/N-90-0690
Elgin	HRS	NDSU	WALWORTH/REEDER
Faller	HRS	NDSU	ND-2857/ND-2814; ND-2710/ND-688/3/KITT/AMIDON//GRANDIN/(SIB)STOA
Forefront	HRS	SDSU	FN-1700-155/FN-1500-074//WALWORTH
Glenn	HRS	NDSU	ND-2831/STEELE-ND
Howard	HRS	NDSU	PARSHALL/5/GRANDIN/3/IAS-20*4/H-567.71//AMIDON/4/ND-674
Jenna	HRS	Syngenta Seeds, Inc.	N-98-0178/97-S-0212-08
Mott	HRS	NDSU	ERNEST/ND-622/KEENE*2/SD-3310/SD-3414
ND 901CL Plus	HRS	NDSU	TEAL11A/3/Grandin/FS2-14//3*Kulm
NDSW 0612	HRS	NDSU	N97-0117//MT9420/3/971//IDO533/9747
Prosper	HRS	NDSU	ND-2857/DAPPS; ND-2857/ND-2814;
RB07	HRS	UoM	NORLANDER/HJ98
Rollag	HRS	UoM	MN-95229-40*2/RL-70-4
Steele-ND	HRS	NDSU	PARSHALL/ND-706
SY Soren	HRS	Syngenta Seeds, Inc.	NORPRO/KELBY
Vantage	HRS	Westbred LLC	KEYSTONE/GRANITE
Velva	HRS	NDSU	DAPPS(PI-633862)/2*REEDER
WB Mayville	HRS	Mon.Tech.	POLARIS/TROOPER

HRS=hard red spring wheat; NDSU=North Dakota State University; SDSU=South Dakota State University; UoM=University of Minnesota; Mon.Tech=Monsanto Technology.

All the whole-wheat flour sample were subjected to bread baking. Baking qualities were characterized by baking absorption, mixing time, loaf volume, oven spring, weight, bread crumb and grain score, and crumb firmness. Baking absorption was determined as the amount of water required for optimum dough baking performance and was expressed as a percent of flour weight on a 14% mb. Loaf volume was determined by rapeseed displacement method (AACCI Approved Method 10-05.01). Subjective analysis of final loaf score was evaluated according to the Guidelines for Scoring Experimental Bread (AACCI Approved Method 10-12.01) using a constant illumination source. The score ranged from 1 to 10, with the higher scores preferred. Firmness of bread was measured using texture analyzer (TA-XT2i, Texture Technologies Corp, NY) according to AACCI Approved Method 74-09.01.

Experimental Design and Data Analyses

Experiment 1 was conducted as Completely Random Design (CRD) with split plot arrangement, where main plot was bread-making method and subplot was whole-wheat flour type. The second experiment was conducted as Randomized Complete Block Design (RCBD) with treating location as replication. Data were subjected to analysis of variance (ANOVA) and variance component analysis assuming cultivar and location effects as random. Means were separated by Fisher's protected Least Significant Difference (LSD). Simple linear correlation (r_s, n=126) was calculated from data across all combinations of 21 cultivars and six locations. Correlation coefficient among cultivars (r_c) (n=21) was estimated using each mean performance of 21 cultivars across six locations. Correlation among growing locations (r_r) (n = 6) was calculated using each mean performance of six locations. All the statistical analyses were performed using the SAS software (Version 9.4, SAS Institute; Cary, NC).

Results and Discussion

Experiment 1: Whole-Wheat Bread-Making Method

Flour and Dough Quality of Commercial Whole-Wheat Flour. The physical and chemical characteristics of commercial whole-wheat flour (CWWF) were determined prior to baking experiment. The particle size distributions of CWWF were given in Figure 34. Their farinograph parameters, gluten quality, gassing power, as well as physical and chemical characteristics were shown in Table 31. Generally, 60-90% of particle size for CWWFs were fall under fine portion (<150μm). The increasing order in fine particle size portion was in the following order: CWWF1 > CWWF4 > CWWF3 > CWWF2. Variation in particle size portion among different CWWF can be associated with milling types used (Prabhasankar and Rao 2001), milling practice (Paper 1 in this dissertation), as well as wheat cultivars (Seyer and Gélinas 2009). Different wheat cultivars were reported to behave differently during grinding due to the mechanical strength needed by the bran portion (Seyer and Gélinas 2009). Therefore, the wheat cultivars could influence the bran particle size distribution during grinding in this CWWF.

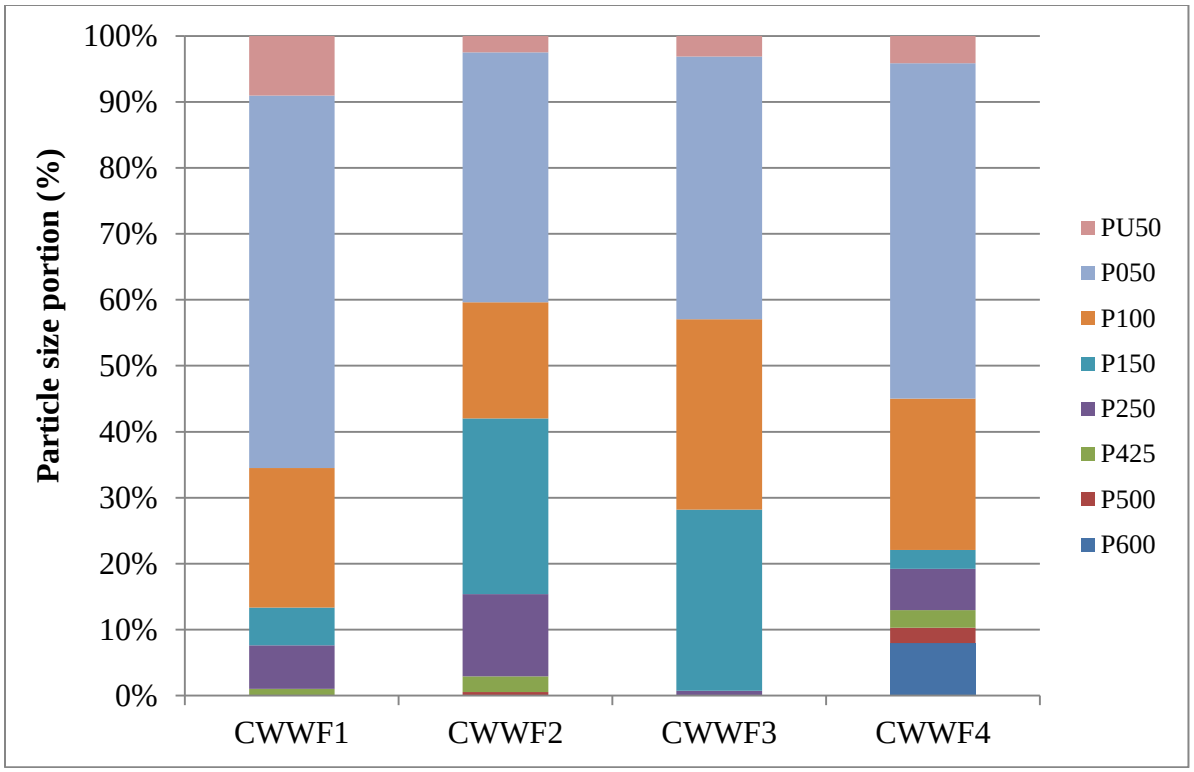

Figure 34. Particle size distributions among commercial whole-wheat flour. CWWF=commercial whole-wheat flour; P600=particle size portion bigger than 600µm; P500=particle size portion between 600-500µm; P425=particle size portion between 500-425µm; P250=particle size portion between 425-250µm; P150=particle size portion between 250-150µm; P100=particle size portion between 150-100µm; P050=particle size portion between 100-50µm; PU50=particle size portion less than 50µm.

There was significant ($P<0.05$) difference in chemical composition for CWWF samples. The protein of CWWF samples ranged from 13.79–15.40% (14%mb). The result was similar to the protein content of whole-wheat flour made from spring wheat (13.91–15.11%) reported previously (Bruckner et al. 2001). The ash content of CWWF ranged between 1.39–1.58% (14%mb). The increase in ash content was in the following order: CWWF1 > CWWF3 > CWWF4 > CWWF2.

Table 31. Flour and dough qualities of commercial whole-wheat flour (CWWF).

Sample	Physical/Chemical Characteristics					Farinograph parameters				Gassing Power at 90 min (psi)
	Protein (14% mb)	Ash (14% mb)	Starch Damage (14% mb)	Gluten Index (1–100)	Wet Gluten (%)	Absorption (14% mb)	Peak (min.)	Stability (min.)	MTI (BU)	
CWWF1	15.40 a	1.58 a	6.49 a	88.94 a	31.14 d	74.25 a	13.20 a	14.70 a	17.50 a	5.77 b
CWWF2	13.79 c	1.39 c	4.43 d	94.30 c	25.70 b	68.50 c	6.85 b	14.70 a	14.50 a	5.70 bc
CWWF3	13.80 c	1.54 b	5.53 b	97.17 d	21.37 a	67.87 d	11.33 a	15.40 a	13.67 a	5.62 c
CWWF4	14.32 b	1.54 b	4.75 c	91.61 b	33.14 c	69.87 b	7.10 b	12.80 b	20.00 a	6.72 a

MTI=mixing tolerance index; BU=brabender unit; mb=moisture basis.

Similar to protein content, starch damage was the highest in CWWF1 (6.49%) and least in CWWF2 (4.43%). The trend in starch damage content was in the following order: CWWF1 > CWWF3 > CWWF4 > CWWF2. Starch damage was affected by grinding mills type (Prabhasankar and Rao 2001), milling practice (Paper 1 in this dissertation), and wheat class (Prabhasankar and Rao 2001). Gluten index for CWWF ranged between 88.94 to 97.17% (Table 3) with increasing in the following order: CWWF3 > CWWF2 > CWWF4 > CWWF1.

CWWF1 recorded the highest farinograph water absorption (74.25%) (Table 31). High portion of fine particle size (87% for less than 150μm) (Fig.34) and high protein (15.4 %) and starch damaged content (6.5%) (Table 31) compared to other samples may attributed to the high water absorption. This was in agreement with various published articles and reports (Khalid et al. 2015; Khalid and Simsek 2015; Lai et al. 1989b; Noort et al. 2010; Prabhasankar and Rao 2001; Tara et al. 1972). Farinograph peak time means time needed to reach fully developed dough/gluten. Variation in peak times were found for CWWF samples (Table 31). Since fiber and other compounds were present in the flour, they might interact with each other (Jackson and Hoseney 1986; Joye et al. 2009; Noort et al. 2010; Pareyt et al. 2011) and cause a longer peak time than usual (if compared to refined flour). Protein content may affect the peak time, too. CWWF1 has high protein content (15.40%); thus, resulted in higher peak time. Whereas, low protein content of CWWF2 (13.79%) exhibited the shortest peak time (6.85 min). It has been shown that wheat class (hard vs. soft wheat; spring vs. winter wheat) affected the protein content of the milled flour (Bruckner et al. 2001; Prabhasankar and Rao 2001); thus, these CWWF may use different types of wheat grains.

High stability was preferred, despite low protein content, CWWF3 possess high stability (15.40 min.) attributed to its protein quality (97% of gluten index, Table 31). Mixing Tolerance

Index (MTI) was expressed as a value in BU or as a percentage of BU lost over time. This is used by bakers to determine the amount that dough will soften over a period of mixing (Brabender 2016b). No significant differences ($P>0.05$) for MTI among CWWF samples. Yeast activity during fermentation was measured as gassing power. Gassing power of CWWF ranged from 5.62 to 6.72 psi. Higher psi value indicates high amount of carbon dioxide were produced by yeast during 90 min of fermentation.

Bread-Making Methods for Whole-Wheat Bread. Three bread baking methods were applied to four types of CWWF with different characteristics. Interaction between baking methods-flour types (CWWF) was significant at $P<0.0001$ for baking absorption, loaf volume, crumb grain score, and symmetry score (Appendix Table D1-D2). The values were given in Table 32. Baking water absorption is the amount of water taken up by the flour to achieve the desired consistency or optimal end result (Osorio et al. 2003). Bakers prefer high level of flour water absorption, as water absorption is a primary quality determinant for bread-making (Morgan et al. 2000). Furthermore, water is economically advantages than any other ingredient (Baasandorj et al. 2015).

In general, straight dough and no-time dough method required higher water absorption than sponge-and-dough method (Table 32). CWWF1 had among the highest baking absorption (78%) (straight dough and no-time dough), as it contains high fine particle portion (87%, Fig.34), high protein content (15.4%, Table 31), as well as high starch damage (6.5%, Table 31) compared to other CWWF. Large portion of fine particle size has been associated with high water absorption (Noort et al. 2010); high starch damage and protein content were also associated with high water absorption (Tara et al. 1972). However, it was not true for sponge-and-dough method, where CWWF1 exhibited only 73% for baking absorption. The range of

baking absorption (70.00–78.60%) of the whole wheat flour varieties in this result is close to the range (79.06–86.81%) reported previously (Bruckner et al. 2001).

Loaf volume and crumb firmness are the main quality characteristics of bread (Katina et al. 2006). Loaf volume of whole-wheat bread made with sponge-and-dough method comparatively higher than whole-wheat bread made with other baking method (Table 32). CWWF1 exhibited highest loaf volume compared to other CWWF at each bread-baking method. The possible reason for this might be due to high proportion of fine particle size portion (87%, Fig.34). This is in agreement with previous observation that flour with finely ground bran and short produced high loaf volume (Khalid et al. 2015; Zhang and Moore 1999). In contrast with high loaf volume of bread made from sponge-and-dough method, straight dough method had the largest ($P<0.05$) bread gas cells size (averaged across flour types = 1.67 mm) compared to other bread-making method (data not shown). This may be evidence that the application of straight dough method (2 h fermentation) could not retain small gas cells in bread crumbs as much as other methods during proofing and baking.

Oven spring is the term used to describe the sudden increases in the volume of fermented dough during the first 10-12 min of baking. It is due to increased rate of fermentation and expansion of gases (Bender 2005). Generally, there were little to no oven spring occurred for whole-wheat bread. The highest oven spring (1.2 inch) was exhibited from whole-wheat bread made from CWWF1 using no-time dough (Table 32), whereas the least (-0.6 inch) was attributed from CWWF2 made using sponge-and-dough method. Generally, sponge-and-dough and straight dough methods produced whole-wheat bread with less oven spring compared to no-time method (Table 32). One common step for straight dough and sponge-and-dough method was long hour fermentation; 2 h for straight dough and 4 h for sponge-and-dough method. Fermentation was

needed in bread-making for bread leavening and flavor development (Pyler and Gorton 2008). Fermentation was meant for making the dough lighter and spongier by the action of proteolytic enzymes, organic and inorganic acids, alcohol, and the acidic environment (Pyler and Gorton 2008). However, in whole-wheat bread system, where approximately 20-25% of bran was incorporated, the acidic condition during fermentation environment may cause changes in gluten network and bran composition. Evidence from Paper 3 could describe the changes. Long fermentation and acidic condition may release bound phenolic acids (Katina et al. 2005), which could disrupt the gluten network via inhibition of disulfide bond formation (Han and Koh 2011b; Koh and Ng 2008). Fiber altered the protein molecular structure via inducing the conformation of two α-helix protein complexes to form antiparallel-β-sheet structures (Nawrocka et al. 2016).

Loaf weight were significantly ($P<0.05$) affected by baking method (Table 32). Whole-wheat bread made using no-time dough had the heaviest weight (156 g). There is no fermentation process in no-time dough method (Fig.32), therefore this will definitely not let the yeast leaven the dough. As Pyler and Gordon (2008) explained the importance, primary fermentation, causes the dough to undergo several physical-chemical changes that result in the desired rheology of the dough, such as lighter and spongier dough. Fermentation also produces unique flavor that desired for bread.

Table 32. Baking qualities as affected by baking methods-flour type interaction

Baking Methods	Flour Type	Baking Absorption (%)	Loaf Volume (cc)	Oven Spring (inch)	Crumb Grain Score (1-10)
No-Time Dough	CWWF1	78.3	686.7	1.2	5.7
	CWWF2	75.0	605.0	0.4	4.7
	CWWF3	77.3	576.7	0.0	3.7
	CWWF4	77.6	613.3	0.4	5.0
	Mean ± SE	77.0 ± 1.4	620.4 ± 46.9	0.5 ± 0.5	4.8 ± 0.8
Sponge-and-Dough	CWWF1	72.6	855.0	0.4	7.3
	CWWF2	70.0	615.0	-0.6	6.0
	CWWF3	75.3	735.0	0.0	7.0
	CWWF4	75.6	820.0	0.5	7.0
	Mean ± SE	73.4 ± 2.6	756.3 ± 106.8	0.1 ± 0.5	6.8 ± 0.6
Straight Dough	CWWF1	78.6	688.3	0.2	8.0
	CWWF2	74.3	591.7	-0.4	6.7
	CWWF3	77.3	496.7	-0.5	3.0
	CWWF4	78.6	666.7	-0.2	7.3
	Mean ± SE	77.2 ± 2.0	610.8 ± 86.6	-0.2 ± 0.3	6.3 ± 2.2
LSD between flour within the same baking method (P<0.05)		0.40	50.80	0.40	0.79
LSD between flour for different baking method (P<0.05)		0.40	49.10	0.40	0.90
LSD between means for baking method (P<0.05)		0.24	27.23	0.20	0.60

CWWF=Commercial Whole Wheat Flour; SE=standard error mean; NS=not significant as in Appendix ANOVA Table D1-D2.

Table 32. Baking qualities as affected by baking methods-flour type interaction (continued).

Baking Methods	Flour Type	Symmetry Score (1-10)	Loaf Weight (g)	Baking Mix Time (sec.)
No-Time Dough	CWWF1	6.0	152.7	300.0
	CWWF2	4.7	153.7	265.0
	CWWF3	3.0	155.3	280.0
	CWWF4	3.7	156.8	265.0
	Mean ± SE	4.3 ± 1.3	154.6 ± 1.8	277.5 ± 16.6
Sponge-and-Dough	CWWF1	6.0	149.1	230.0
	CWWF2	2.0	147.7	200.0
	CWWF3	3.0	149.6	210.0
	CWWF4	6.0	148.7	200.0
	Mean ± SE	4.3 ± 2.1	148.8 ± 0.8	210.0 ± 14.1
Straight Dough	CWWF1	7.0	143.2	300.0
	CWWF2	2.7	144.7	270.0
	CWWF3	2.0	146.7	270.0
	CWWF4	7.0	146.9	245.0
	Mean ± SE	4.7 ± 2.7	145.4 ± 1.8	271.3 ± 22.5
LSD between flour within the same baking method (P<0.05)		0.52	NS	NS
LSD between flour for different baking method (P<0.05)		0.50	NS	NS
LSD between means for baking method (P<0.05)		0.24	2.97	9.99

CWWF=Commercial Whole Wheat Flour; SE=standard error mean; NS=not significant as in Appendix ANOVA Table D1-D2.

Effects of Baking Methods on Bread Quality Characteristics. Figure 35 showed the cross section images of whole-wheat bread made by different types of whole-wheat flour using different bread-making method. In glimpse, sponge-and-dough method produced whole-wheat bread with similar loaf volume and cell distribution, even though different types of whole-wheat flour were used. No-time dough exhibited generally low loaf volume as the method itself does not allow yeast to work extra and gluten network could not fully develop. No-time dough baking method was created to fulfill the industry requirement to shorten the time and space required for bread baking (Pyler and Gorton 2008). As a result, no-time dough was associated with enrichment usage of ingredients such as oxidizing agents and chemical dough development (Pyler and Gorton 2008) to produce dough with the same rheology as dough undergo fermentation process. In contrast with straight dough, fermentation time of sponge-and-dough was reduced by 50% to achieve desirable fully raised fermented dough. Furthermore, O'Donnell (1996) suggested that whole-wheat bread should be baked using straight dough method as it requires medium fermentation time to reduce the bitterness flavor produced by bran fraction yet it allows yeast to leaven the dough.

Straight dough recorded highest variation in baking mix time, baked weight, crumb grain score, and symmetry score as indicated by standard error values (Table 32). Higher variation indicated that this bread-making method was suitable to identify differences among flour types as well as wheat cultivars for whole-wheat bread-making quality. Although Maeda et al. (2004) and Sahli (2015) suggested sponge-and-dough and sourdough method was the best for whole-wheat bread production respectively, their purpose and material used for the experiment were different. Maeda et al (2004) used polished wheat flour (similar to polished rice) to utilize the rice milling equipment/facilities in their country, while Sahli (2015) used one type of

commercially available whole grain flour to investigate the changes in phytonutrient and antioxidant quality of whole-wheat bread throughout storage.

Relationship Between Whole-Wheat Bread and Flour Quality Characteristics for Different Bread-Making Methods. Flour composition is very important parameters in bread making because it leads to end product quality. In this experiment, different types of whole-wheat flour were used to produce whole-wheat bread using different types of bread baking method. It is important to distinguish the difference between whole-wheat bread produced from different whole-wheat flour using different bread-baking methods. Correlation coefficients between flour/dough and baking quality parameters are shown in Table 33. Generally, protein contents of whole-wheat flour had a positive and significant ($P<0.05$) correlation with most of the bread quality characteristics. However, the flour protein content exhibited significant and negative correlation with bread firmness in straight dough ($r = -0.72$) and no-time dough ($r = -0.74$) methods and non- significant ($P>0.05$) correlation in sponge-and-dough method.

Gluten index equally exhibited negative and significant ($P<0.05$) correlations with most of the bread baking quality parameters, except bread firmness, which exhibited positive and significant ($P<0.05$) correlations in whole-wheat bread made with straight dough ($r = 0.93$) and no-time dough ($r = 0.84$) method, but not significant ($P>0.05$) with sponge-and-dough method. Wet gluten showed higher correlations with most of whole-wheat bread quality characteristics made with straight dough method compared to sponge-and-dough method. Farinograph stability showed no significant correlation ($P>0.05$) for almost all bread quality parameters made with all three bread-baking method, except with whole-wheat bread symmetry score, which had negative association with straight dough ($r = -0.64$) and sponge-and-dough ($r = -0.58$) methods.

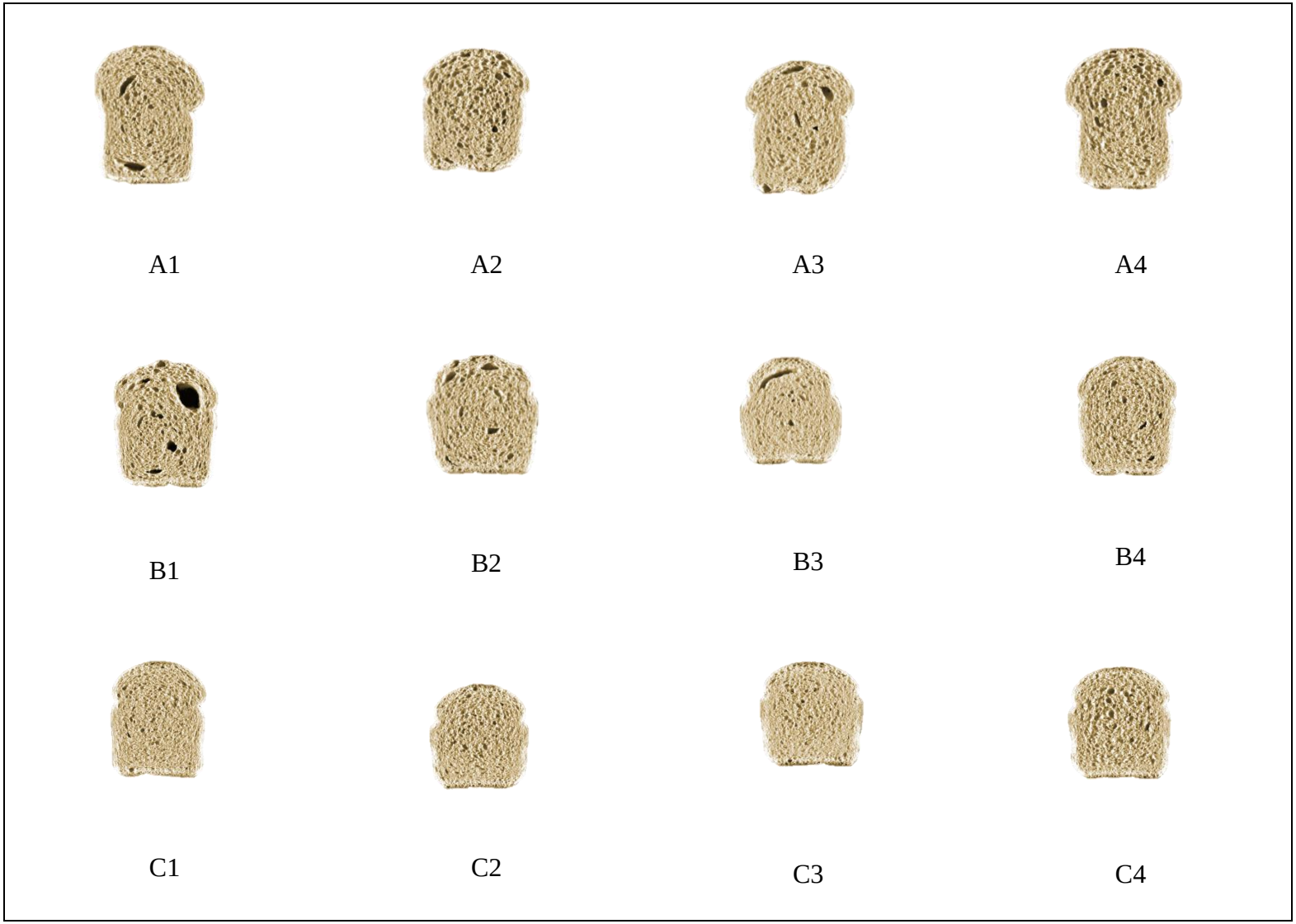

Figure 35. Images of cross section of whole-wheat bread made from sponge-and-dough (A), straight dough (B), and no-time dough (C) using CWWF1 (1), CWWF2 (2), CWWF3 (3) and CWWF4 (4).

In general, bread quality characteristics evaluated by straight dough method showed higher or similar correlations with whole wheat flour traits when compared to those from sponge-and-dough and no-time dough methods. This indicated that straight dough might be more suitable to see influence of whole wheat flour characteristic on bread making quality than other methods.

Presence of bran in flour matrix impacted flour, dough and bread quality parameters. In general, the correlation coefficient suggests that small increase in proportion of bran particles impacted significantly on bread qualities using straight dough method. Sponge-and-dough method is a great equalizer as it could produce whole-wheat bread with similar firmness and crumb grain texture regardless of whole-wheat flour quality (Table 33). In our case, we would like to see the differences on whole-wheat bread samples made with different whole-wheat flour types and bread-making methods. Therefore, we conducted another correlation analyses between bread-baking methods and bread qualities as given in Table 34. "StD vs SpD" exhibited significant correlation for baking absorption (r=0.74, P<0.01), baking mix time (r=0.66, P<0.05), and loaf symmetry (r=0.001). However, SpD did not show significant correlation with StD and NoD for loaf volume indicating that SpD might not be sensitive enough to segregate whole wheat flour samples based on loaf volume. Whereas, "StD vs NoD" poses positive correlation for most of the bread qualities evaluated suggesting that StD and NoD might similar trend for evaluation of whole wheat flour breadmaking quality (Table 34).

Graybosch and his team (2013) investigate straight dough and sponge-and-dough method on wheat cultivars that produce over- or super-strong dough. They agreed that straight dough method exhibited the most obvious effect of overexpression such as dramatic increase in mix time than that of sponge-and-dough method. They concluded that "Comparison of the two

methods suggests that the straight dough procedure was actually more sensitive to differences". However, it should be noted that Graybosch et al. (2013) were using refined flour whereas, we were using whole-wheat flour. Another two articles also reported that greater loaf volumes were produced from transgenic flours made using straight dough method (Popineau et al. 2001; Vasil et al. 2001). It should also be pointed out that these two articles were using refined flour for their experiment. Although there might be some differences between refined flour and whole-wheat flour, based on our findings and agreement with other articles reviewed, we concluded that straight dough is the best experimental bread-making method for whole-wheat bread in order to see the differences between whole-wheat flour samples.

Table 33. Correlation coefficients between bread and flour qualities among different bread-baking methods.

	OS	LV	SV	CG	SY	FM
			Straight dough method			
PC	0.77^{**}	0.73^{**}	0.77^{**}	0.63^{*}	0.80^{**}	-0.72^{**}
GI	-0.75^{**}	-0.95^{***}	-0.95^{***}	-0.87^{***}	-0.89^{***}	0.93^{***}
WG	0.57^{NS}	0.90^{***}	0.89^{***}	0.86^{***}	0.94^{***}	-0.95^{***}
FWA	0.75^{**}	0.75^{**}	0.79^{**}	0.68^{*}	0.77^{**}	-0.73^{**}
FST	-0.21^{NS}	-0.56^{NS}	-0.52^{NS}	-0.52^{NS}	-0.64^{*}	0.66^{*}
GP	0.17^{NS}	0.52^{NS}	0.45^{NS}	0.43^{NS}	0.65^{*}	-0.61^{*}
P150	-0.63^{*}	-0.85^{***}	-0.83^{***}	-0.71^{*}	-0.99^{***}	0.90^{***}
P100	-0.33^{NS}	-0.56^{NS}	-0.59^{*}	-0.75^{**}	-0.24^{NS}	0.53^{NS}
P050	0.72^{**}	0.81^{**}	0.81^{**}	0.65^{*}	0.94^{***}	-0.82^{**}
PU50	0.72^{**}	0.66^{*}	0.70^{*}	0.53^{NS}	0.72^{**}	-0.63^{*}
			Sponge and dough method			
PC	0.64^{*}	0.75^{**}	0.75^{**}	0.56^{NS}	0.79^{**}	0.22^{NS}
GI	-0.51^{NS}	-0.61^{*}	-0.61^{*}	-0.40^{NS}	-0.78^{**}	-0.15^{NS}
WG	0.63^{*}	0.64^{*}	0.66^{*}	0.30^{NS}	0.84^{***}	-0.06^{NS}
FWA	0.57^{NS}	0.70^{*}	0.70^{*}	0.52^{NS}	0.74^{**}	0.25^{NS}
FST	-0.46^{NS}	-0.36^{NS}	-0.38^{NS}	-0.03^{NS}	-0.58^{*}	0.29^{NS}
GP	0.52^{NS}	0.44^{NS}	0.45^{NS}	0.20^{NS}	0.63^{*}	-0.32^{NS}
P150	-0.79^{**}	-0.82^{**}	-0.83^{***}	-0.56^{NS}	-0.97^{***}	0.04^{NS}
P100	0.29^{NS}	0.30^{NS}	0.27^{NS}	0.52^{NS}	0.05^{NS}	-0.18^{NS}
P050	0.80^{**}	0.89^{***}	0.89^{***}	0.68^{*}	0.96^{***}	0.09^{NS}
PU50	0.58^{*}	0.74^{**}	0.73^{**}	0.65^{*}	0.73^{**}	0.26^{NS}

OS=oven spring; LV=loaf volume; SV=specific volume; CG=crumb and grain; SY=symmetry; FM=firmness; PC=protein content; GI=gluten index; WG=wet gluten; FWA=farinograph water absorption; FST=farinograph stability; GP=gassing power; P150=particle size portion between 250-150µm; P100=particle size portion between 150-100µm; P050=particle size portion between 100-50µm; PU50=particle size portion less than 50µm; * Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; and NS=not significant.

Table 34. Correlation coefficients between bread-making methods and whole-wheat bread qualities.

	BAB	BMT	OS	LV	LW	SV	CG	CC	SY	FM
StD vs SpD	0.74**	0.66*	0.33NS	0.50NS	0.24NS	0.51NS	0.04NS	x.NS	0.95***	-0.09NS
SpD vs NoD	0.68*	0.65*	0.34NS	0.49NS	0.06NS	0.43NS	0.27NS	x.NS	0.32NS	-0.18NS
StD vs NoD	0.95***	0.71*	0.61*	0.68*	0.83**	0.68*	0.79**	-0.16NS	0.46NS	0.72**

StD=straight dough; SdD=sponge-and-dough; NoD=no-time dough; x=could not be calculated since the SpD method recorded the same crumb color score for all flour types; OS=oven spring; LV=loaf volume; SV=specific volume; CG=crumb and grain; SY=symmetry; FM=firmness; * Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; NS=not significant.

Experiment 2: Effect of Location and Cultivar on Whole-Wheat Bread-Making Quality

Environmental Conditions. Environmental conditions with respect to temperature and rain fall in growing season are given in Table 35. Each location received lower than average rainfall in the early part of the growing season. Temperatures were equal and slightly higher than normal average at each location in the early part of the growing season. Carrington had higher than average normal total precipitation compared to other location in the end of the harvesting period. Heavy rainfall during harvesting may result in sprouting wheat. Total precipitation was highest in Langdon and Hettinger with 276 and 272 mm respectively. Hettinger had the huge temperature variation compared to others (4 to 32°C). Whereas, Prosper had the driest condition for crop growth, with an average growing season temperature from 8 to 32°C and growing season total precipitation of 153 mm.

Location and Cultivars Effect on Whole-Wheat Bread Qualities. Straight dough was used to evaluate the 21 cultivars planted at 6 locations. Effect of location and cultivar on whole-wheat bread qualities were given in Table 36. Locations varied moderately in whole-wheat bread baking quality. Carrington had the lowest baking absorption (65%) while Casselton needed more water for baking absorption (79%) among other location. Whole-wheat bread baked from wheat planted in Minot and Hettinger had the lowest (654 cc) and highest (792 cc) loaf volume respectively. Crumb grain and texture for whole-wheat bread baked from wheat planted at 6 locations were not significantly ($P>0.05$) difference. In contrast, whole-wheat crumb color score possess high (7.6) and low (6.5) score for Hettinger and Dickinson respectively. Whole-wheat dough handling properties were the best for Hettinger with 9.6 score, with characteristics of easy-to-handle, easy-to-seam, and good machinability. Hettinger exhibited the better whole-

wheat bread baking qualities for loaf volume (792 cc), loaf symmetry (4.6), crumb color (7.6), and dough handling (9.6) compared to other locations evaluated.

Among cultivars, crumb color and dough handling properties for whole-wheat were not significantly difference (P>0.05). Generally, wide ranges for other whole-wheat bread quality parameters were observed across 21 cultivars. Baking absorption was highest in Barlow (77%) and lowest in Forefront (72%). Baking mix time was longest in Glenn (4.7 min) and shortest in ND901CL Plus (4.2 min). Brennan exhibited the lowest loaf volume (624 cc) while Faller was the highest (814 cc). Velva poses the lowest score (3.9) for crumb grain and texture while Breaker had the highest score (5.5). Low crumb grain and texture score was characterized by open grain, big rounded cells crumb, gummy and coarse texture. WB Mayville had lack of bread symmetry (2.9) compared to Glenn, which poses high bread symmetry (5.1) among all 21 cultivars.

About 89% of the variability in baking absorption in the present sample set can be related to location factor and only 7% to cultivar, and another 4% was error (Fig.36). Preston et al. (2001) and Finlay et al. (2007) found the effects of environment to be greater than that of genotype for farinograph absorption and baking water absorption, which is in agreement with our results for HRS water absorption. However, their findings were related to refined flour. In 2009, a study was done with 21 wheat cultivars to evaluate whole-wheat bread quality in Canada (Gélinas et al. 2009). They found that farming site was not significant (P>0.05) for farinograph absorption, and it should be point out that the study was done to evaluate organic farming practices on whole-wheat bread quality. Baking absorption is highly correlated with flour protein content, and wet and dry gluten content (Ohm and Chung 1999) for hard winter wheat refined flour samples. For dough strength (farinograph stability), Preston et al. (2001) found genotypic

effects were greater than those of environment. This is in contrast with our findings that error attributed by cultivar x location was greater (61% variability) than that of cultivar (16%) and location (23%) itself for baking mix time (Fig.36). This was likely due to the effect arising from bran component. Dough strength is largely determined by the interactions between polymeric proteins causing from disulfide linked proteins, and hydrogen-bonding aggregates play the main role in this structure (Aussenac et al. 2001). Among bran components, phenolic acids are known to disrupt the gluten network via inhibition of disulfide bond formation (Han and Koh 2011; Koh and Ng 2008). Fiber was also found to alter the protein molecular structure via inducing the changes in conformation of two α-helix protein complexes to form antiparallel-β-sheet structures (Nawrocka et al. 2016).

There was nearly half of variability for whole-wheat bread loaf volume was contributed by cultivars (47%), and only 25% to locations (Fig.36). Panozzo and Eagles (2000) was in agreement with our findings when they studied wheat cultivars in Australian environments. Kolster et al. (1991) found that differences in loaf volume between genotypes with different allele depending on environment in The Netherlands. Whole-wheat loaf symmetry variability was contributed by cultivars (41%), locations (24%), and residual error (35%). Whole-wheat loaf symmetry was correlated with whole-wheat flour fine particle size portion (Paper 1). Fine bran particle size resulted in relatively high loaf volume (Khalid et al. 2015; Noort et al. 2010). Bran from different cultivars possesses different physical and chemical character (Greffeuille et al. 2006) as well as different mechanical strength needed for size reduction (Greffeuille et al. 2007)

Table 35. Rainfall and temperature for the growing season at Carrington, Dickinson, Hettinger, Langdon, Minot, and Prosper, North Dakota in 2012.

Environment	Month	Rainfall (mm)		Temperature (^{o}C)			
		Total	Normal Total τ	Max.	Min.	Avg.	Normal Avg. τ
Carrington	May	51	61	20	7	13	12
	June	74	91	25	12	19	17
	July	31	81	29	16	22	20
	August	81	56	26	11	18	19
Dickinson	May	49	61	20	5	12	12
	June	56	81	26	11	19	17
	July	39	58	32	17	25	21
	August	32	34	28	12	20	21
Hettinger	May	56	61	20	4	12	12
	June	60	75	27	11	19	17
	July	100	51	32	16	24	21
	August	57	38	29	11	20	21
Langdon	May	37	69	18	6	12	11
	June	109	94	23	11	17	16
	July	87	76	28	15	21	19
	August	42	60	26	11	18	18
Minot	May	45	75	18	6	12	12
	June	78	91	24	12	18	17
	July	18	63	29	16	23	20
	August	25	46	27	13	20	20
Prosper ττ	May	46	68	23	8	15	13
	June	67	101	27	13	20	19
	July	16	81	32	17	24	20
	August	23	57	29	11	20	20

τ Based on 1990-2012 average; ττ Due to proximity of the location, data used for Casselton; Source: North Dakota Agriculture Weather Network (NDAWN 2016)

Table 36. Locations and genotypes effect on whole-wheat bread baking qualities

	BAB	BMT	LV	GT	SY	CC	DO
Locations							
Carrington	65.42	4.61	715.65	4.59	3.42	7.28	9.54
Casselton	79.47	4.14	734.78	4.91	4.41	7.51	9.27
Dickinson	76.81	4.58	731.90	4.62	4.16	6.47	9.60
Hettinger	74.81	4.40	792.20	4.80	4.61	7.58	9.62
Langdon	73.79	4.31	736.45	4.70	4.20	7.41	9.46
Minot	74.44	4.34	654.04	4.76	3.29	7.28	9.51
LSD (P=0.05)	0.62	0.18	28.98	NS	0.40	0.55	0.35
Genotypes							
Alsen	73.97	4.43	712.74	4.93	4.28	7.27	9.42
Barlow	76.58	4.43	775.17	5.05	4.51	7.24	9.55
Breaker	73.75	4.48	786.52	5.53	4.72	7.25	9.49
Brennan	73.60	4.36	623.84	4.25	3.05	7.28	9.61
Elgin	74.94	4.33	709.34	4.87	3.56	7.27	9.55
Faller	72.87	4.36	813.75	5.18	4.88	7.24	9.55
Forefront	71.87	4.43	780.46	5.24	4.22	7.25	9.45
Glenn	76.06	4.66	788.03	4.68	5.10	7.23	9.66
Howard	73.62	4.41	775.92	4.99	4.88	7.21	9.55
Jenna	73.77	4.38	725.99	4.62	4.07	7.25	9.55
Mott	73.01	4.53	703.69	4.70	3.86	7.24	9.53
ND 901CL Plus	75.31	4.16	659.11	4.07	3.27	7.26	9.47
NDSW 0612	75.00	4.21	709.34	4.68	3.71	7.27	9.34
Prosper	73.29	4.41	810.73	5.12	4.66	7.27	9.45
RB07	72.36	4.46	750.96	5.05	4.44	7.22	9.50
Rollag	76.07	4.28	652.60	4.37	3.05	7.30	9.18
Steele-ND	73.34	4.28	703.29	4.43	3.56	7.24	9.50
SY Soren	74.13	4.58	789.54	4.93	4.51	7.23	9.50
Vantage	74.95	4.36	657.89	3.94	3.27	7.29	9.50
Velva	73.15	4.48	716.15	4.43	3.78	7.26	9.55
WB Mayville	74.94	4.29	632.50	4.22	2.91	7.28	9.59
LSD (P=0.05)	1.11	0.28	52.99	0.69	0.71	NS	NS

BAB=baking absorption (14%mb); BMT=baking mix time (min.); LV=loaf volume (cc); GT=crumb grain and texture score (1-10); SY=loaf symmetry score (1-10); CC=crumb color score (1-10); DO=dough handling score (1-10); LSD=least significant difference.

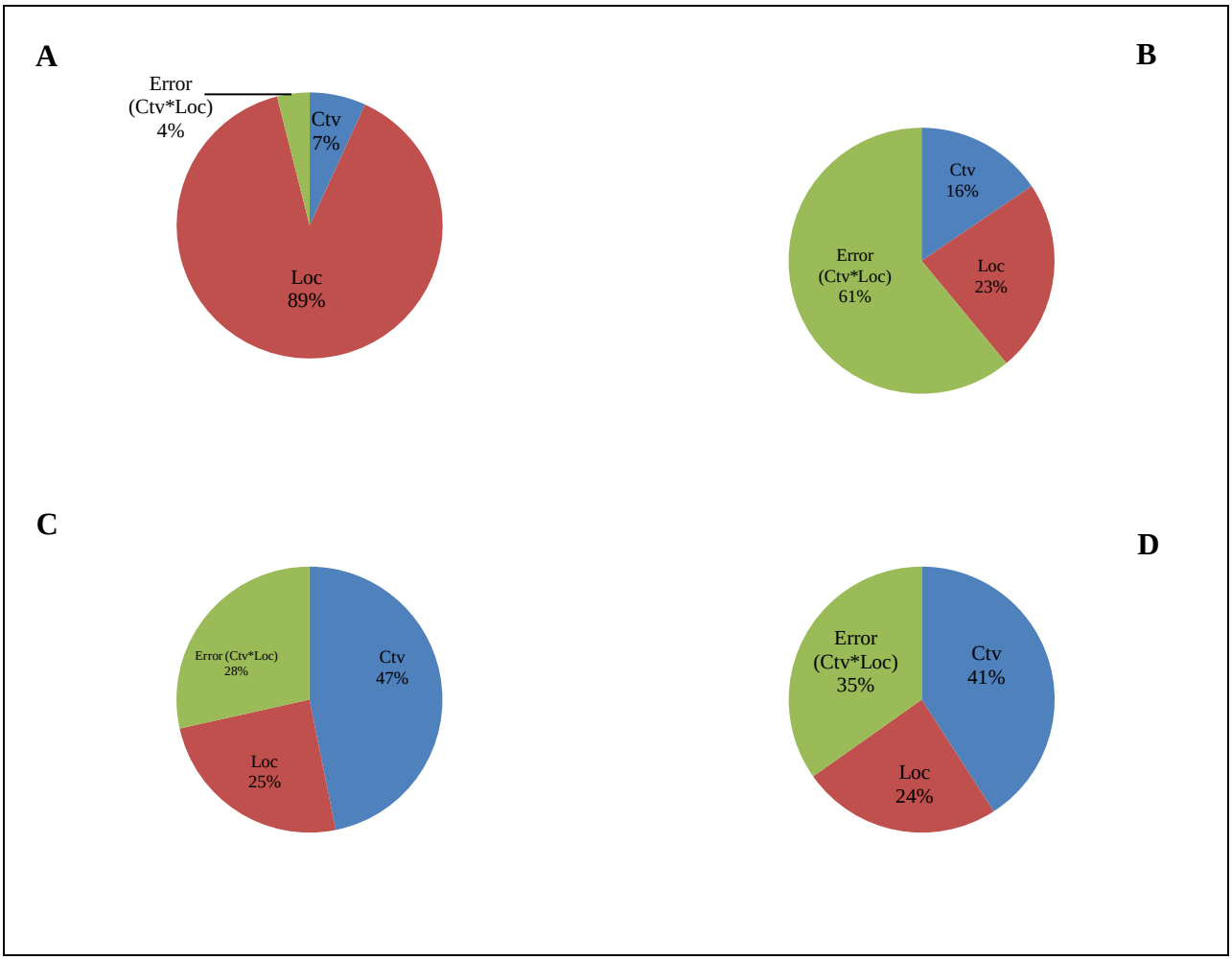

Figure 36. Contribution (%) of cultivars (Ctv), location (Loc), and the residual (error) variability (Ctv*Loc) to the whole-wheat bread baking qualities. (A) baking absorption; (B) baking mix time; (C) loaf volume; (D) bread symmetry.

The relatively high contribution of the cultivar to the whole-wheat bread quality observed here indicates that whole-wheat loaf volume and symmetry are stable evaluation parameters for evaluation of hard spring wheat genotypes in breeding program. A tendency toward high loaf volume and symmetry can be observed for the cultivars Faller (813 cc, 4.9), Prosper (811 cc, 4.7), Glenn (788 cc, 5.1), and SY Soren (789 cc, 4.5). However, more research needs to be conducted in multiple years of growing seasons before definitive conclusions can be made.

Relationship between Whole-Wheat Bread Baking Qualities. Significant linear correlation coefficients (r_s for simple, and r_c for cultivar) occurred among whole-wheat bread baking quality characteristics (Table 37) for phenotype and cultivars. Correlation among growing locations was not significant (P>0.05) among whole-wheat bread baking qualities, therefore no coefficient values were shown. The effects in cultivar and cultivar by locations had significant effects on association between loaf volume and symmetry (r_s=0.80, r_c=0.95, P<0.001). The high r_c value indicated that the correlations was influenced by variations caused by cultivars. Graybosch et al. (2013) found that loaf symmetry score for white bread was significantly higher in transgenic (very high in HMW-glutenin-subunits) wheat cultivars than that of the nontransgenic sample. Finlay et al. (2007) found highly significant genotypic effects (P<0.0001) for white bread loaf volume both within and across all growing locations. As explained above, cultivars Faller, Prosper, Glenn, and SY Soren tend to demonstrate high loaf volume across 6 locations. This is a classical indication of a relative high in the protein quality are the key factors in wheat bread-making performance (Bushuk and Scanlon 1993).

Table 37. Correlation coefficient between whole-wheat bread baking qualities

	BAB	BMT	DO	LV	SY	GT	CC
	Simple correlations						
BAB	-	-0.34 ***	-0.14 NS	0.02 NS	0.22 *	0.09 NS	-0.11 NS
BMT	-0.18 NS	-	0.39 ***	0.13 NS	0.18 NS	0.00 NS	0.15 NS
DO	-0.12 NS	0.42 NS	-	0.09 NS	0.14 NS	-0.05 NS	0.07 NS
LV	-0.29 NS	0.57 **	0.17 NS	-	0.80 ***	0.58 ***	0.27 **
SY	-0.23 NS	0.66 **	0.26 NS	0.95 ***	-	0.47 ***	0.29 **
GT	-0.38 NS	0.45 *	0.01 NS	0.85 ***	0.80 ***	-	0.25 **
CC	0.33 NS	-0.52 *	-0.47 *	-0.65 **	-0.71 ***	-0.50 *	-
	Correlations for cultivars						

Key

r_s
r_c

BAB=baking absorption (14%mb); BMT=baking mix time (min.); LV=loaf volume (cc); GT=crumb grain and texture score (1-10); SY=loaf symmetry score (1-10); CC=crumb color score (1-10); DO=dough handling score (1-10); * Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; and NS=not significant.

The simple correlations and correlations for cultivar showed opposite direction between whole-wheat bread loaf volume and crumb color score. The correlation between loaf volume and crumb color was positively for simple correlations (r_s=0.27, P<0.01) and negatively significant for cultivar correlation (r_c=-0.65, P<0.01). High protein content tends to have less whitish crumb color; however this is true for white bread. As for our case the whole-wheat bread, high protein content may be contributed by bran components apart from protein in endosperm, although the bran's protein may not be functional. Bran tends to gives bread crumb darker color or unpleasant appearance to consumer in markets. Bran chemical and physical characteristics associated with color were mainly ascribed to cultivars (Brouns et al. 2012; Finney et al. 1985; Gebruers et al. 2010b; Greffeuille et al. 2006; Greffeuille et al. 2007; Li et al. 2009; Mendis et al. 2013). Specifically, low score in whole-wheat bread crumb color was associated with high protein content for cultivars as indicated by the high r_c value between them.

Conclusion

The present investigation indicated that straight dough method with extra 10 min of proofing time was suitable for experimental whole-wheat bread-making in order to differentiate hard red spring wheat samples. Sponge-and-dough method is widely used commercially in the United States. However, it was not thought to be a suitable experimental bread-making method for quality evaluation of whole wheat flour since it is a great equalizer and showed no significant difference for crumb firmness and crumb grain and texture among whole-wheat bread made from different flour types. We also investigated variability of whole-wheat bread-making quality for 21 hard spring wheat cultivars grown at 6 locations across North Dakota. Whole-wheat bread made from grains planted in Hettinger region exhibited the highest whole-wheat bread baking qualities for loaf volume, loaf symmetry, crumb color, and dough handling compared to other locations evaluated. Cultivars were shown to have high contribution to the variability of whole-wheat loaf volume and loaf symmetry, showing 47% and 41% contribution respectively. Locations greatly contribute to the variability of whole-wheat baking absorption, showing 89% contribution. These results indicated that the whole-wheat bread quality, in terms of loaf volume and crumb color were largely under genetic control, and breeders can aim at achieving high loaf volume in hard spring wheat. However, more research needs to be conducted in multiple years of growing seasons before definitive conclusions can be made.

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OVERALL CONCLUSIONS

When examining the whole-wheat flour production, whole-wheat bread quality, and whole-wheat bread baking methods, several interesting conclusions can be made. These conclusions are related to the end-product quality, as well as bran component that affect the most on bread quality.

Tempering, rotor speed, and feed-rate influence the quality of whole-wheat flour produced by centrifugal mill. These factors altered the fine particle size distribution in the whole-wheat flour, varied the damaged starch content, changed the mixograph dough strength, and affected the baking parameters, which were dough handling score, loaf volume, and crumb score. However, with the proper utilization of milling procedure, it was possible to optimize the manufacturing of whole-wheat flour with desirable bread baking qualities.

Results of the whole-wheat milling indicated that a high fraction (70-90%) of fine particle size of whole-wheat flour produced from centrifugal mill resulted in whole-wheat bread with desirable bread qualities, such as high loaf volume, smooth crumb texture, and good dough handling properties. Combinations of low tempering moisture and high rotor speed on a centrifugal mill produced whole-wheat flour with low starch damage, low flour temperature, and low flour moisture content. Size reduction of bran, byproduct from roller milling, was successful with low tempering moisture, high rotor speed and low feed-rate. The ground bran had high yield of fine particle size portion. Flour adhering to bran impacted ground bran protein content, ground bran temperature, and total starch.

This study was also able to investigate the effect of bran components on flour, dough, and bread qualities. Extracted bran components (oil, extractable phenolics, hydrolysable phenolics, and fiber) showed prominent effect on quality parameters. Dough and bread made from whole-

wheat flour had low gluten index and loaf volume respectively, compared to white dough and bread. Bran's fiber component disturbed the protein conformation thus altered its functionality and possess low loaf volume and low gluten index. Bran's fiber also impacted the protein solubility in bread crumb. Interaction between fiber and hydrolysable phenolics resulted in low loaf volume. The protein solubility was greater with the interaction between fiber, hydrolysable phenolics, and extractable phenolics. Fiber altered the protein conformation, and phenolics prevent the disulfide linkages in gluten matrix. These resulted in fail functionality of gluten matrix thus provide low loaf volume and gluten index.

Three whole-wheat bread baking methods were evaluated in this study. Straight dough method with extra 10 min of proofing was the best method for producing whole-wheat bread for research purposes. This method recorded the highest variation in baking mix time, loaf weight, crumb grain score, and symmetry score for whole-wheat bread when using different types of whole-wheat flour. The high variation is needed to unveil differences between flour especially cultivars. Differences are needed to distinguish which flour or cultivars poses the best or worst for bread characteristics. In reference to effect of cultivar and location, cultivars showed high variability for loaf volume and symmetry. Location term contributed high variability to baking absorption.

Generally, whole-wheat flour with fine particle size, which is similar to refined flour particle size, can be produced using centrifugal mill with acceptable flour and bread qualities. Influence of bran components (oil, extractable and hydrolysable phenolics, and fiber) were very complex as they showed significant ($P<0.05$) interactions for all the components on flour, dough and bread qualities. The bran components impacted the protein solubility thus affecting the dough and bread parameters. Straight dough method with an extra 10 min of proof time was

suitable for whole-wheat bread baking for research purposes. This method was able to distinguish the differences between flour types or wheat cultivars used. Spring wheat cultivar contributed highly to the variability of whole-wheat loaf volume and loaf symmetry. Whereas, planting location contributed greatly to the variability of whole-wheat baking absorption.

FUTURE RESEARCH AND APPLICATIONS

To complement the study of bran milling, it would be interesting to blend the ground bran with the refined flour (producing whole-wheat flour) and evaluate its performance towards the flour, dough, and bread baking quality. For the whole-wheat milling study, lipoxygenase activities may be an interesting parameter to be measured along with analysis of phenolics content. Prediction of shelf life study on whole-wheat flour is another area to be focused on in the future as whole-wheat flour is known for a short shelf life due to lipoxygenase activity. Most studies were focused on blended whole-wheat flour and stone and/or hammer mill whole-wheat flour. Therefore, the use of different mills to produce whole-wheat flour would be another interesting area to explore.

For the reconstitution study on whole-wheat bread, it would be interesting to investigate further on protein changes during bread baking process. It would be interesting to see how the protein changes at each processing step (such as flour, after mixing, fermented dough, and bread). On the other hand, this particular study may be complemented by use of Scanning Electron Microscopic (SEM) imaging for each dough treatment, in order to see, at the microscopic level, how the gluten matrix, starch granules, and other bran components interact.

To complement the bread baking method study, sourdough method would be another interesting bread baking method to be evaluated. Sourdough method was normally used for rye pan bread, and rye was known with high in fiber content. More in depth visual evaluation with SEM images on mixed and fermented dough may reveal how the gluten matrix and starch granules appear to be in the dough system with different baking methods.

APPENDIX A. WHOLE-WHEAT FLOUR MILLING EXPERIMENT TABLES

Table A1. F-value for milling qualities obtained by centrifugal mill on whole-wheat flour milling experiment.

Dependent variable	Source of variation	Df	F-value	
Flour temperature (^{o}C)	Rep	2	5.94	**
	Feed Rate (FR)	1	4.06	*
	Rotor Speed (RS)	3	0.94	
	Seed Moisture Content (SMC)	3	11.55	****
	FR*RS	3	0.27	
	FR*SMC	3	0.27	
	RS*SMC	9	0.24	
	FR*RS*SMC	9	0.25	
Mill temperature (^{o}C)	Rep	2	9.41	***
	Feed Rate (FR)	1	25.22	****
	Rotor Speed (RS)	3	4.86	**
	Seed Moisture Content (SMC)	3	1.68	
	FR*RS	3	2.81	*
	FR*SMC	3	0.93	
	RS*SMC	9	1.50	
	FR*RS*SMC	9	1.53	
Particle Size – Coarse (%)	Rep	2	0.10	
	Feed Rate (FR)	1	11.76	**
	Rotor Speed (RS)	3	1.65	
	Seed Moisture Content (SMC)	3	2.43	
	FR*RS	3	1.65	
	FR*SMC	3	2.43	
	RS*SMC	9	2.69	*
	FR*RS*SMC	9	2.69	*
Particle Size – Medium (%)	Rep	2	0.33	
	Feed Rate (FR)	1	7.29	**
	Rotor Speed (RS)	3	2105.60	****
	Seed Moisture Content (SMC)	3	40.45	****
	FR*RS	3	1.42	
	FR*SMC	3	1.42	
	RS*SMC	9	15.10	****
	FR*RS*SMC	9	1.93	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom.

Table A1. F-value for milling qualities obtained by centrifugal mill on whole-wheat flour milling experiment (continued).

Dependent variable	Source of variation	Df	F-value	
Particle Size – Fine (%)	Rep	2	0.42	
	Feed Rate (FR)	1	7.25	**
	Rotor Speed (RS)	3	1067.67	****
	Seed Moisture Content (SMC)	3	26.85	****
	FR*RS	3	0.24	
	FR*SMC	3	3.9	*
	RS*SMC	9	13.29	****
	FR*RS*SMC	9	1.82	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom.

Table A2. F-value for flour qualities obtained by centrifugal mill on whole-wheat flour milling experiment.

Dependent variable	Source of variation	Df	F-value	
Flour moisture content (%)	Rep	2	1.16	
	Feed Rate (FR)	1	1.81	
	Rotor Speed (RS)	3	0.66	
	Seed Moisture Content (SMC)	3	25.26	****
	FR*RS	3	1.72	
	FR*SMC	3	1.63	
	RS*SMC	9	1.14	
	FR*RS*SMC	9	1.09	
Flour ash content (%)	Rep	2	22.08	****
	Feed Rate (FR)	1	0.64	
	Rotor Speed (RS)	3	4.62	**
	Seed Moisture Content (SMC)	3	11.14	****
	FR*RS	3	1.61	
	FR*SMC	3	1.08	
	RS*SMC	9	1.80	
	FR*RS*SMC	9	2.07	*
Flour protein content (%)	Rep	2	0.99	
	Feed Rate (FR)	1	4.04	*
	Rotor Speed (RS)	3	2.29	
	Seed Moisture Content (SMC)	3	2.14	
	FR*RS	3	3.13	*
	FR*SMC	3	1.33	
	RS*SMC	9	0.99	
	FR*RS*SMC	9	1.10	
Starch damage (14%mb)	Rep	2	75.12	****
	Feed Rate (FR)	1	201.43	****
	Rotor Speed (RS)	3	283.5	****
	Seed Moisture Content (SMC)	3	381.23	****
	FR*RS	3	59.11	****
	FR*SMC	3	4.17	**
	RS*SMC	9	21.48	****
	FR*RS*SMC	9	6.8	****

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom.

Table A2. F-value for flour qualities obtained by centrifugal mill on whole-wheat flour milling experiment (continued).

Dependent variable	Source of variation	Df	F-value	
Total starch (14%mb)	Rep	2	34.55	****
	Feed Rate (FR)	1	293.18	****
	Rotor Speed (RS)	3	3.63	*
	Seed Moisture Content (SMC)	3	7.12	***
	FR*RS	3	13.90	****
	FR*SMC	3	2.81	*
	RS*SMC	9	2.25	*
	FR*RS*SMC	9	10.66	****
Damaged starch in Total starch (14%mb)				
	Rep	2	25.89	****
	Feed Rate (FR)	1	772.87	****
	Rotor Speed (RS)	3	302.4	****
	Seed Moisture Content (SMC)	3	380.01	****
	FR*RS	3	110.88	****
	FR*SMC	3	3.92	*
	RS*SMC	9	25.85	****
	FR*RS*SMC	9	9.4	****

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom.

Table A3. F-value for mixogram data on whole-wheat flour milling experiment.

Dependent variable	Source of variation	Df	F-value	
Mid line Peak Time (Min.)	Rep	2	0.06	
	Feed Rate (FR)	1	1.91	
	Rotor Speed (RS)	3	5.04	**
	Seed Moisture Content (SMC)	3	9.54	****
	FR*RS	3	2.22	
	FR*SMC	3	1.95	
	RS*SMC	9	0.47	
	FR*RS*SMC	9	0.98	
Mid line Peak Value (%)	Rep	2	5.38	**
	Feed Rate (FR)	1	9.70	**
	Rotor Speed (RS)	3	5.05	**
	Seed Moisture Content (SMC)	3	12.5	****
	FR*RS	3	6.68	***
	FR*SMC	3	0.33	
	RS*SMC	9	0.45	
	FR*RS*SMC	9	0.43	
Mid line Right Value (%)	Rep	2	7.62	**
	Feed Rate (FR)	1	9.99	**
	Rotor Speed (RS)	3	4.02	*
	Seed Moisture Content (SMC)	3	11.01	****
	FR*RS	3	7.08	***
	FR*SMC	3	0.37	
	RS*SMC	9	0.44	
	FR*RS*SMC	9	0.53	
Total area under the midline curve (%Torque*Min)	Rep	2	1.97	
	Feed Rate (FR)	1	8.59	**
	Rotor Speed (RS)	3	6.15	**
	Seed Moisture Content (SMC)	3	19.39	****
	FR*RS	3	3.57	*
	FR*SMC	3	0.70	
	RS*SMC	9	0.24	
	FR*RS*SMC	9	0.24	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom.

Table A3. F-value for mixogram data on whole-wheat flour milling experiment (continued).

Dependent variable	Source of variation	Df	F-value	
Midline curve width measured after peak at 6min (%)	Rep	2	21.33	****
	Feed Rate (FR)	1	16.31	***
	Rotor Speed (RS)	3	8.72	****
	Seed Moisture Content (SMC)	3	1.31	
	FR*RS	3	2.58	
	FR*SMC	3	2.40	
	RS*SMC	9	1.04	
	FR*RS*SMC	9	1.10	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom.

Table A4. F-value for baking data on whole-wheat flour milling experiment.

Dependent variable	Source of variation	Df	F-value	
Baking Absorption (14% mb)	Rep	2	2.94	
	Feed Rate (FR)	1	2.99	
	Rotor Speed (RS)	3	3.43	*
	Seed Moisture Content (SMC)	3	1.63	
	FR*RS	3	1.77	
	FR*SMC	3	0.54	
	RS*SMC	9	2.27	*
	FR*RS*SMC	9	1.10	
Baking Mixing Time (Min.)	Rep	2	14.71	****
	Feed Rate (FR)	1	0.22	
	Rotor Speed (RS)	3	0.08	
	Seed Moisture Content (SMC)	3	2.29	
	FR*RS	3	1.14	
	FR*SMC	3	0.26	
	RS*SMC	9	0.23	
	FR*RS*SMC	9	0.91	
Dough Handling Properties	Rep	2	2.32	
	Feed Rate (FR)	1	0.19	
	Rotor Speed (RS)	3	5.11	**
	Seed Moisture Content (SMC)	3	2.33	
	FR*RS	3	1.83	
	FR*SMC	3	3.09	*
	RS*SMC	9	0.86	
	FR*RS*SMC	9	1.20	
Holes	Rep	2	0.95	
	Feed Rate (FR)	1	2.78	
	Rotor Speed (RS)	3	0.59	
	Seed Moisture Content (SMC)	3	1.18	
	FR*RS	3	0.69	
	FR*SMC	3	0.40	
	RS*SMC	9	0.60	
	FR*RS*SMC	9	0.74	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom.

Table A4. F-value for baking data on whole-wheat flour milling experiment (continued).

Dependent variable	Source of variation	Df	F-value	
Loaf Volume	Rep	2	0.47	
	Feed Rate (FR)	1	1.69	
	Rotor Speed (RS)	3	3.03	*
	Seed Moisture Content (SMC)	3	2.53	
	FR*RS	3	2.69	
	FR*SMC	3	1.19	
	RS*SMC	9	0.60	
	FR*RS*SMC	9	0.49	
Crumb Texture	Rep	2	10.10	***
	Feed Rate (FR)	1	2.93	
	Rotor Speed (RS)	3	1.46	
	Seed Moisture Content (SMC)	3	0.98	
	FR*RS	3	7.06	***
	FR*SMC	3	1.56	
	RS*SMC	9	0.73	
	FR*RS*SMC	9	0.31	
Symmetry	Rep	2	2.36	
	Feed Rate (FR)	1	0.10	
	Rotor Speed (RS)	3	6.31	***
	Seed Moisture Content (SMC)	3	0.70	
	FR*RS	3	0.45	
	FR*SMC	3	0.30	
	RS*SMC	9	0.85	
	FR*RS*SMC	9	0.59	

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom.

APPENDIX B. BRAN MILLING EXPERIMENT TABLES

Table B1. F-value for ground bran temperature (°C) and mill surface temperature (°C) on bran milling experiment.

Dependant Variable	Source	Df	F-Value	
Ground Bran	Rep (R)	2	0.71	
Temperature (°C)	Bran Cleaning (BC)	1	1.86	
	R*BC	2	45.47	****
	Feed rate (FR)	1	213.49	****
	Rotor speed (RS)	3	163.41	****
	Tempering moisture (TM)	3	14.84	****
	BC*FR	1	9.28	**
	BC*RS	3	1.49	
	BC*TM	3	2.01	
	FR*RS	3	3.95	**
	FR*TM	3	1.38	
	RS*TM	9	1.16	
	BC*FR*RS	3	5.85	***
	BC*FR*TM	3	6.68	***
	BC*RS*TM	9	1.69	
	FR*RS*TM	9	1.49	
	BC*FR*RS*TM	9	0.91	
Mill Surface	Rep (R)	2	1.03	
Temperature (°C)	Bran Cleaning (BC)	1	3.53	
	R*BC	2	106.98	****
	Feed rate (FR)	1	34.65	****
	Rotor speed (RS)	3	63.58	****
	Tempering moisture (TM)	3	21.78	****
	BC*FR	1	0.11	
	BC*RS	3	7.17	***
	BC*TM	3	3.46	*
	FR*RS	3	0.8	
	FR*TM	3	0.36	
	RS*TM	9	2.04	*
	BC*FR*RS	3	2.61	
	BC*FR*TM	3	7.11	***
	BC*RS*TM	9	3.78	***
	FR*RS*TM	9	3.82	***
	BC*FR*RS*TM	9	2.19	*

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom.

Table B2. F-value for medium (%) and fine (%) particle size portion on bran milling experiment.

Dependent Variable	Source	Df	F-Value	
Medium Particle Size (%)	Rep (R)	2	0.63	
	Bran Cleaning (BC)	1	0.80	
	R*BC	2	49.43	****
	Feed rate (FR)	1	82.31	****
	Rotor speed (RS)	3	127.94	****
	Tempering moisture (TM)	3	63.03	****
	BC*FR	1	0.01	
	BC*RS	3	4.88	**
	BC*TM	3	3.97	**
	FR*RS	3	12.40	****
	FR*TM	3	10.95	****
	RS*TM	9	5.48	****
	BC*FR*RS	3	10.21	****
	BC*FR*TM	3	1.05	
	BC*RS*TM	9	5.35	****
	FR*RS*TM	9	2.75	**
	BC*FR*RS*TM	9	6.11	****
Fine Particle Size (%)	Rep (R)	2	0.57	
	Bran Cleaning (BC)	1	0.54	
	R*BC	2	51.09	****
	Feed rate (FR)	1	127.01	****
	Rotor speed (RS)	3	249.29	****
	Tempering moisture (TM)	3	79.70	****
	BC*FR	1	1.26	
	BC*RS	3	4.68	**
	BC*TM	3	2.31	
	FR*RS	3	6.86	***
	FR*TM	3	9.62	****
	RS*TM	9	6.29	****
	BC*FR*RS	3	12.94	****
	BC*FR*TM	3	1.16	
	BC*RS*TM	9	5.67	****
	FR*RS*TM	9	2.79	**
	BC*FR*RS*TM	9	6.34	****

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom.

Table B3. F-value for ground bran moisture (%) and ash content (14% mb) on bran milling experiment

Dependent Variable	Source	Df	F-Value	
Ground Bran Moisture Content (%)	Rep (R)	2	0.03	
	Bran Cleaning (BC)	1	1.28	
	R*BC	2	114.01	****
	Feed rate (FR)	1	51.78	****
	Rotor speed (RS)	3	253.97	****
	Tempering moisture (TM)	3	249.07	****
	BC*FR	1	3.29	
	BC*RS	3	6.94	***
	BC*TM	3	4.69	**
	FR*RS	3	2.57	
	FR*TM	3	0.07	
	RS*TM	9	7.12	****
	BC*FR*RS	3	7.43	***
	BC*FR*TM	3	0.65	
	BC*RS*TM	9	2.29	*
	FR*RS*TM	9	0.88	
	BC*FR*RS*TM	9	0.82	
Ground Bran Ash Content (14% mb)	Rep (R)	2	3.13	
	Bran Cleaning (BC)	1	0.04	
	R*BC	2	45.72	****
	Feed rate (FR)	1	4.17	*
	Rotor speed (RS)	3	7.20	***
	Tempering moisture (TM)	3	2.83	*
	BC*FR	1	5.04	*
	BC*RS	3	4.05	**
	BC*TM	3	24.21	****
	FR*RS	3	1.87	
	FR*TM	3	1.20	
	RS*TM	9	2.24	*
	BC*FR*RS	3	4.61	**
	BC*FR*TM	3	3.42	*
	BC*RS*TM	9	2.06	*
	FR*RS*TM	9	1.05	
	BC*FR*RS*TM	9	0.86	

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom.

Table B4. F-value for protein (14% mb) and starch damaged (14% mb) content of ground bran on bran milling experiment.

Dependent Variable	Source	Df	F-Value	
Ground Bran Protein Content (14% mb)	Rep (R)	2	10.84	
	Bran Cleaning (BC)	1	1048.28	**
	R*BC	2	0.60	
	Feed rate (FR)	1	6.29	*
	Rotor speed (RS)	3	8.15	****
	Tempering moisture (TM)	3	40.97	****
	BC*FR	1	2.59	
	BC*RS	3	12.58	****
	BC*TM	3	69.83	****
	FR*RS	3	3.71	*
	FR*TM	3	17.11	****
	RS*TM	9	4.36	****
	BC*FR*RS	3	5.12	**
	BC*FR*TM	3	17.20	****
	BC*RS*TM	9	7.66	****
	FR*RS*TM	9	5.29	****
	BC*FR*RS*TM	9	1.60	
Ground Bran Starch Damaged (14% mb)	Rep (R)	2	1.72	
	Bran Cleaning (BC)	1	11.51	
	R*BC	2	14.18	****
	Feed rate (FR)	1	0.23	
	Rotor speed (RS)	3	29.45	****
	Tempering moisture (TM)	3	1.52	
	BC*FR	1	39.24	****
	BC*RS	3	0.78	
	BC*TM	3	1.04	
	FR*RS	3	2.11	
	FR*TM	3	0.57	
	RS*TM	9	1.49	
	BC*FR*RS	3	0.90	
	BC*FR*TM	3	1.18	
	BC*RS*TM	9	1.49	
	FR*RS*TM	9	1.37	
	BC*FR*RS*TM	9	1.16	

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom.

Table B5. F-value for starch damaged (14%mb) and total starch (14%mb) on bran milling experiment.

Dependent Variable	Source	Df	F-Value	
Ground Bran Total Starch (14% mb)	Rep (R)	2	14.54	
	Bran Cleaning (BC)	1	3107.18	***
	R*BC	2	2.08	
	Feed rate (FR)	1	4.69	*
	Rotor speed (RS)	3	17.19	****
	Tempering moisture (TM)	3	44.50	****
	BC*FR	1	97.39	****
	BC*RS	3	41.44	****
	BC*TM	3	51.62	****
	FR*RS	3	10.37	****
	FR*TM	3	3.93	*
	RS*TM	9	15.67	****
	BC*FR*RS	3	26.20	****
	BC*FR*TM	3	15.73	****
	BC*RS*TM	9	13.15	****
	FR*RS*TM	9	25.68	****
	BC*FR*RS*TM	9	21.30	****

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square.

Table B6. Ground bran particle size distribution as affected by four main factors[a].

Factors	COA (%, w/w)	MED (% w/w)	FINE (%, w/w)
Main plot bran cleaning process			
CB	1.69a	58.46a	38.22a
NC	1.44a	55.17a	40.89a
LSD	1.22	15.80	15.70
Tempering level			
10%	1.04d	52.67c	44.02a
12%	1.38c	55.53b	41.16b
14%	1.79b	56.53b	39.77b
16%	2.04a	62.53a	33.25c
LSD	0.22	1.46	1.43
Rotor speed			
6,000 rpm	5.61a	64.46a	28.37d
9,000 rpm	0.21b	58.41b	39.02c
12,000 rpm	0.17b	52.97c	44.68b
15,000 rpm	0.26b	51.43d	46.14a
LSD	0.22	1.46	1.43
Feed-rate			
6 g/min	0.85b	54.45b	42.43a
12 g/min	2.27a	59.18a	36.67b
LSD	0.16	1.03	1.01

[a]Mean ± standard deviation; n = 96 for main plot bran cleaning; n = 48 for tempering level; n = 48 for rotor speed; n = 96 for feed rate; values followed by the same letters within factors in the same column are not significantly different; CB = cleaned bran; NC = non-clean bran; COA = coarse (>425µm) particle size portion; MED = medium ($425 < x > 150$µm) particle size portion; FINE = fine (<150µm) particle size portion; LSD=least significant difference.

Table B7. Chemical composition of ground bran as affected by four main factors[a].

Factors	Moisture content (%)	Ash content (14%mb)	Protein content (14%mb)	Total starch (14%mb)
Main plot bran cleaning process				
CB	7.39a	5.02a	15.17b	11.17b
NC	7.77a	5.00a	15.73a	18.12a
LSD	1.44	0.40	0.07	0.54
Tempering level				
10%	6.93d	4.99b	15.31c	14.19c
12%	7.49c	5.01ab	15.36c	14.12c
14%	7.79b	4.99b	15.52b	15.29a
16%	8.09a	5.04a	15.61a	14.98b
LSD	0.09	0.04	0.06	0.24
Rotor speed				
6,000 rpm	8.11	4.98b	15.53a	14.48b
9,000 rpm	7.81	4.98b	15.45b	14.46b
12,000 rpm	7.41	5.06a	15.45b	14.46b
15,000 rpm	6.96	5.02a	15.37c	15.18a
LSD	0.09	0.04	0.06	0.24
Feed-rate				
6 g/min	7.46b	4.99b	15.48a	14.74a
12 g/min	7.69a	5.02a	15.42b	14.55b
LSD	0.06	0.03	0.04	0.17

[a]Mean ± standard deviation; n = 96 for main plot bran cleaning; n = 48 for tempering level; n = 48 for rotor speed; n = 96 for feed rate; values followed by the same letters within factors in the same column are not significantly different; CB = cleaned bran; NC = non-clean bran; LSD=least significant difference.

APPENDIX C. RECONSTITUTION EXPERIMENT TABLES

Table C1. F-value for farinograph water absorption (%) and development time (min.) on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Farinograph Water Absorption (14%mb)	OIL	1	63.79	****
	EP	1	38.18	****
	HP	1	411.67	****
	FB	1	2196.55	****
	OIL*EP	1	0.29	
	OIL*HP	1	4.87	*
	OIL*FB	1	29.58	****
	EP*HP	1	0.70	
	EP*FB	1	8.52	*
	HP*FB	1	15.68	***
	OIL*EP*HP	1	8.36	*
	OIL*EP*FB	1	0.08	
	OIL*HP*FB	1	17.69	***
	EP*HP*FB	1	0.03	
	OIL*EP*HP*FB	1	6.99	*
Farinograph Development Time (min.)	OIL	1	0.00	
	EP	1	0.57	
	HP	1	15.98	***
	FB	1	71.75	****
	OIL*EP	1	0.04	
	OIL*HP	1	2.20	
	OIL*FB	1	4.66	*
	EP*HP	1	0.15	
	EP*FB	1	26.79	****
	HP*FB	1	1.14	
	OIL*EP*HP	1	0.01	
	OIL*EP*FB	1	0.06	
	OIL*HP*FB	1	3.67	
	EP*HP*FB	1	1.45	
	OIL*EP*HP*FB	1	0.09	

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C2. F-value for farinograph stability (min.) and mixing tolerance index (BU) on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Farinograph Stability (min.)	OIL	1	0.06	
	EP	1	10.04	**
	HP	1	53.14	****
	FB	1	152.18	****
	OIL*EP	1	1.73	
	OIL*HP	1	0.26	
	OIL*FB	1	0.15	
	EP*HP	1	8.70	*
	EP*FB	1	6.93	*
	HP*FB	1	0.13	
	OIL*EP*HP	1	7.06	*
	OIL*EP*FB	1	2.28	
	OIL*HP*FB	1	0.26	
	EP*HP*FB	1	0.50	
	OIL*EP*HP*FB	1	13.55	***
Farinograph mixing tolerance index (BU)	OIL	1	2.04	
	EP	1	39.49	****
	HP	1	1.34	
	FB	1	102.84	****
	OIL*EP	1	1.34	
	OIL*HP	1	0.01	
	OIL*FB	1	0.19	
	EP*HP	1	8.04	**
	EP*FB	1	55.71	****
	HP*FB	1	21.21	****
	OIL*EP*HP	1	19.97	****
	OIL*EP*FB	1	3.72	
	OIL*HP*FB	1	0.87	
	EP*HP*FB	1	0.32	
	OIL*EP*HP*FB	1	28.42	****

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C3. F-value for farinograph time to breakdown (min.) and gluten index (%) on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Farinograph Time to Breakdown (min.)	OIL	1	0.46	
	EP	1	0.01	
	HP	1	4.99	*
	FB	1	7.63	**
	OIL*EP	1	0.06	
	OIL*HP	1	2.81	
	OIL*FB	1	3.79	
	EP*HP	1	0.24	
	EP*FB	1	22.59	****
	HP*FB	1	0.18	
	OIL*EP*HP	1	1.30	
	OIL*EP*FB	1	0.02	
	OIL*HP*FB	1	4.25	*
	EP*HP*FB	1	0.00	
	OIL*EP*HP*FB	1	1.82	
Gluten Index	OIL	1	47.61	****
	EP	1	51.63	****
	HP	1	6.05	*
	FB	1	43.78	****
	OIL*EP	1	0.39	
	OIL*HP	1	2.56	
	OIL*FB	1	21.39	****
	EP*HP	1	12.48	**
	EP*FB	1	41.70	****
	HP*FB	1	3.49	
	OIL*EP*HP	1	0.50	
	OIL*EP*FB	1	84.16	****
	OIL*HP*FB	1	7.47	*
	EP*HP*FB	1	3.13	
	OIL*EP*HP*FB	1	49.53	****

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C4. F-value for wet gluten and baking absorption (%) on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Wet Gluten (as is)	OIL	1	3.95	
	EP	1	0.09	
	HP	1	12.68	**
	FB	1	170.47	****
	OIL*EP	1	8.85	**
	OIL*HP	1	7.54	**
	OIL*FB	1	6.77	*
	EP*HP	1	1.83	
	EP*FB	1	0.08	
	HP*FB	1	5.45	*
	OIL*EP*HP	1	0.77	
	OIL*EP*FB	1	41.87	****
	OIL*HP*FB	1	0.28	
	EP*HP*FB	1	0.94	
	OIL*EP*HP*FB	1	0.04	
Baking Absorption (%)	OIL	1	21.91	****
	EP	1	25.78	****
	HP	1	162.20	****
	FB	1	1411.58	****
	OIL*EP	1	0.47	
	OIL*HP	1	0.36	
	OIL*FB	1	7.21	*
	EP*HP	1	0.04	
	EP*FB	1	1.67	
	HP*FB	1	7.77	**
	OIL*EP*HP	1	3.52	
	OIL*EP*FB	1	3.20	
	OIL*HP*FB	1	9.28	**
	EP*HP*FB	1	1.99	
	OIL*EP*HP*FB	1	0.55	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C5. F-value for baking mix time (min.) and dough handling score on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Baking Mixing Time (min.)	OIL	1	0.38	
	EP	1	2.96	
	HP	1	0.74	
	FB	1	0.97	
	OIL*EP	1	0.54	
	OIL*HP	1	1.22	
	OIL*FB	1	0.24	
	EP*HP	1	3.86	
	EP*FB	1	0.38	
	HP*FB	1	13.58	***
	OIL*EP*HP	1	0.24	
	OIL*EP*FB	1	0.14	
	OIL*HP*FB	1	0.06	
	EP*HP*FB	1	0.14	
	OIL*EP*HP*FB	1	2.55	
Baking Dough Handling Score	OIL	1	1.03	
	EP	1	0.11	
	HP	1	11.43	**
	FB	1	25.71	****
	OIL*EP	1	1.83	
	OIL*HP	1	0.11	
	OIL*FB	1	0.46	
	EP*HP	1	19.31	***
	EP*FB	1	0.46	
	HP*FB	1	0.11	
	OIL*EP*HP	1	0.00	
	OIL*EP*FB	1	2.86	
	OIL*HP*FB	1	0.00	
	EP*HP*FB	1	1.83	
	OIL*EP*HP*FB	1	1.03	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C6. F-value for baked weight (g) and loaf volume (cc) on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Baked Weight (g.)	OIL	1	0.25	
	EP	1	13.26	***
	HP	1	0.08	
	FB	1	903.82	****
	OIL*EP	1	1.77	
	OIL*HP	1	0.06	
	OIL*FB	1	0.72	
	EP*HP	1	0.00	
	EP*FB	1	0.46	
	HP*FB	1	15.23	***
	OIL*EP*HP	1	0.11	
	OIL*EP*FB	1	0.08	
	OIL*HP*FB	1	0.21	
	EP*HP*FB	1	0.00	
	OIL*EP*HP*FB	1	1.14	
Corrected Loaf Volume (cc)	OIL	1	0.77	
	EP	1	0.09	
	HP	1	14.53	***
	FB	1	186.35	****
	OIL*EP	1	0.24	
	OIL*HP	1	0.09	
	OIL*FB	1	0.24	
	EP*HP	1	1.09	
	EP*FB	1	0.47	
	HP*FB	1	1.16	
	OIL*EP*HP	1	1.02	
	OIL*EP*FB	1	0.42	
	OIL*HP*FB	1	0.42	
	EP*HP*FB	1	0.56	
	OIL*EP*HP*FB	1	0.38	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C7. F-value for specific volume (cc/g) and crumb score on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Specific Volume (cc/g)	OIL	1	0.51	
	EP	1	0.60	
	HP	1	13.92	***
	FB	1	271.32	****
	OIL*EP	1	0.49	
	OIL*HP	1	0.04	
	OIL*FB	1	0.13	
	EP*HP	1	1.08	
	EP*FB	1	0.08	
	HP*FB	1	3.25	
	OIL*EP*HP	1	0.83	
	OIL*EP*FB	1	0.56	
	OIL*HP*FB	1	0.26	
	EP*HP*FB	1	0.62	
	OIL*EP*HP*FB	1	0.22	
Crumb Score	OIL	1	4.35	*
	EP	1	0.00	
	HP	1	11.13	**
	FB	1	238.09	****
	OIL*EP	1	0.17	
	OIL*HP	1	1.57	
	OIL*FB	1	2.78	
	EP*HP	1	0.70	
	EP*FB	1	0.17	
	HP*FB	1	14.09	***
	OIL*EP*HP	1	0.17	
	OIL*EP*FB	1	0.70	
	OIL*HP*FB	1	6.26	*
	EP*HP*FB	1	1.57	
	OIL*EP*HP*FB	1	0.70	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C8. F-value for oven spring (inch) and proof height (inch) on reconstitution experiment.

Dependent Variable	Source	Df	F-Value	
Baking Oven Spring (inch)	OIL	1	0.43	
	EP	1	6.45	*
	HP	1	10.84	**
	FB	1	39.73	****
	OIL*EP	1	0.22	
	OIL*HP	1	1.99	
	OIL*FB	1	0.43	
	EP*HP	1	0.08	
	EP*FB	1	2.56	
	HP*FB	1	8.50	**
	OIL*EP*HP	1	5.53	*
	OIL*EP*FB	1	0.72	
	OIL*HP*FB	1	1.99	
	EP*HP*FB	1	0.22	
	OIL*EP*HP*FB	1	0.22	
Proof Height (inch)	OIL	1	0.57	
	EP	1	1.52	
	HP	1	5.11	*
	FB	1	265.62	****
	OIL*EP	1	0.04	
	OIL*HP	1	4.22	*
	OIL*FB	1	3.42	
	EP*HP	1	0.23	
	EP*FB	1	0.08	
	HP*FB	1	0.57	
	OIL*EP*HP	1	2.27	
	OIL*EP*FB	1	2.48	
	OIL*HP*FB	1	1.88	
	EP*HP*FB	1	0.38	
	OIL*EP*HP*FB	1	0.02	

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

Table C9. F-value for gassing power at 90 min. on reconstitution experiment

Dependent Variable	Source	Df	F-Value	
Gassing Power at 90 min. (psi)	OIL	1	25.77	****
	EP	1	1109.11	****
	HP	1	545.36	****
	FB	1	420.66	****
	OIL*EP	1	319.34	****
	OIL*HP	1	163.67	****
	OIL*FB	1	0.37	
	EP*HP	1	103.09	****
	EP*FB	1	23.75	****
	HP*FB	1	489.94	****
	OIL*EP*HP	1	10.56	**
	OIL*EP*FB	1	42.23	****
	OIL*HP*FB	1	4.99	*
	EP*HP*FB	1	47.67	****
	OIL*EP*HP*FB	1	42.23	****

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; **** Significant at P<0.0001; Df = degrees of freedom; MS = mean square; OIL=oil component; EP=extractable phenolics component; HP=hydrolysable phenolics component; FB=high fiber bran.

APPENDIX D. WHOLE-WHEAT BREAD BAKING METHOD EXPERIMENT TABLES

Table D1. F-value for baking absorption (%), mix time (sec.), loaf volume (cc), oven spring (inch), baked weight (g) and specific volume (cc) on whole wheat bread baking method experiment.

Dependent Variable	Source	F-Value	
Baking Absorption (%)	Method (M)	1014.00	****
	Rep*M	1.00	ns
	Flour (F)	568.50	****
	M*F	65.33	****
	Error	.	
Baking Mix Time (sec.)	Method (M)	166.94	****
	Rep*M	0.80	ns
	Flour (F)	21.38	****
	M*F	1.08	ns
	Error	.	
Loaf Volume (cc)	Method (M)	106.83	****
	Rep*M	0.85	ns
	Flour (F)	51.04	****
	M*F	10.54	****
	Error	.	
Oven Spring (inch)	Method (M)	41.72	***
	Rep*M	0.64	ns
	Flour (F)	20.08	****
	M*F	3.83	*
	Error	.	
Baked Weight (g)	Method (M)	29.67	***
	Rep*M	6.44	***
	Flour (F)	10.29	***
	M*F	2.49	ns
	Error	.	
Specific Volume (cc)	Method (M)	107.68	****
	Rep*M	0.88	ns
	Flour (F)	51.68	****
	M*F	10.56	****
	Error	.	

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom; MS = mean square.

Table D2. F-value for crumb grain score, color score, loaf symmetry and firmness on whole wheat bread baking method experiment.

Dependent Variable	Source	F-Value	
Crumb Grain Score (1-10)	Method (M)	38.38	****
	Rep*M	1.70	ns
	Flour (F)	46.78	****
	M*F	19.00	****
	Error	.	
Crumb Color Score (1-10)	Method (M)	6.00	*
	Rep*M	0.75	ns
	Flour (F)	0.75	ns
	M*F	1.13	ns
	Error	.	
Symmetry Score (1-10)	Method (M)	10.50	*
	Rep*M	0.60	ns
	Flour (F)	315.50	****
	M*F	57.50	****
	Error	.	
Firmness (g force)	Method (M)	0.51	ns
	Rep*M	0.93	ns
	Flour (F)	2.59	ns
	M*F	1.73	ns
	Error	.	

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom; MS = mean square.

Table D3. F-value for baking properties on genotype by location experiment.

Dependent Variable	Source	F-Value	
GT	Cultivar (C)	3.76	****
	Location (L)	2.18	ns
	Error (C*L)	.	
Absorption (%)	Cultivar (C)	10.88	****
	Location (L)	448.02	****
	Error (C*L)	.	
Mix Time (sec.)	Cultivar (C)	2.45	**
	Location (L)	8.62	****
	Error (C*L)	.	
Loaf Volume (cc)	Cultivar (C)	10.37	****
	Location (L)	18.15	****
	Error (C*L)	.	
Symmetry Score (1-10)	Cultivar (C)	7.70	****
	Location (L)	14.84	****
	Error (C*L)	.	
Crumb Color Score (1-10)	Cultivar (C)	0.95	ns
	Location (L)	4.93	***
	Error (C*L)	.	
Dough Handling Properties Score (1-10)	Cultivar (C)	1.45	ns
	Location (L)	2.38	*
	Error (C*L)	1.45	

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; **** Significant at $P < 0.0001$; Df = degrees of freedom; MS = mean square.