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New Technologies for Whole Wheat Processing: Addressing Milling and Storage Issues

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NEW TECHNOLOGIES FOR WHOLE WHEAT PROCESSING: ADDRESSING MILLING AND STORAGE ISSUES

by

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Whole wheat flour production and demand has increased dramatically during the last decade due to evidence supporting the benefits of whole grains in the diet. Hence, the food industry has provided a wide variety of new whole grain products. There are unique challenges that accompany whole wheat flour production, especially related to milling and storage. The present thesis provides new strategies on the adaptation of new technologies to overcome whole wheat processing issues. These issues are first discussed in a literature review and then followed by three research studies. In the first study, retail whole wheat flours were evaluated for particle size distribution to determine variations in currently available products. Significant differences were found for particle size distribution among and within brands. Compositional data elucidated differences in the degree to which the bran fraction of the kernel was milled. In the second study we aimed to produce whole wheat flour in the laboratory that could be used for end-use quality testing. We varied the moisture content during milling to produce flours with different particle size distributions and evaluated the functional properties of the flours. Mean particle size of the coarse fraction (>230 μm) decreased as moisture content decreased.

Wheat milled at lower moisture contents (i.e., 6.89-7.98%) provided flours with better functionality and mixing properties. In the third study, salts were added to wheat during tempering to reduce lipolytic activity in an effort to extend shelf life of whole wheat flour. This strategy was effective at inhibiting lipase, and provided flour with better baking properties than the control after 6 months of storage. The outcomes of these studies serve as new strategies for the production and evaluation of whole wheat flour.

To my family, my friends and to all the people who

believe in me as a promising scientist…

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"Keep your dreams alive. Understand that to achieve anything requires faith and belief in yourself, vision, hard work, determination, and dedication. Remember all things are possible for those who believe."

-Gail Devers

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All things are possible for those who believe…

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Preface

Introduction

Consumers have demanded more whole grain products during the last decade due to the known benefits associated with its consumption (e.g., the prevention of cardiovascular diseases and certain types of cancer such as colon cancer, reduction of the risk of type-2 diabetes and its prebiotic potential; McKeown, 2002; Vardakou, et al., 2008; USDA, 2010; Broekaert et al., 2011). Indeed, about 56% of grocery shoppers switched to whole grain bread, and the consumption of other whole grain options (e.g. black rice, whole grain crackers, and cereal bars) have been increasing as well (Sloan, 2011).

Due to this demand, the food industry has begun offering more whole grain products, with a 1960% increase in whole grain product launches in 2011 compared with 2000 (Mintel, 2011). Whole wheat flour production was about 2% of total wheat flour production in 2000 (Vocke et al., 2008) compared to 5% in 2010 (the latest data available; Sosland, 2011).

One of the issues of whole wheat flour production is the lack of well-established milling procedures. Whole grain flours are produced by a variety of techniques and result in flours with widely different particle sizes and functionalities (Kilhberg et al., 2004). Studies have established that particle size of the bran fraction has a remarkable influence on functional properties of the flour (Robertson and Eastwood, 1981; Mongeau and Brassard, 1982; Anderson and Estwood, 1987; Galliard and Gallagher, 1988, de Kock et al., 1999, Zhang and Moore, 1999).

Another issue with whole wheat flour utilization is shelf-life. Whole wheat flour is highly susceptible to rancidity due to the presence of lipolytic enzymes (Every et al., 2006), especially lipase (Pomeranz, 1988; Galliard, 1994; Tait and Galliard, 1988). This affects end-use and storage properties (Bell et al., 1979; Galliard, 1986a, 1986b, 1994; Galliard and Gallagher, 1988; Tait and Galliard, 1988; Hansen and Rose, 1996; Wang et al., 2004; Every et al., 2006), as it causes loss in flour functionality, nutritional quality and sensory acceptability during whole wheat flour storage. Thus, different strategies have been proposed to stabilize lipids in whole wheat flour. Most of them aim to the inactivation of lipase, as it would stop the degradation at early stages of storage (Galliard, 1994), although many of these strategies initiate non-enzymatic degradation of lipids (Cuendet et al., 1954; Vetrimani et al., 1990; Lehtinen et al., 2003; Rose et al., 2008).

Objectives and Hypotheses

The overall objective of this thesis was to address two important concerns of the milling industry: milling and shelf life of whole wheat flour, by the evaluation of available milling technologies and the implementation of new processing approaches. It was hypothesized that:

1) Since the production of whole wheat flour is not a standard process in the milling industry (Kihlberg et al., 2004; Doblado-Maldonado et al., 2012); it was hypothesized that a wide variation in particle size distribution among retail flour samples will be observed.

2) Particle size highly influences flour functionality (Robertson and Eastwood 1981; Mongeau and Brassard, 1982; Anderson and Estwood, 1987; Galliard and Gallagher, 1988; de Kock et al., 1999; Zhang and Moore, 1999). For instance, very large

bran particles (>600 μm) reduce breadmaking quality (Zhang and Moore, 1999). Since it is known that wheat with low moisture content provides flours with smaller particle size (Delcour and Hoseney, 2010); it was hypothesized that milling wheat kernels with low moisture content will provide whole wheat flour with enhanced baking quality compared with the traditional moisture content.

3) Metal ions have been shown to affect lipase activity (Barros et al., 2010). Hence, it was hypothesized that the addition of different salts at normal usage levels would reduce lipase activity and prolong shelf life of whole wheat flour by reducing hydrolytic rancidity.

To address these hypotheses, our first objective was to determine the differences in particle size distribution and composition of retail whole wheat flours, to determine variation in currently available products. Our second objective was to determine the functional properties of whole wheat flour that had been roller milled at different moisture contents in an effort to develop a method to produce whole wheat flour in the laboratory for end-use quality evaluation. The third objective was to design a method to inactivate lipase that did not also initiate non-enzymatic oxidation of whole wheat flour and prolonged functionality during storage.

Organization

This thesis is organized as follows: a literature review (Chapter 1) followed by manuscripts describing three research projects (Chapters 2, 3, and 4). Chapter 1 was published previously (Doblado-Maldonado et al 2012). Chapter 2 describes an assessment of retail brands of whole wheat flours in terms of particle size distribution and composition of fractions separated by sieving. Chapter 3 describes the effect of moisture

content prior to milling on the functional properties and particle size distribution of whole wheat flour. Chapters 2 and 3 have been formatted using the guidelines for *Cereal Chemistry*. Chapter 4 describes the development of a new strategy for the stabilization of whole wheat flour during storage by the inhibition of lipase. Chapter 4 has been formatted using the guidelines for *Food Chemistry*. References can be found at the end of each chapter.

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CHAPTER 1. KEY ISSUES AND CHALLENGES IN WHOLE WHEAT FLOUR MILLING AND STORAGE

1. ABSTRACT

Whole wheat flour is increasingly popular as research continues to reveal the benefits of whole grains and the food industry offers more whole grain options for consumers. The purpose of this review is to address milling and shelf-life issues that are unique to whole wheat flour. No standard methods are available for whole wheat flour milling, resulting in very different bran particle sizes. Literature suggests that moderate bran particle size is the best for bread production, while small particle size is better for non-gluten applications. Shelf-life of whole wheat flour is shorter compared to white flour due to the presence of lipids and lipid-degrading enzymes. Lipolytic degradation leads to reduction in functionality, palatability and nutritional properties. Strategies to stabilize whole wheat flour have focused on controlling lipolytic enzyme activity and have marginally succeeded.

Key words: bread, nutrition, bran, shelf-life, enzymes, stability.

2. INTRODUCTION

AACC International has defined whole wheat flour as being prepared from wheat (other than durum) such that the proportions of the intact grain—the bran, germ, and endosperm—remain unaltered (AACC International, 1999). Whole wheat flour contains substantially more vitamins, minerals, antioxidants and other nutrients than regular wheat flour, since these compounds are concentrated in the outer portions of the grain (Weaver, 2001). Some of these nutrients are replaced in the enrichment process of wheat flour, which is mandatory in 64 countries around the world (Flour Fortification Initiative, 2012), although many nutritional components are still lower, especially minerals and dietary fibre.

With the advent of modern roller mills during the industrial revolution, whole wheat flour production all but disappeared during much of the twentieth century. In the US, whole wheat flour production was about 2% of total wheat flour production in 2000 (Vocke et al., 2008) and only about 7% of the population consumed at least 3 servings of whole grains per day [US Department of Agriculture (USDA), 2000].

Food companies worldwide have responded to the mounting evidence supporting the benefits of whole grains with a 1960% increase in whole grain product launches in 2011 compared with 2000 (Mintel, 2011). In the US, the increase in whole grain food production nearly tripled whole wheat flour production from 2002-2011: 3.13×10^{8} kg (6.91×10^{6} cwts) in 2002-03 compared with 9.33×10^{8} kg (2.05×10^{7} cwts) in 2010-11 (1.8% and 5% of total wheat flour production, respectively; Sosland, 2011).

Whole wheat flour possesses several unique challenges to the milling and baking industries. For instance, whereas milling procedures for traditional flours have been well-

established, whole grain flours are produced by a variety of techniques and result in flours with widely different particle sizes and functionalities (Kihlberg et al., 2004). Furthermore, whole wheat flour contains more enzymatic activity (Every et al., 2006a), lipids (Chung et al., 2009), and antioxidants (Adom et al., 2005) than wheat flour, which can affect end-use (Galliard and Gallagher, 1988; Tait and Galliard, 1988; Wang et al., 2004; Every et al., 2006a) and storage properties (Bell et al., 1979; Tait and Galliard, 1988; Galliard, 1986a, 1986b, 1994; Hansen and Rose, 1996). The purpose of this review is to address these key issues—milling and shelf-life—and discuss strategies to overcome new challenges relative to increased whole wheat flour production.

3. WHOLE WHEAT FLOUR MILLING

3.1 Wheat selection

Wheat kernel physical characteristics, such as uniformity in kernel hardness and size, are important for milling traditional wheat flour because they maximize separation of the bran from the endosperm during roller milling (Li and Posner, 1987; Posner and Hibbs, 2005). These parameters may not be important for milling whole wheat flour, since separation of kernel components is not the goal.

Bran colour is one kernel attribute that is not often considered when milling wheat flour, but has a substantial impact on whole wheat flour. Wheat kernel pericarp color can vary from white to black or from red to blue, although most commercial wheats are classified as red or white. Most wheat produced in the US is red wheat; therefore, most flour, including whole wheat flour, is milled from red wheat. Although chemically similar (Table 1), whole grain flour produced from white wheats produces bread with a lighter colour and less bitter flavour, which is generally favored by consumers, compared

with red wheat flours (McGuire and Opalka, 1995). However, there are other factors to consider. For instance, in a sensory analysis of whole grain muffins made from white or red wheat, consumers perceived muffins made from red wheat as more healthy, even though nutritional composition of the two muffins was nearly identical (Camire et al., 2006).

On a genotypic level, selection of varieties of wheat for whole wheat flour production may pose some challenges. End-use quality attributes, such as water absorption and gluten strength, are an important part of wheat selection, although these analyses are typically performed only on wheat flour. Data from these tests may not accurately predict the performance of a variety of wheat in a whole wheat flour application. Bruckner et al. (2001) analyzed mixograph and baking properties in 11 winter and 12 spring wheat varieties grown in four locations using both wheat flour and whole grain flour. They found that, while correlations between flour and whole flour for many variables were significant, correlation coefficients varied widely. For instance, water absorption and loaf volume correlation coefficients ranged from 0.17 to 0.81 and from 0.08 to 0.72, respectively, depending on variety and crop year. Clearly the outer portions of the wheat kernel exert physical and chemical effects on dough properties that vary among different types of wheat (de Kock et al., 1999; Seyer and Gelina, 2009; Noort et al., 2010).

Because the outer portions of the wheat kernel affect baking quality by both physical and chemical means, quantifying these attributes may be important in selecting wheat varieties that are most appropriate for whole grain baking. For instance, bran friability, its ability to be reduced to small particle sizes, varies among cultivars

(Greffeuille et al., 2006) and cultivars with low friability produce higher quality bread (Seyer and Gelina, 2009). The outer portions of the kernel also contain various chemical compounds and enzymes that can affect baking properties (Joye et al., 2009), such as glutathione (Every et al., 2006b), phytate (Lehrfeld and Wu, 1991), ferulic acid (Adom et al., 2005), and lipoxygenase (Every et al., 2006a). Because these constituents are concentrated in the outer portions of the wheat grain, a given wheat variety may be acceptable for use in traditional baking but may adversely affect whole wheat baking.

Consumers of whole grain products are generally more health conscious than those that do not consume whole grain products. Therefore, it may be worthwhile to select wheat genotypes for whole grain applications based on desirable nutritional properties such as phytochemical and dietary fibre content (Ward et al., 2008; Gebruers et al. 2010). This is already done for some other grains, such as oats, where millers often select particular varieties of grain with high β-glucan content to support health-claims for heart health and cholesterol reduction.

3.2 Milling process

Perhaps the most important consideration in producing whole grain flour is selecting the milling process that will be used. Indeed milling technique may have a greater impact on whole wheat bread quality than the quality of wheat used for producing the flour or the formulation of the bread itself (Kihlberg et al., 2004). The two predominant techniques for grinding whole grain flours are stone and roller mills. Whole grain flours could also notionally be produced with an impact or hammer mill but this is rarely used (Kent and Evers, 1994).

3.2.1 Stone milling

Stone mills are the oldest attrition mills used for making whole grain flours, which simultaneously use compression, shear, and abrasion to grind wheat kernels between two stones and produce a theoretical extraction rate of 100% (Kihlberg et al., 2004). Modern stone mills are metal plates with composition stones attached (Posner and Hibbs, 2005).

Stone mills generate considerable heat due to friction. This can result in considerable damage to starch, protein, and unsaturated fatty acids in comparison with other milling techniques (Prabhasankar and Rao, 2001). Furthermore, in large, continuous milling operations, heat generated from stone milling can pose a fire risk.

Interestingly, there appears to be a marketing advantage by using the term "stone ground" with consumers, as evidenced by the preponderance of whole wheat flour products making this claim in both retail and commercial markets (Posner and Hibbs, 2005). Thus, some mills will "crack" the grain using a stone mill with the plates situated far enough apart to not generate excessive heat. Additional capital costs may be required to equip existing mills with such a set up. The cracked wheat is then reduced to flour on a roller mill.

3.2.2 Roller milling

The process of roller milling involves separation of the endosperm from the bran and germ followed by gradual size reduction of endosperm (Ziegler and Greer, 1971). In this process, wheat is passed through a series of corrugated and smooth rollers accompanied by sifting between stages. Producing flour that fulfills the requirement for being whole grain is achieved by blending bran and germ back with the endosperm flour

in the naturally-occurring proportions. Feeding the bran and germ milling streams with the endosperm flour stream is most often achieved in a continuous process, rather than collecting all fractions in separate bins and recombining at the end of milling. In this case, production of whole wheat flour would not involve additional capital expense beyond what is required for regular roller milling. Sometimes whole wheat flour is made by physically separating flour millstreams and then recombining at the end of the milling process. This is usually done when the bran will undergo some post-milling such as ultra fine grinding or heating. In these cases, capital costs would be required for the post-milling, plus equipment for recombining the fractions.

When producing whole wheat flour on roller mills, a number of conditions are different from those used for wheat flour (Kent and Evers, 1994). First, conditioning (tempering) is less important when milling whole wheat flour. While wheat flour relies on proper conditioning to facilitate endosperm and bran separation, this is not required for whole wheat milling. Thus, in theory no conditioning should be required, although many mills will add 1-2% moisture to soften the grain and improve efficiency in terms of the energy required to produce the flour. Efficiency can also be improved by tightening the roll gap and using more open scalp covers to increase the break release, as well as changing some of the smooth rolls to corrugated during reduction. The purifier air valves should also be adjusted so that the bran and germ are not rejected but are returned to the reduction system (Kent and Evers, 1994).

There are several noteworthy advantages of making whole grain flour from roller mills as opposed to stone mills. First, the amount of grinding and reduction at each roll can be adjusted to accommodate variations in raw materials, which makes roller milling

both economical and flexible (Posner and Hibbs, 2005). Second, the use of selective corrugations and differential speeds subjects the endosperm fraction to minimal shear and compressive forces during the grinding and reduction, which allows less heat to build on reduction rolls and results in less destruction to chemical components in the flour (Prabhasankar and Rao, 2001). A third advantage of making whole grain flours from roller mills is that wheat bran and germ can be separated from the endosperm fraction and subjected to further processing such as heating or fine grinding to affect the storage or functional properties of the flour (Posner and Hibbs, 2005).

3.2.3 Particle size

Differences in whole wheat milling practices are evident in a survey of 5 national brands of whole wheat flour (Figure 1). As shown, 43% of whole wheat flour from brand 1 passed through a sieve with a 0.230 mm opening, while <6% passed through this sieve in the other brands; brand 2 contained >10% of particles >0.841 mm, while the other brands had 1% or less of this particle size.

Particle size of the bran fraction in whole wheat flour has an amazing influence on functional properties of the flour. In general, large wheat bran particles (mean particle size of more than about 500 μm) lead to higher water absorption (Robertson and Eastwood, 1981; Mongeau and Brassard, 1982; Anderson and Eastwood, 1987) and loaf volume (Galliard and Gallagher, 1988; de Kock et al., 1999; Zhang and Moore, 1999) compared with finer bran particle sizes (mean particle size less than about 500 μm). However, if bran particles are too coarse (>600 μm), bread possesses a rough crust appearance and gritty texture (Zhang and Moore, 1999). Small particles have a greater negative impact on bread quality because chemical components in the bran can interact

more readily with gluten and inhibit development (Noort et al., 2010). However, from a nutritional standpoint, smaller particles could help in the release of vitamins and other components from the outer cells of the kernel (Kahlon et al. 1986). Thus, a moderate particle size (mean bran particle size of about 400-500 μm) may be the most desirable in whole wheat flour for bread production.

Products that do not require gluten development may have different particle size requirements compared with those that do. In an evaluation of 69 soft wheat cultivars for whole wheat cookie baking quality, cookie spread was influenced by whole wheat flour particle size (Gaines and Donelson, 1985). Small particles produced large cookies (more spread), while larger bran particles produced smaller cookies (less spread). Furthermore, bran particle size influences cake quality. For instance, cakes produced with up to 36% (flour weight) wheat bran of different particle sizes (50, 80, 250 μm) showed the greatest increase in firmness, chewiness, and yellowness when more coarse particle sizes were used. In a sensory evaluation, these changes were not favored by consumers; the best sensory acceptability was reached when finer particle sizes were used (Gomez et al., 2010).

4. WHOLE WHEAT FLOUR STORAGE

While not enough data exist to suggest a definitive shelf-life for whole wheat flour, it is well accepted that the shelf-life of whole wheat flour is considerably shorter than regular wheat flour. Flour millers stamp use-by dates of 3-9 months after milling on whole wheat flour packages, while regular wheat flour use-by dates range from 9-15 months after milling. Although these dates can be helpful, actual shelf-life could be shorter or longer depending on temperature and humidity during storage and on failure

endpoints (i.e., the point at which the flour is deemed unacceptable as determined by the company or the experimenter)

Whole grain flour storage is accompanied by a cascade of biochemical changes that lead to reduced flour functionality. The most unstable components in whole wheat flour are the lipids (Pomeranz, 1988). Lipid degradation is the predominant cause of the loss in flour functionality during whole wheat flour storage. Indeed, Tait and Galliard (1988) demonstrated that exchanging the lipids in fresh and stored whole wheat flour could completely account for the changes in flour functionality as a result of storage. Therefore, this section will emphasize whole wheat flour lipid degradation, with brief discussions on changes in other flour components.

4.1 Lipid degradation during whole wheat flour storage

Despite being a minor constituent of wheat flour, endogenous lipids contribute substantially to flour functionality. Bread made from defatted flour is inferior to bread with endogenous lipids, even when shortening is added during the mixing process (Bell et al., 1979; Moore et al., 1986). Upon mixing flour with water, lipids cannot be extracted from dough with common solvents due to binding with gluten proteins, which is essential for proper gluten development (Goesaert et al., 2005).

Lipids begin to break down in whole wheat flour by hydrolytic rancidity, which can be followed by oxidative rancidity. These changes can occur enzymically or non-enzymically and affect flour quality (Figure 2).

4.1.1 Hydrolytic rancidity

Hydrolytic rancidity in whole wheat flour proceeds through the action of lipase (O'Connor et al., 1992). Lipase (EC 3.1.1.3) hydrolyzes triacylglycerols to non-esterified

fatty acids and diglycerides, monoglycerides, and eventually glycerol; thus the release of non-esterfied fatty acids in whole wheat flour is related to lipase activity. Wheat lipase activity is mostly located in the bran fraction of the grain (Galliard, 1986a). There is an enzyme termed ‘wheat germ lipase’ that catalyzes deesterification of triacetin and other artificial water-soluble substrates (O'Connor et al., 1992) and thus is technically an esterase. True lipase activity (i.e., activity on water-insoluble substrates) of wheat germ lipase is likely a result of contamination with lipase from wheat bran (Galliard, 1994). Lipase exhibits maximum activity in wheat at about 17% moisture content; however, at moisture contents commonly observed in flour during storage (10-14%), lipase activity continues at about 50% of maximum (Figure 3). This property makes lipase unique among hydrolytic enzymes, i.e., lipase only requires a catalytic amount of water to act, whereas excessive amounts of water protect the lipid from being exposed to the catalytic site of the enzyme and reduce activity (Galliard, 1994).

Hydrolytic rancidity in whole wheat flour can lead to a decrease in sensory quality (Hansen and Rose, 1996) and functional properties of whole grain flour (Pomeranz, 1988; Galliard, 1994; Tait and Galliard, 1988). Weekly sensory evaluation of whole wheat flour for 11 weeks demonstrated that hydrolytic rancidity was inversely related to the acceptability of bread made from these flours (Hansen and Rose, 1996). Whole wheat flour with a high content of non-esterified fatty acids has been described as musty, bitter, and rancid (Heinio et al., 2002).

Products of hydrolytic rancidity have an effect on baking quality (Bell et al., 1979; Tait and Galliard, 1988). At low concentrations, non-esterified polyunsaturated fatty acids have a positive effect on loaf volume through co-oxidation of gluten protein

sulfhydryl groups during mixing. However, at high concentrations non-esterified polyunsaturated fatty acids affect dough mixing by reducing lipid binding capacity of gluten. This reduces gas holding capacity and elasticity of gluten (Miller et al., 1948; Carr et al., 1992). Interestingly, saturated fatty acids seem to have no effect on dough and baking properties (Bell et al., 1979). In addition to direct effects on bread quality, non-esterified polyunsaturated fatty acids are substrates for lipoxygenase, an enzyme that generates oxidation products that decrease the quality and acceptability of whole wheat flour (Loiseau et al., 2001).

4.1.2 Oxidative rancidity

Lipids can be oxidized in whole wheat flour enzymically (Galliard, 1986a, 1986b; Brash, 1999) or through autoxidation (Robards and Kerr, 1988). Enzymic lipid oxidation occurs through the action of lipoxygenase (EC 1.13.11.12). Lipoxygenase in wheat is located in the germ and bran of the grain (Galliard, 1994; Loiseau et al., 2001). It consists of a group of isozymes with a molecular mass of ~110 kDa and optimal activity at pH between 4.5 and 6.0 (Loiseau et al., 2001). Lipoxygenase attacks the methylene group between two double bonds in polyunsaturated fatty acids, preferentially non-esterified polyunsaturated fatty acids (Morrison and Panpaprai, 1975; Galliard, 1986a). Autoxidation can occur by non-enzymic reaction of grain lipids with atmospheric oxygen. Under both mechanisms, lipid oxidation involves addition of oxygen to polyunsaturated fatty acids, forming hydroperoxides (Loiseau et al., 2001), followed by fissure of the carbon chain into smaller, volatile compounds (e.g., epoxyaldehydes, ketones, lactones, furans; Robards and Kerr, 1988; McWilliams, 2005).

Lipid oxidation during storage of whole wheat flour is a much slower process than lipid hydrolysis (Galliard, 1994). This is because, unlike lipase, lipoxygenase exhibits very little activity at moisture contents typically found during storage (Wang et al., 1987), and because whole wheat flour contains high levels of protective antioxidants (Adom et al., 2005).

Despite being a slower process than lipid hydrolysis during storage, lipid oxidation can contribute substantially to loss of product quality. While minimally active in dry flour, lipoxygenase becomes active when stored flour is mixed with water and rapidly oxidizes non-esterified fatty acids present in the flour from the action of lipase (Galliard, 1986b). This is evident in Figure 4, which shows that whole wheat flour containing high non-esterified fatty acid content exhibits high oxygen consumption from lipid oxidation when the flour is mixed with water.

Oxidation of lipids can lead to a decrease in nutritional quality and consumer acceptability of whole wheat flour and whole wheat flour-based products. Lipid oxidation reduces nutritional quality through loss of essential fatty acids (Pokorny and Vilisek, 1995), although, more significantly, reduced nutritional quality is affected through co-oxidation of other flour components. Free radicals that are generated can denature proteins (Warwick et al., 1979) and convert essential amino acids into unavailable derivatives (Pokorny and Vilisek, 1995). Lipoxygenase activity also causes significant losses of carotenoids (Leenhardt et al., 2006) and vitamin E (Lentinen et al., 2003). Consumer acceptability of whole wheat flour declines as a result of lipid oxidation (Galliard and Gallagher, 1988; Tait and Galliard, 1988), which can generate undesirable

odor components that affect sensory acceptability of whole wheat flour-based products (Galliard and Gallagher, 1988; Heinio et al., 2002).

4.2 Protein degradation during whole wheat flour storage

Wheat proteins are unique due to their ability to form a viscoelastic dough. Wheat storage proteins (i.e., gliadin and glutenin) contain intra- and inter-molecular disulfide bonds that are important contributors to their functionality: gliadin is responsible for the cohesiveness of the dough and contains intra-molecular disulfide bonds, while glutenin provides elasticity to the dough and contains inter- and intra-molecular disulfide bonds (Veraverbeke and Delcour, 2002). Right after milling, flour proteins contain a high proportion of sulfhydryl groups and exhibit poor quality for bread making. Short-term (months) aging or chemical bleaching improves flour functionality through sulfhydryl/disulfide interchange among gluten proteins (mainly glutenin; Veraverbeke and Delcour, 2002; Goesaert et al., 2005).

During long-term storage, however, protein functionality is reduced. Wilkes and Copeland (2008) found an increase in wheat flour protein solubility over 270 d of storage at 30 °C. The most substantial increase was in the high molecular weight glutenin fraction. This may be a result of sulfhydryl/disulfide interchange with low molecular weight sulfhydryl compounds such as glutathione, which would decrease elasticity of dough. These effects may be more pronounced in whole wheat flour due to a higher glutathione content compared with wheat flour (Every et al., 2006b).

Changes in gluten functionality could also be a result of co-oxidation with lipids, due to close interactions between protein and lipid radicals (Dean et al., 1997). The properties of gluten are dependent on binding of lipid components from flour (Goesaret et

al., 2005); thus degradation of the lipids in whole wheat flour may result in poor gluten development. In addition, lipid oxidation can convert lysine, cysteine, methionine, and tryptophan into unavailable derivatives (Pokorny and Vilisek, 1995; Rehman and Shah, 1999).

4.3 Carbohydrate degradation during whole wheat flour storage

In wheat flour, resistance to stretching decreased in doughs made from three different flours stored for 24 months (Bell et al., 1979). This phenomenon could indicate modifications in protein, but also could be attributed to changes in the starch as a result of endogenous amylolytic activity (Rehman and Shah, 1999). Indeed, an increase in low molecular weight carbohydrates has been reported in whole wheat flour during storage (Marthe et al., 2002). Low molecular weight carbohydrates in dough increase bread crust coloration due to the Maillard reaction (Pomeranz, 1988). However, whole wheat bread made from flour or wheat stored for extremely long periods of time may exhibit more pale crust rather than a darker crust (unpublished observations while preparing bread for Rose et al., 2011). This may be due to reduction in amino acids available for the Maillard reaction. While this has not been shown conclusively, Rehman and Shah (1999) reported a reduction in total available lysine by 18% after storage of wheat at 25 ºC for 6 months.

Non-esterified fatty acids may also modify starch characteristics during storage. Salman and Copeland (2007) reported an increase in final viscosity of wheat flours heated in a Rapid Visco Analyzer over 12 months of storage at 20 and 30 °C. Iodine binding and non-esterified fatty acid analyses suggested the formation of amylose-fatty acid complexes during storage.

4.4 Degradation of other components during whole wheat flour storage

Wennermark and Jägerstad (1992) demonstrated a decrease in vitamin E activity by 40% during the storage of whole wheat flour at 20 °C for 12 months. Nielsen and Hansen (2008) showed similar results: 32% decrease in vitamin E over 297 d of storage at room temperature. Decrease of vitamin E content is associated with lipid oxidation (Lehtinen et al., 2003; Nielsen and Hansen, 2008). Carotenoids also oxidize during flour storage as a result of lipid oxidation (Arya and Parihar, 1981; Farrington et al., 1981). There was a 7.2-11.5 % reduction in thiamin during 12 months of storage of whole wheat flour under varying conditions (8-12 % moisture content; 10-32 °C; 25-55% relative humidity; Franz, 1968).

4.5 Strategies to improve whole wheat flour storage stability

As the above discussion illustrates, lipid degradation in whole wheat flour during storage is the major contributor to loss of product quality. The rate of lipid degradation can be reduced by cold storage. Indeed, compared with 20 °C, storage of whole wheat flour at -20 °C for 20 weeks has been shown to prevent the deleterious changes in lipids that accompany loss in whole wheat flour functionality (Tait and Galliard, 1988). Unfortunately, refrigerated transport and storage is probably cost prohibitive for the flour industry.

Other strategies to stabilize lipids in foods have included modified atmosphere and addition of antioxidants. These strategies may be more cost effective, but would likely be minimally effective. The former would be less effective because degradation of whole wheat flour lipids begins with hydrolytic rancidity, which is enzymatic and does not require oxygen. For instance, over 6 months of storage of whole wheat flour, no differences in non-esterified fatty acid development were observed at 25 °C and 35 °C

(Figure 5). When the flour was stored under abusive conditions (45 °C) a slight reduction in non-esterified fatty acids was observed after 6 months of storage without oxygen, although most likely a result of oxidation of non-esterified fatty acids in the sample with atmospheric oxygen in the headspace, rather than an actual decrease in release of non-esterified fatty acids (Rose et al. 2005).

Therefore, other strategies to stabilize lipids in whole wheat flour must be employed. The obvious strategy to control rancidity of whole wheat flour would be inhibition of lipase activity, the first step of lipid degradation (Figure 2). This would inhibit the generation of substrates for lipoxygenase during oxidative rancidity (Galliard, 1994).

A number of heat processing approaches have been explored to inhibit lipolytic activity in whole wheat flour. Since the lipase activity is concentrated in the bran, this fraction can be heated separately and then added to wheat flour in the proper proportions to make whole wheat flour (Rose et al., 2008). This allows for the inhibition of lipase without risking influencing the flour functional properties. Furthermore, in wheat, rice, and oats, lipase is more stable than lipoxygenase (O'Connor et al., 1992). Therefore, if lipase is denatured, lipoxygenase is also denatured. Vetrimani and Haridas Rao (1990) reduced lipase activity in wheat bran by 40% by heating at 175 °C for 40 min. Rose et al. (2008) reported 74, 93 and 96% reduction in lipase activity when wheat bran was dry heated at 175 °C for 25 min, 60 s of microwave (1000 W), or 60 s of steam, respectively.

The challenge with heat treatments to inactivate lipase is that they can easily promote autoxidation; that is, heat treatments that totally inactive lipase have resulted in flours that oxidize more rapidly than control flour (Cuendet et al., 1954; Molteberg et al.,

1995). Lehtinen et al. (2003) demonstrated that the majority of oxidation in heat treated oats was from polar, membrane-bound lipids, rather than storage triacylglycerols; thus, they suggested that premature lipid oxidation in heat treated flours was due to disintegration of membrane structures and inactivation of heat labile antioxidants.

As a result, other strategies that do not involve heat have been employed to inhibit lipase activity. Because the activity of lipase can be influenced by the presence of metal ions (Barros et al., 2010), Munshi et al. (1993) treated rice bran with $ZnCl_2$, $NiCl_2$, $FeCl_3$, or $CuCl_2$, by dissolving each salt in HCl or methanol and spraying the solution in a fine mist over the bran. The salts were applied at 25-200 µg of the metal ion/g bran. They found that the effectiveness of these salts against lipase activity during 10 d of storage was $NiCl_2$>$FeCl_3$>$ZnCl_2$> $CuCl_2$. The practical applicability of this approach may be limited, however, since $NiCl_2$ addition to whole wheat flour for food use would be unacceptable, and $FeCl_3$, while perhaps more nutritionally acceptable, would probably promote lipid oxidation (Huma et al., 2007). In a similar approach, Prabhakar et al. (1986) treated rice bran with HCl to reduce the pH. This decreased the non-esterified fatty acid concentration by about 80% comparing with an untreated bran after 30 d of storage at 25-30 ºC and 50-65% relative humidity. However, this treatment would likely affect flour functionality. Champagne and Hron (1994) treated rice bran with boiling ethanol vapors to denature lipase. Rice bran was placed in a vessel affixed with a condenser above and boiling ethanol beneath. They found that the vapors were effective in denaturing lipase, but the vapor extracted antioxidants as it passed through the bran, thus increasing susceptibility to lipid oxidation. Addition of citric acid or butylated hydroxytoluene to the ethanol helped stave off premature lipid oxidation.

Besides lipase inactivation, other strategies involving new processing technologies for food applications could be used as solutions to increase shelf-life of whole wheat flour. Marathe et al. (2002) tested gamma irradiation to extend shelf life of whole wheat flour. After 6 months of storage, chapaties, an Indian unleavened bread, made with whole wheat flour that had been treated with 0.25 kGy irradiation were significantly preferred over those made with untreated whole wheat flour.

5. CONCLUSIONS

Selection of wheat and milling technique may be different when producing whole wheat flour compared to wheat flour. Chemical components and physical properties of the outer portions of the wheat kernel influence baking properties. We are only beginning to understand these effects; more research is necessary to identify components with the greatest influence that can be manipulated to create whole wheat flours with optimum functionality.

During whole wheat flour storage, the products of lipase and lipoxygenase activity are the major culprits in the loss of sensory acceptability, nutritional value and functional quality. The strategy to control rancidity of whole wheat flour has been inhibition of lipase activity, thus halting or slowing the early steps of lipids degradation. Unfortunately, these approaches have been met with only marginal success.

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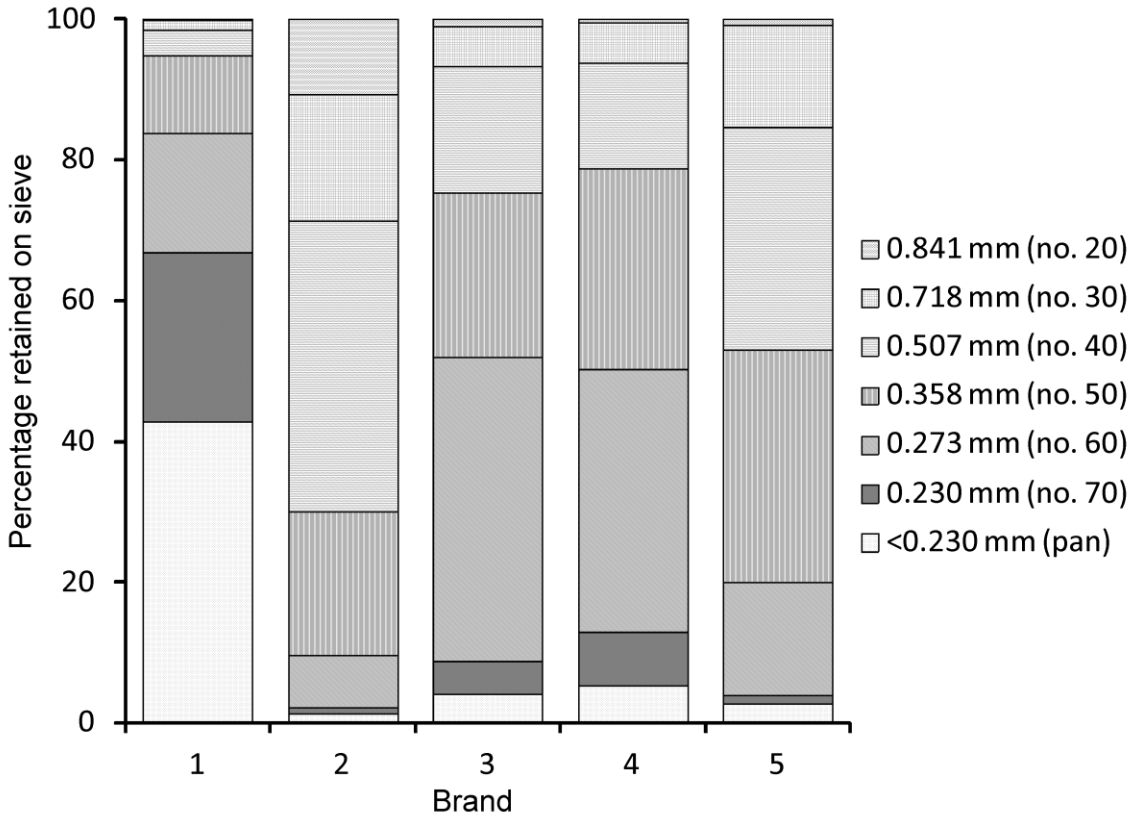

Figure 1. Particle size distribution of five brands of whole wheat flour obtained from a local market. From the packaging for each flour: brand 1 was "All natural premium whole wheat flour"; brand 2 was "Old-fashioned 100% stone ground all natural whole wheat flour"; brand 3 was "All natural whole wheat flour"; brand 4 was "100% stone ground whole wheat flour"; and brand 5 was "Premium 100% whole wheat flour". Flour (100 g) was separated on a sieve shaker (Model SS-15, Gilson Company, Lewis Center, OH) for 15 min and then the weight retained on each sieve was recorded according to the American Society for Testing and Materials standard methods (Pope and Ward, 1998); values represent the average of three replications.

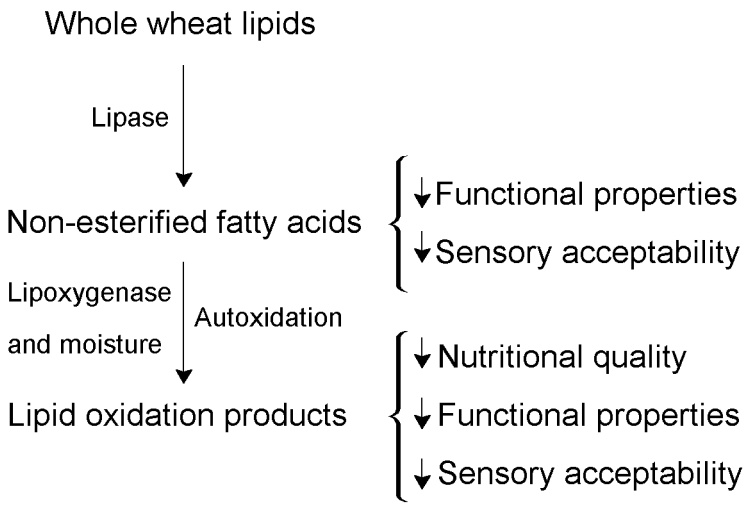

Figure 2. Causes and consequences of lipid degradation in whole wheat flour during storage.

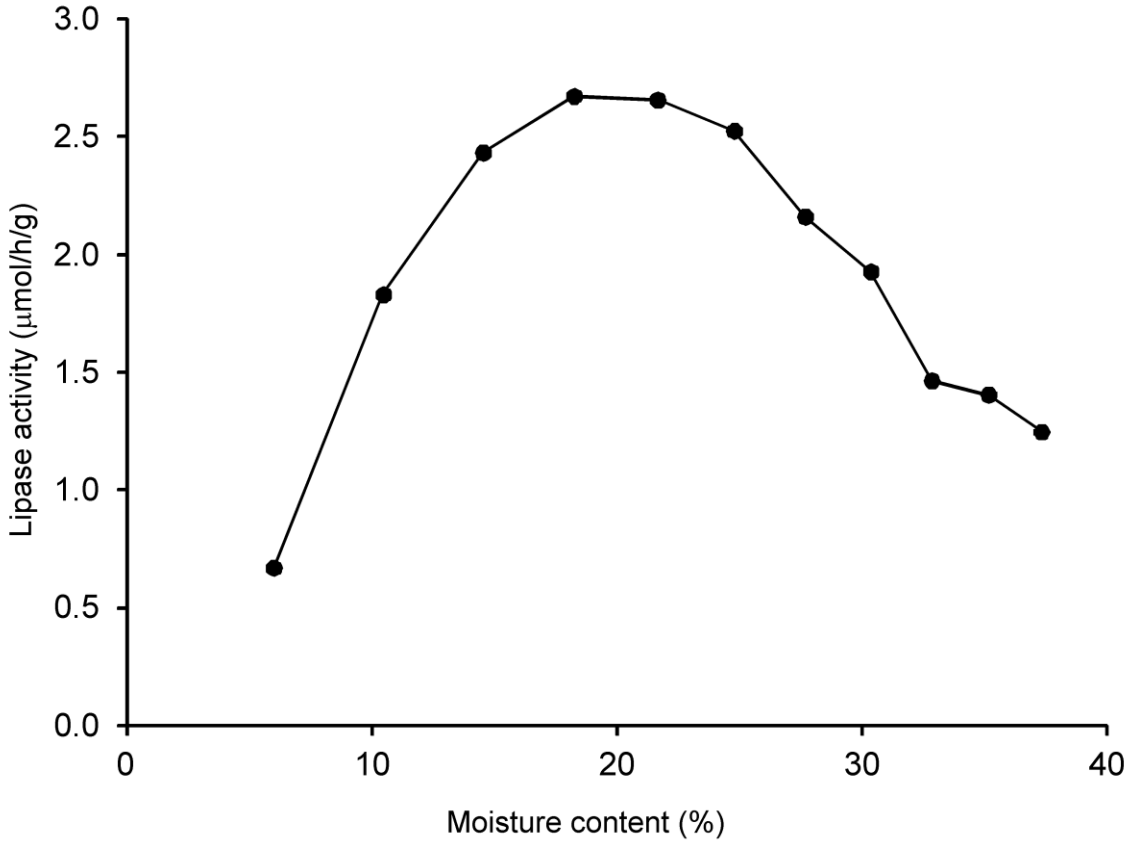

Figure 3. Lipase activity as a function of wheat bran moisture content; reprinted with permission from Rose and Pike (2006); copyright 2006 Springer.

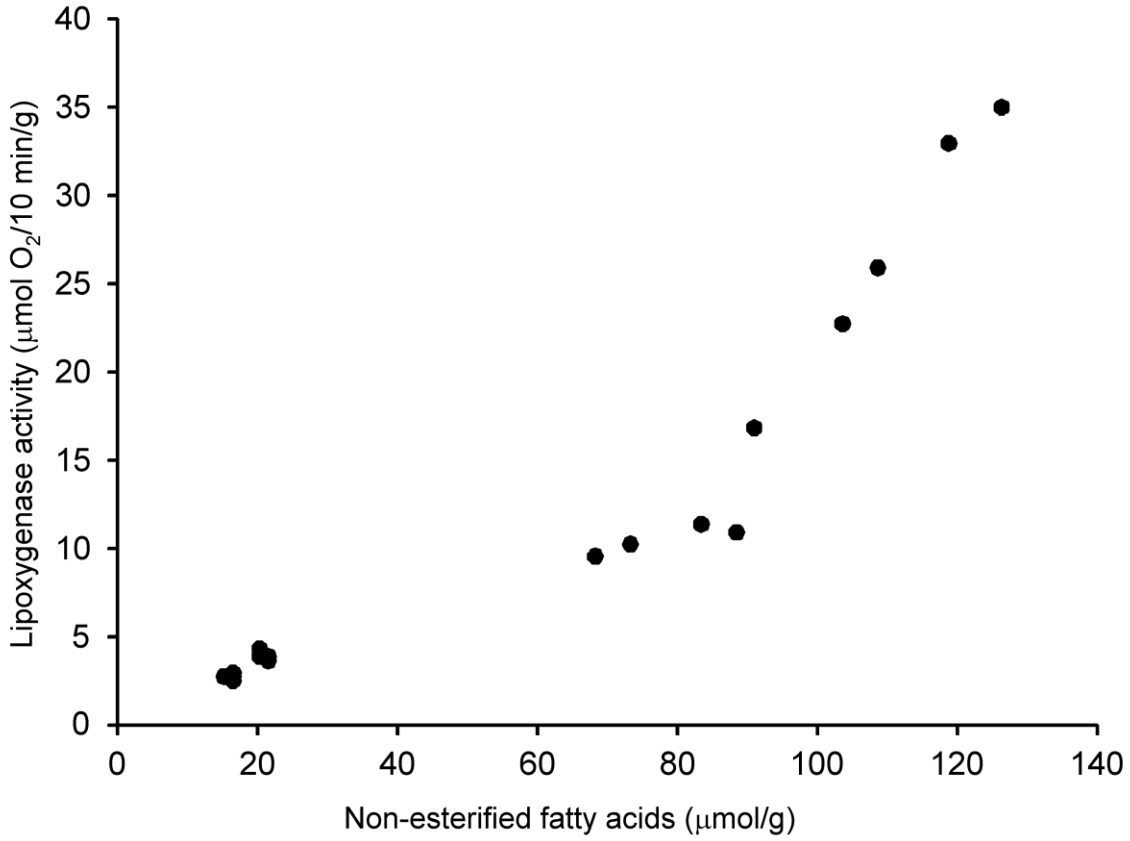

Figure 4. Lipoxygenase activity (LOX) in aqueous suspensions of whole wheat flour as a function of non-esterified fatty acid (NEFA) content; for linear relationship: LOX = 0.238*NEFA – 2.15 (R^2 = 0.87; p<0.0001); for quadratic relationship: LOX = 0.00365*$NEFA^2$ – 0.223*NEFA + 6.13 (R^2 = 0.98; p<0.0001); figure produced from data in Galliard (1986b).

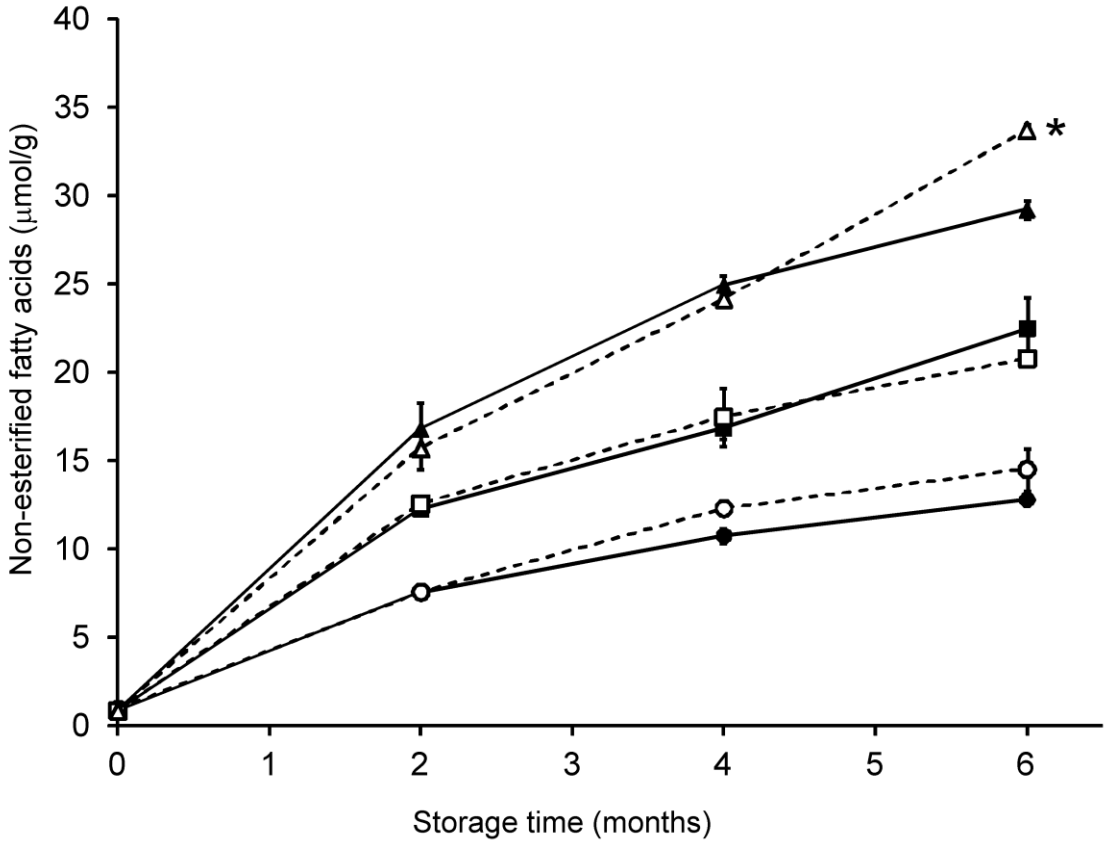

Figure 5. Non-esterified fatty acids in whole wheat flour (10.6% moisture) stored at 25 °C (circles), 35 °C (squares), and 45 °C (triangles) in foil-lined laminate pouches sealed with atmospheric oxygen (filled shapes) or with an oxygen absorber packet (Ageless, Mitsubishi Gas Chemical America, New York, NY USA) that reduced oxygen to <0.1% (as measured by a 3500-series oxygen analyzer, Illinois Instruments, Johnsburg, IL USA; open shapes); error bars show standard deviation; n = 2; some error bars were too small to plot; *indicates significantly different (Student's t-test; $p<0.05$) from the sample stored at that temperature under atmospheric oxygen at each time point (Rose et al., 2005).

CHAPTER 2. PARTICLE DISTRIBUTION AND COMPOSITION OF RETAIL WHOLE WHEAT FLOURS SEPARATED BY SIEVING

1. ABSTRACT

The purpose of this study was to evaluate the differences among four retail whole wheat flours with respect to particle size distribution and composition of fractions separated by sieving. Interestingly, not only were significant differences discovered among the brands for particle size distribution, but lots within two of the brands were significantly different ($p<0.05$), suggesting that flour particle size produced by the same company is not always consistent. Starch damage ranged from 5.72-7.69%. As expected, darker colors were associated with the larger particle size fractions, and the colors lightened as particle size decreased. This suggested that the differences in particle size were due to differences in the degree to which the bran fraction of the kernel was milled, an observation substantiated by the distribution of ash in each fraction, which ranged from 0.37-31.56%. Distribution of protein ranged from 0.19-61.81%. These data are relevant because differences in particle size distribution and composition affect functionality, sensory acceptability, nutritional properties, and shelf-life of whole wheat flour.

2. INTRODUCTION

Whole wheat flour is produced by many techniques (Kihlberg et al 2004; Doblado-Maldonado et al 2012). For instance, target milling moisture contents may differ; some whole wheat flours may be milled entirely on roller mills, while others may be produced by first cracking the grain on a stone mill; and the bran and shorts may be milled along with the endosperm or they may be physically removed from the endosperm and recombined after the milling process.

One of the key differences in whole wheat flours produced by different milling techniques is the different particle sizes produced (Kihlberg et al 2004). Particle size distribution affects functionality and baking quality of whole wheat flour-based products (Galliard and Gallagher 1988). For instance, coarse bran has been reported to result in higher loaf volume (de Kock et al 1999; Zhang & Moore 1999; Noort et al 2010), whereas fine bran provides cookies with greater spread (Gaines & Donelson 1985) and cakes with better texture and acceptability (Gomez et al 2010). Particle size may also influence glycemic response (Heaton et al 1988; Hallfrisch & Behall 2000), dietary fiber fermentation (Jenkins et al 1999; Stewart & Slavin 2009), and bioavailability of nutrients such as vitamins A and E (Kahlon et al 1986).

Whole wheat flour is defined in the US Code of Federal Regulations (Title 21, part 137B) as being prepared by grinding cleaned wheat (other than durum) so that at least 90% passes through a 2.36 mm sieve and at least 50% passes through an 850 μm sieve. However, from personal observation it was evident that particle size distributions of whole wheat flours on the market were dramatically smaller than would be required to pass this standard of identity. Therefore, the objective of this research was to assess the

differences among four retail whole wheat flours with respect to particle size distribution and composition (i.e., color, protein, and ash content) of fractions separated by sieving.

3. MATERIALS AND METHODS

3.1 Samples

Three lots from four different national brands of whole wheat flour [packaged in 5 lb. (2.2 kg) paper bags] were purchased from different markets in Lincoln, Nebraska, U.S.A. From the packaging for each flour: brand 1 was “all natural whole wheat flour”, brand 2 was “premium 100% whole wheat flour”, brand 3 was “old-fashioned 100% stone ground all natural whole wheat flour”, and brand 4 was “100% stone ground whole wheat flour”. Thus, based on the description and the mentioned milling technique, there were two noticeable groups of samples: “roller-milled” and “stone-milled”, each of them with two brands and three lots per brand. Samples were stored at room temperature for about one week prior to analysis.

3.2 Separation by sieving

Three samples of whole wheat flour (200g each) from each lot were separated on a sieve shaker (Model SS-15, Gilson Company, Lewis Center, OH) equipped with US Standard Sieves No 20, 30, 40, 50, 60 and 70 (i.e., >0.841mm, 0.718-0.841mm, 0.507-0.718 mm, 0.358-0.507 mm, 0.273-0.358 mm, 0.230-0.273 mm, <0.230 mm). Sample was shaken for 10 min and the weight retained on each sieve was recorded as g/kg of the total (Pope & Ward 1998). Weight distribution on each sieve was used to calculate mean particle size and geometric standard deviation (Ensor et al 1970).

3.3 Composition

Moisture content was determined in triplicate flour using Approved Method 44-19.01 (AACC International 2012). In a preliminary experiment, moisture did not differ among different sieved fractions from the same whole wheat flour (data not shown). Thus, moisture content of the whole flour was used to calculate dry weight of particles on each sieve.

Ash and protein contents were determined in triplicate on whole wheat flour starting material and in all seven particle size fractions (Approved Methods 08-01.01 and 46-30.01; AACC International 2012). A conversion factor of 5.85 was used to convert %nitrogen to %protein.

Color was determined by a Minolta Chroma Meter CR-300 (Minolta Camera Co., Ltd., Osaka, Japan). L*, a*, and b* scores were converted to white index after Chen et al (1999) and to RGB values using the EasyRGB Color Calculator software (Logicol, Color Technology).

Starch damage was determined in triplicate according to Approved Method 76-31.01 (AACC International 2012) using a starch damage assay kit (Megazyme, Bray, Ireland).

3.4 Experimental design and data analysis

Data were analyzed using SAS software (version 9.2, SAS Institute, Cary, NC, U.S.A.) using a generalized linear mixed model analysis of variance with Tukey's test, to determine significant differences between and within brands. To fit the response, a completely randomized design was used with three experimental units per brand (i.e., lots). Significance was defined as $P<0.05$.

4. RESULTS

4.1 Particle size distribution

Mean particle size of the tested brands of whole wheat flour were in the sequence of brand 3>2>1=4 (Table I). Brands 2 and 3 showed a lower percentage of particles <200 μm compared to the other brands, while about 60-65% and 45-50% of particles from brands 1 and 4, respectively, were <200μm (Figure 1A). Thus, brands 2 and 3 were coarser than brands 1 and 4. Although the mean particle size of roller milled samples (i.e., brands 1 and 2) was significantly lower than stone milled (i.e., brands 3 and 4) (mean particle size=193 and 274 μm, respectively; p=0.02), the brands did not appear to group by mill type: brands 1 and 4 and brands 2 and 3 appeared more similar than 1 and 2 (roller) and 3 and 4 (stone) (Figure 1).

Differences were also identified within brands. Mean particle size was significantly different within brands 2 and 3, ranging from 168-262 μm in brand 2 to 325-458 μm in brand 3 (Table I). The consistency in particle size distribution for brands 1 and 4 and the inconsistency in brands 2 and 3 were readily apparent (Figure 1).

4.2 Composition

Starch damage varied between and within brands (Table I). Brand 1 had the highest percent of starch damage and brand 3 had the lowest. Lower starch damage was obtained for the samples with the highest mean particle size and vice versa.

Color measurements showed that finer particles, in general, had a whiter color (Table II). The correlation coefficients between the particle size of a fraction and its white index for brands 1-4 were -0.82 ($p<0.001$), -0.45 ($p<0.05$), -0.95 ($p<0.001$) and -0.86 ($p<0.001$), respectively. Color distributions among particle size fractions were fairly

consistent among lots from the same brand, except for brand 2, where lot 2 was whiter than either of the other lots in four of the seven particle size fractions.

Ash retained per fraction varied considerably between brands (Table III). For instance, >30% of the total ash content was retained in the fraction with the smallest particle size in brands 1 and 4, while 4.61% of total ash was retained in the same fraction of brand 3. In larger particle size fractions, brand 3 showed ash retention of 24.9% for particles >841μm, whereas brands 2 and 4 presented values <2% in the same fraction. When evaluating the relationship between particle size and ash content, significant positive correlations were found for brands 1, 3 and 4 ($r=0.96$, $p<0.001$; $r=0.93$, $p<0.001$; $r=0.83$, $p<0.001$; respectively).

Protein content was higher than 16% in brands 1, 2 and 4; brand 3 had less than 13% protein (Table III). After comparing the different particle size fractions, differences were found within brand. In the case of brand 3, larger particle size fractions carried a higher content of protein, whereas smaller particle size fractions showed lower protein content. This trend was not as clear in the other brands.

5. DISCUSSION

In this study we evaluated the particle size distribution and composition of four nationally (US) available retail brands of whole wheat flour. As mentioned, according to the packaging of each whole wheat flour, brands 1 and 2 did not specify the type of mill used to produce the flour and were therefore assumed to be “roller milled” (since this is the most common industrial practice), while brands 3 and 4 indicated that they were “stone ground”. Upon inspection of the particle size distributions among brands, however, brand 1, for instance, was clearly more similar to brand 4 than brand 2 (Figure

1 and Table I). Thus, greater differences were found within mill type rather than between them. This is in opposition to Kihlberg et al (2004), who reported that roller milled whole wheat flour contained a greater proportion of the smallest and the largest particles compared with stone milled samples. The disparity between the present study and Kihlberg et al (2004) is likely because the distinction between roller milled and stone milled was taken from the packaging in the present study and from a controlled trial in Kilhberg et al (2004). Since "stone ground" is not a regulated term, the flours in this study could have been milled by any number of techniques, including coupling stone and roller milling (Doblado-Maldonado et al 2012). Because the roller milled and stone milled distinction did not appear to have a great influence on particle size distribution, this classification was not used further.

Differences in particle size distribution between brands may be attributed to many factors, including the type of wheat used, the milling technology, and the moisture content and environmental conditions during milling. For instance, bran friability, or the ability to be reduced to small particle size, inherently differs among wheat cultivars (Greffeuille et al 2006). Bran friability has been correlated with whole wheat flour functionality, with low friability providing bread with higher quality (Seyer & Gélinas 2009). Secondly, milling technology may also affect particle size distribution. Whereas roller mills include a reduction system to reduce particle size of flour while maintaining the bran particles in relatively large pieces, stone and hammer mills lack this system (Ziegler & Greer 1971; Posner & Hibbs 2005). Thirdly, wheat moisture content affects the friability of the bran, with high moisture content providing a pliable bran fraction that resists shattering (Delcour & Hoseney 2010).

Significant particle size differences were found within brands 2 and 3 (i.e., among lots; Figure 1 and Table I), suggesting that whole wheat flours produced by the same company are not always consistent. The differences found within brand may be attributed to the differences described above, but color data suggested that the class of wheat used may not even be consistent (Table II). For brand 2, lot 2 had a greater white index in most evaluated particle size fractions compared to the other two lots. These results suggested that brand 2 lot 2 may have been milled from white wheat, while the other two may have been milled from red wheat.

Decreasing percent ash as particle size decreased suggested that smaller particles were predominantly endosperm, while larger particles were dominated by bran. This was most evident in brands 1, 3, and 4 (Table III). Protein is also more concentrated in the outer layers of the wheat kernel (Piot et al 2000), but only brand 3 showed a consistent increase in protein content as particle size increased. This is likely because the difference in protein content between the outer kernel layers and the endosperm is not as great as the difference in ash content.

This study suggests that the production of whole wheat flour is not a standardized technique in the US, therefore different particle size distributions were observed between brands, and interestingly within some of them. Greater differences were found within mill type than between mill type, as indicated on the packaging material. Compositional data showed that the bran fraction of the kernel is milled to different extents among milling companies. Special attention towards this issue should be paid as functionality, shelf-life, acceptability, and nutritional properties may be affected.

6. ACKNOWLEDGMENTS

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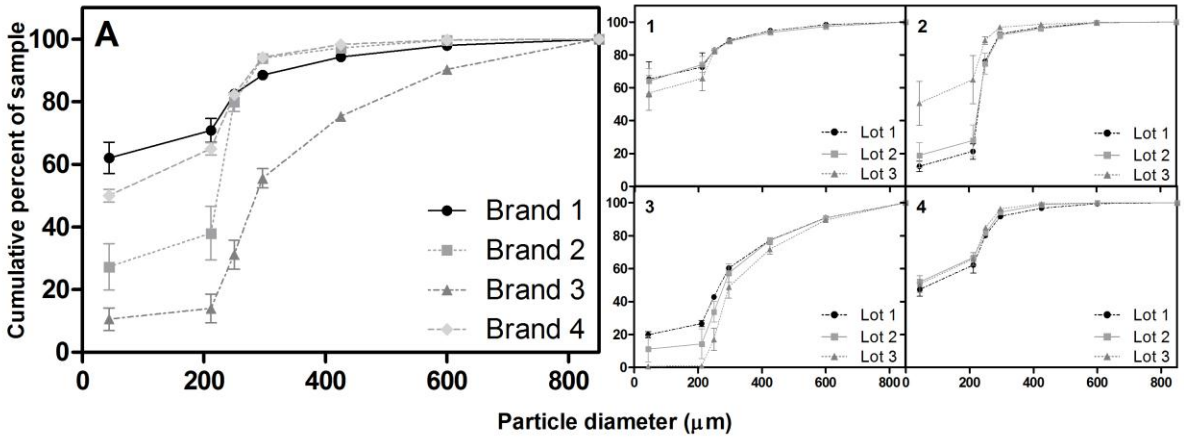

Figure 1. Differences in particle size distribution of four different national brands (A) and within three lots of each brand (1, 2, 3, and 4) of whole wheat flour.

Table 1. Mean Particle Size and Starch Damage in Whole Wheat Flour [a,b]

	Mean particle size (µm)				Starch damage (%)			
Brand	Lot 1	Lot 2	Lot 3	Mean	Lot 1	Lot 2	Lot 3	Mean
1	155 ± 2A	156 ± 2A	168 ± 2A	160 ± 23c	7.84 ± 0.61A	7.43 ± 0.40A	7.81 ± 0.38A	7.69 ± 0.19a
2	262 ± 2A	250 ± 2AB	169 ± 2B	227 ± 53b	5.67 ± 0.64B	7.66 ± 0.31A	5.46 ± 0.40B	6.26 ± 0.99b
3	325 ± 2B	376 ± 2A	458 ± 2A	387 ± 69a	5.50 ± 0.54A	3.94 ± 0.15B	4.56 ± 0.11B	4.67 ± 0.64c
4	179 ± 2A	137 ± 2A	167 ± 2A	161 ± 31c	5.74 ± 0.12A	5.26 ± 0.82A	6.17 ± 0.73A	5.72 ± 0.37b

[a]Means of triplicates ± standard deviation. Lot standard deviation includes the variation of triplicate measurements from the same lot, whereas the standard deviation for the mean value of each brand was calculated based upon the variation in the three lots.

[b]Means with different letters are significantly different ($P<0.05$). Upper case letters indicate differences between the lots of a given brand, whereas lower case letters indicate differences between the mean values of the four brands.

Table 1. Differences in color[a] and white indices[b] of whole wheat flour particle size fractions[c]

		White index[c]		
	Particle size	Lot 1	Lot 2	Lot 3
Brand 1	> 841 µm	57.85 ± 0.24Bd	58.86 ± 0.09Ae	57.47 ± 0.10Bd
	718 – 841 µm	58.66 ± 0.58Ad	58.47 ± 0.67Ae	58.02 ± 0.38Ad
	507 – 718 µm	60.70 ± 0.62Ad	61.35 ± 0.21Ad	59.18 ± 0.23Bcd
	358 – 507 µm	63.95 ± 1.58Ac	63.63 ± 0.45Ac	61.85 ± 0.88Ac
	273 – 358 µm	82.47 ± 3.52Ab	83.92 ± 0.36Ab	81.29 ± 3.64Ab
	230 – 273 µm	86.78 ± 0.26Aa	87.04 ± 0.04Aa	86.03 ± 0.07Ba
	< 230 µm	87.45 ± 0.08Aa	87.40 ± 0.11Aa	86.98 ± 0.13Ba
	Whole sample	82.97 ± 0.48	83.20 ± 0.64	83.13 ± 0.33
Brand 2	> 841 µm	64.08 ± 0.24Bd	83.82 ± 0.01Aa	62.23 ± 0.23Ce
	718 – 841 µm	81.39 ± 0.20Bab	83.23 ± 0.17Aa	81.45 ± 0.53Ba
	507 – 718 µm	80.49 ± 1.56Ab	79.35 ± 1.19Ac	76.00 ± 0.80Bc
	358 – 507 µm	73.25 ± 1.17Bc	79.77 ± 0.31Abc	68.44 ± 0.66Cd
	273 – 358 µm	81.00 ± 1.14Bab	84.22 ± 0.12Aa	78.44 ± 1.19Cb
	230 – 273 µm	81.99 ± 0.14Aab	81.94 ± 0.73Aabc	77.04 ± 2.02Bbc
	< 230 µm	82.77 ± 0.21Aa	82.17 ± 3.05Aab	82.70 ± 0.14Aa
	Whole sample	81.19 ± 0.24	82.52 ± 0.07	81.38 ± 0.06
Brand 3	> 841 µm	58.36 ± 0.69Be	60.01 ± 1.20ABe	61.27 ± 1.47Ad
	718 – 841 µm	62.56 ± 1.27Bd	64.64 ± 1.19Bd	69.60 ± 1.07Ac
	507 – 718 µm	70.87 ± 2.97Ac	78.28 ± 3.38Ac	78.11 ± 2.80Ab
	358 – 507 µm	80.63 ± 1.55Bb	83.18 ± 1.09ABb	85.66 ± 0.30Aa
	273 – 358 µm	86.67 ± 0.18Ba	87.77 ± 0.05Aa	87.28 ± 0.30Aa
	230 – 273 µm	85.89 ± 0.31Aa	86.03 ± 0.38Aab	85.08 ± 0.69Aa
	< 230 µm	87.87 ± 0.18Ba	88.63 ± 0.19Aa	87.47 ± 0.22Ba
	Whole sample	84.54 ± 0.90	85.24 ± 0.44	84.89 ± 0.45
Brand 4	> 841 µm	55.90 ± 0.37Ce	57.73 ± 0.17Bf	60.79 ± 1.08Ae
	718 – 841 µm	57.16 ± 0.15Bde	58.37 ± 0.60Af	57.70 ± 0.56ABf
	507 – 718 µm	58.83 ± 0.21Ad	59.51 ± 0.18Ae	58.99 ± 1.23Aef
	358 – 507 µm	67.26 ± 2.62Ac	64.91 ± 0.55ABd	62.79 ± 0.20Bd
	273 – 358 µm	77.07 ± 1.06Ab	73.59 ± 0.58Bc	75.61 ± 1.20ABc
	230 – 273 µm	78.20 ± 0.31Ab	77.40 ± 0.34Ab	77.63 ± 0.99Ab
	< 230 µm	84.48 ± 0.31Ba	84.99 ± 0.08Aa	84.34 ± 0.02Ba
	Whole sample	81.39 ± 0.65	82.14 ± 0.17	81.10 ± 0.57

[a]Color of cell (online version) represents the actual color of the obtained fraction.

[b]White index=$100-\sqrt{((100-L)^2+a^2+b^2)}$

[c]Means of triplicates ± standard deviation; means with different letters are significantly different ($P<0.05$); upper case letters indicate differences between the lots of a given brand fraction, whereas lower case letters indicate differences between the fractions of a given lot.

Table 2. Differences in ash and protein of fractions of retail whole wheat flour [a,b]

	Particle size	Ash content (%)	Ash retained (% of total ash)	Protein content (%)	Protein retained (% of total protein)
Brand 1	> 841 µm	7.36 ± 0.21a	8.16	17.89 ± 0.25c	2.22
	718 – 841 µm	6.79 ± 0.09ab	13.4	18.75 ± 0.15b	4.13
	507 – 718 µm	6.26 ± 0.06b	19.7	19.50 ± 0.31a	6.57
	358 – 507 µm	4.14 ± 1.01c	14.0	19.84 ± 0.06a	7.26
	273 – 358 µm	1.57 ± 0.25d	9.46	16.97 ± 0.04d	11.9
	230 – 273 µm	0.75 ± 0.23d	3.61	16.31 ± 0.16e	9.17
	< 230 µm	0.95 ± 0.26d	31.6	16.45 ± 0.12e	61.8
	Total content (%)	1.63 ± 0.13	100	16.50 ± 0.13	100
Brand 2	> 841 µm	1.28 ± 0.04bc	0.37	16.59 ± 0.09abcd	0.41
	718 – 841 µm	1.65 ± 0.09b	3.01	17.09 ± 0.29ab	2.61
	507 – 718 µm	2.37 ± 0.27a	5.49	17.17 ± 0.24a	3.36
	358 – 507 µm	2.22 ± 0.31a	21.7	16.55 ± 0.39bcd	14.0
	273 – 358 µm	1.28 ± 0.12bc	38.0	16.22 ± 0.34cd	41.5
	230 – 273 µm	1.29 ± 0.09bc	10.2	15.97 ± 0.29d	10.5
	< 230 µm	1.03 ± 0.03c	21.2	16.71 ± 0.17abc	27.7
	Total content (%)	1.42 ± 0.02	100	16.50 ± 0.14	100
Brand 3	> 841 µm	4.58 ± 0.13a	24.9	17.08 ± 0.49a	12.9
	718 – 841 µm	3.51 ± 0.16b	29.3	16.33 ± 0.32a	18.9
	507 – 718 µm	1.77 ± 0.12c	19.5	13.40 ± 0.43b	20.5
	358 – 507 µm	0.85 ± 0.14d	11.7	11.95 ± 0.51c	22.3
	273 – 358 µm	0.90 ± 0.09d	8.80	11.14 ± 0.12c	14.9
	230 – 273 µm	0.86 ± 0.33d	1.20	11.08 ± 0.09c	1.46
	< 230 µm	0.54 ± 0.30d	4.61	11.70 ± 0.41c	9.56
	Total content (%)	1.66 ± 0.04	100	12.57 ± 0.07	100
Brand 4	> 841 µm	9.53 ± 3.74a	1.67	17.93 ± 0.30a	0.19
	718 – 841 µm	5.59 ± 1.85b	5.54	18.29 ± 1.02a	1.55
	507 – 718 µm	5.49 ± 0.65b	14.1	18.29 ± 1.04a	4.64
	358 – 507 µm	2.59 ± 0.21bc	18.3	16.02 ± 0.47b	11.5
	273 – 358 µm	1.63 ± 0.33c	16.5	14.99 ± 0.06b	15.5
	230 – 273 µm	1.39 ± 0.28c	12.4	15.34 ± 0.26b	13.8
	< 230 µm	1.06 ± 0.17c	31.6	17.62 ± 0.87a	52.9
	Total content (%)	1.61 ± 0.14	100	16.62 ± 0.50	100.00

[a] Means of triplicates ± standard deviation; means with different letters within column and brand are significantly different ($P<0.05$)

CHAPTER 3. LOW MOISTURE LABORATORY MILLING OF WHEAT (*Triticum aestivum*) FOR THE PRODUCTION OF WHOLEGRAIN FLOUR

1. ABSTRACT

The purpose of this study was to determine the functional properties of whole wheat flour that had been roller milled at different moisture contents. The moisture contents of four cultivars (i.e., three hard red winter and one hard white winter) and a composite (i.e., combination of the red cultivars) were adjusted to 6.90-7.98% (by drying), 9.00-10.6% (by no treatment), and 15.6% (by tempering) and then milled on either a Buhler or a Quadramat Jr mill. All milling fractions were collected and combined after milling. As moisture content decreased, mean particle size (MPS) of the coarse fraction (>230 μm) decreased. No appreciable starch damage was observed. Bread baking trials showed that wheat milled at lower moisture contents provided flours with better functionality and mixing properties. These results suggest that milling wheat with low moisture (i.e., 6.89-7.98%) produces a whole wheat flour with good functionality.

2. INTRODUCTION

In the literature, whole wheat flour has been produced on a laboratory scale by many techniques, including: stone mills (Prabhasankar and Haridas Rao 2001; Shogren et al 2003; Kilhberg et al 2004), cyclone mills (Bruckner et al 2001; Anjum et al 2002), plate mills (Prabhasankar and Haridas Rao 2001), hammer mills (Blandino et al 2001; Prabhasankar and Haridas Rao 2001; Koutinas et al 2003), and roller mills (Prabhasankar and Haridas Rao 2001; Kilhberg et al 2004; Leenhardt et al 2005). Some researchers have used combinations of technologies like sifting at the end of milling to remove coarse particles (Gélinas et al 2009) and re-milling coarse bran with a hammer mill after milling on a roller mill (Prabhasankar and Haridas Rao 2001).

Kilhberg et al (2004) reported that milling technique for production of whole wheat flour has a greater influence on functionality (for bread) than farming technique and the formulation of the bread itself. The key reason for this was the difference in particle size distribution of the flours resulting from using different mills. Particle size has a remarkable influence on functional properties of whole wheat flour, including water absorption (Robertson and Eastwood 1981; Mongeau and Brassard 1982; Anderson and Estwood 1987), baking performance (Galliard and Gallagher, 1988; de Kock et al 1999; Zhang and Moore 1999), and sensory acceptability (Gomez et al 2010).

When milling refined flour, wheat is typically tempered to allow the bran to flake into large particles that are easy to separate from the endosperm (Posner and Hibbs 2005). Without tempering the bran fractures more easily, which is desirable in whole wheat flour production where separation of the botanical parts of the grain is not necessary and reduction of the bran particle size is important (Delcour and Hoseney

2010**)**. Therefore, the purpose of this study was to use moisture content of wheat prior to milling to affect changes in the particle size distribution of the resulting whole wheat flour and determine the effects on functional properties.

3. MATERIALS AND METHODS

3.1 Materials

Four wheat cultivars: three hard red winter (i.e., Overland, McGill, and Wesley) and one hard white winter (i.e., Anton), were obtained from Husker Genetics, the University of Nebraska-Lincoln Foundation Seed Division. A composite (i.e., equal combination of the red cultivars) was also used for this study.

3.2 Whole wheat flour milling

Moisture content of wheat was determined in triplicate after approved method 44-19.01 (AACC International 2012). In preparation for milling, wheat was subjected to three treatments: 1) placed in a convection oven (GCA/Precision Scientific, Chicago, IL) at 40 ºC for 14 h (overnight); 2) no treatment; and 3) tempered to 15.6% moisture according to approved method 26-10.02 (AACC International 2012).

Treated and untreated wheat were milled using either an experimental mill (Buhler, Minneapolis, MN) after approved method 26-21.02 (AACC International 2012), or a Quadrumat Jr laboratory mill (CW Brabender, South Hackensack, NJ) after approved method 26-50.01 (AACC International 2012). When wheat was milled in the Buhler mill, fractions (i.e., flour, bran, and shorts) were collected and recombined in a tumbling mixer for 3 min to obtain 100% extraction. The tumbling mixer consisted of a cylindrical

container wherein the flour, bran, and shorts were placed. The cylindrical container measured 0.22 m x 0.19 m (height x diameter) and was held at a 45^{o} angle and rotated at 6.7 rpm on a circular axis. When wheat was milled in the Quadrumat Jr mill, the sifting roller following milling was removed and wholegrain flour was obtained in the collection drawer. Seven hundred fifty g of each wheat cultivar were milled on the Buhler experimental mill and 50g were milled on the Quadrumat Jr mill, each in triplicate.

3.3 Separation by sieving

The three replicates of whole wheat flour (100g of samples milled on a Buhler mill, and 15g of samples milled on a Quadrumat Jr mill) from each treatment*cultivar combination were separated on a sieve shaker (Model SS-15, Gilson Company, Lewis Center, OH) equipped with US standard sieve numbers 20, 30, 40, 50, 60 and 70, followed by a collection pan (i.e., >0.850mm, 0.600-0.850mm, 0.425-0.600 mm, 0.297-0.425 mm, 0.250-0.297 mm, 0.212-0.250 mm, <0.212 mm). Sample was shaken for 10 min and the weight retained on each sieve and in the pan was recorded as g/kg of the total (Pope and Ward 1998). Sieve sizes were chosen such that the "flour" size particles would pass through to the pan (i.e., smaller than 0.212 mm; Codex Alimentarius Commission 1995), while the larger bran particles would be separated by size on the larger screens. Weight of flour passing through all screens and ending up in the pan was deemed "fine particles" and recorded as % of the total weight; "coarse particles" were retained on the sieves and weight distribution on each sieve was used to calculate mean particle size (MPS) of the coarse fraction (i.e., particles >0.212 mm; Ensor et al 1970).

3.4 Starch damage, protein and moisture content

Starch damage of each wholegrain flour was determined in triplicate according to Approved Method 76-31.01 (AACC International 2012) using a starch damage assay kit (Megazyme, Bray, Ireland). Protein content was determined in triplicate using approved method 46-30.01 (AACC International 2012). A conversion factor of 5.85 was used to convert %nitrogen to %protein. Moisture content was determined in triplicate using the approved method 44-19.01 (AACC International 2012).

3.5 Evaluation of functional properties

Ten g Mixographs (National Manufacturing, Lincoln, NE, USA) were run on all flours in triplicate after approved method 50.40.02 (AACC International 2012). Water absorption used during the experiment was determined using the regression equation based on the protein content provided in the method with a +1.8% adjustment for whole flour (Bruinsma et al 1978). Mixing time, peak height, and right of peak slope were calculated by the Mixograph software (National Manufacturing, Lincoln, NE, USA). Predicted adjustment for water absorption was also calculated using the Mixograph software, which used equations from Hazelton-Menary et al (2004).

Baking quality of samples milled on the Buhler mill was determined using approved method 10-13.02 (AACC International 2012), except water absorption and mixing time were estimated from Mixograph data and by making test doughs. Loaf volume was determined according to approved method 10-05.01 (AACC International 2012) following cooling for about 1 h. Then, loaves were sliced 12.5 mm thick per slice using an electric knife and bread slicing guide (Black & Decker Corporation, Towson, MD USA), bread firmness was determined according to approved method No 74-10.02 (AACC International 2012), and image analysis was performed using a C-Cell imaging

system (Calibre Control International Ltd., UK) following the manufacturer's instructions.

3.6 Experimental design and data analysis

Data were analyzed using SAS software (version 9.2, SAS Institute, Cary, NC USA.) using a generalized linear mixed model analysis of variance with Tukey's test to determine significant differences between and within all cultivar and treatment combinations. To fit the response, a completely randomized design was used, with five experimental units per treatment (i.e., 4 wheat cultivars and 1 composite), replicated 3 times. Significance was defined as $P<0.05$.

4. RESULTS AND DISCUSSION

4.1 Samples

Wesley contained the most protein and McGill the least (Table I). This was in agreement with the breeder classifications of baking quality. Without any treatment, the moisture contents of the samples ranged from 9.00 to 10.6%. Upon drying, the moisture contents decreased to 6.90 to 7.98%. Slightly different moisture contents were evident between those samples that were to be subjected to Quadrumat Jr milling versus Buhler milling. This was due to different seasons of the year and thus slightly different relative humidity in the room where the samples were stored (although the milling laboratory was maintained at 65% relative humidity).

4.2 Particle size distribution

Mean particle size of the coarse fraction was directly proportional to the moisture content of wheat prior to milling ($r=0.87$ and 0.88, for Buhler and Quadrumat Jr. mills,

respectively; $p<0.05$). When samples were grouped into the treatment received prior to milling, the highest coarse mean particle sizes and lowest percent fine particles were obtained when wheat was tempered prior to milling, whereas dried wheat provided flour with significantly smaller coarse particle size, independent of the type of mill used during the study (Figure 1). Tempered samples in particular contained a noticeably greater proportion of very large particles (i.e., >600μm) compared with the other treatments (Figure 2). Very coarse bran particles affect acceptability and physical properties of baked goods in a detrimental way (Zhang and Moore 1999). Notably, however, very small bran particles can also be undesirable (Noort et al. 2010), which were present in greater proportions in the dried and untreated wheats (Figures 1 and 2). Therefore, evaluation of these flours for functional properties was critical to evaluating their quality.

4.3 Starch damage

Milling technology influences starch damage in flour (Delcour and Hoseney 2010), which has an impact on functional properties of wheat flour (Yamamoto et al 1996). Factors that influence degree of starch damage are roller spacing, feed rate, and moisture content (Kihlberg et al 2004; Ghodke et al 2009). Relevant to this study in particular was the effect of milling moisture content on starch damage.

Some differences in starch damage were found as a function of wheat treatment of the wheat prior to milling (Table II). Although no trends were found in the Buhler milled samples, results from the Quadrumat Jr mill showed that wheat with higher moisture content result in higher starch damage. This is consistent with previous reports (Ghodke et al 2009). An analysis of three lots from four brands of commercial whole wheat flour provided starch damage values between 5.72-7.69% (Doblado-Maldonado et al 2013).

Hence, milling at the moisture contents evaluated in this study did not promote unusual amounts of starch damage in wholegrain flour, as the values of the flour produced on a lab scale were similar to those found in the commercial samples.

4.4 Functional properties

Treatments did not result in dramatic differences in mixing properties (Table III), although some significant differences were evident. Overland showed significant variations between treatments in terms of peak time and height: wholegrain flour with larger coarse particles (i.e., from tempered wheat) required shorter times for optimum dough development (peak time) with lower maximum force (peak height). A similar result was found for McGill for maximum force, but not peak time. Flour produced from tempered Anton and Wesley wheats provided doughs with lower mixing tolerance (more negative right of peak slope) than dried and untreated flours. No significant differences were found for McGill, Overland and the composite cultivars for this particular parameter. Some studies suggest that large bran particles lead to higher water absorption (Mongeau and Brassard 1982; Anderson and Eastwood 1987); however, no significant differences were found in the water absorption between the different treatments in our study. This is perhaps because researchers evaluated bran or bran-flour mixtures instead of whole wheat flour (Mongeau and Brassard 1982; Anderson and Eastwood 1987).

Our results suggested that the treatments did not affect considerably or consistently the mixing properties. Indeed, more significant variations in Mixograph properties were found among cultivars rather than treatments. Such observation suggests that mixing properties are more dependent on the type of wheat than the particle size distribution of the whole flour.

Visually, bread slices made from the whole wheat flours showed a slight trend downward in height from dried to tempered wheat (Figure 3). Differences in cell structure between untreated and tempered wheats were not readily apparent, whereas flours from dried wheats provided slices with more cell uniformity and tempered wheat bread showed greater predominance of large air cells.

Quantitatively, the greatest loaf volumes were achieved when dried wheat was used for milling (Table IV). Thus, flours with the lowest mean particle size of the coarse fraction produced breads with the greatest volumes. This is in opposition to previous reports (Galliard and Gallagher 1988; de Kock et al 1999; Zhang and Moore 1999). However, these studies used unusually large bran flakes (>2 mm). Also of note is that Zhang and Moore (1999) reported that the presence of coarse particles affects not only loaf volume but appearance of bread, which is consistent with our study (Figure 3).

Bread firmness was higher when tempered wheat was used (Table IV). Firmness values did not vary significantly between dried and untreated wheat. These data were in accordance with the volume data.

Tempered wheat provided brighter bread slices (Table IV). Brightness is related to reflectance of the bread, while blackness is related to the occurrence of air cells. Thus, greater brightness implied less occurrence of air cells. Tempered wheat had fewer incidences of air cells, which is in agreement with the volume data.

The number of cells and cell average elongation did not present many notable differences among treatments (Table IV). However, as the quality of wheat (according to the breeder classification) decreased, the variations between the evaluated parameters on the image analysis were more significant. For instance, the number of cells on Wesley

(i.e., good quality cultivar) did not vary between treatments, whereas on Overland (i.e., poor quality cultivar) the higher the moisture content of wheat the lower was the number of cells.

5. CONCLUSION

When wheat was milled at low moisture contents (i.e., 7-8%), wholegrain flour of superior quality breadmaking ability was produced in the laboratory compared to traditionally tempered wheat (at 15.6% moisture). The low moisture content was achieved by drying wheat under moderate conditions for several hours (e.g., 40 °C for 14 h to overnight). The bran fraction fractured into finer particles when milled at low moisture. Bran particles produced under low moisture conditions were not ultrafine, as in Noort et al (2010), rather the mean particle sizes were 315-330 μm. Thus this method for milling circumvented the detrimental effects of ultrafine grinding of wheat bran on bread quality (Noort et al 2010), while also avoiding undesirable effects of very coarse bran on bread quality (Zhang and Moore 1999). It is hoped that this will provide a practical way to produce functional whole wheat flour in the laboratory.

6. ACKNOWLEDGMENTS

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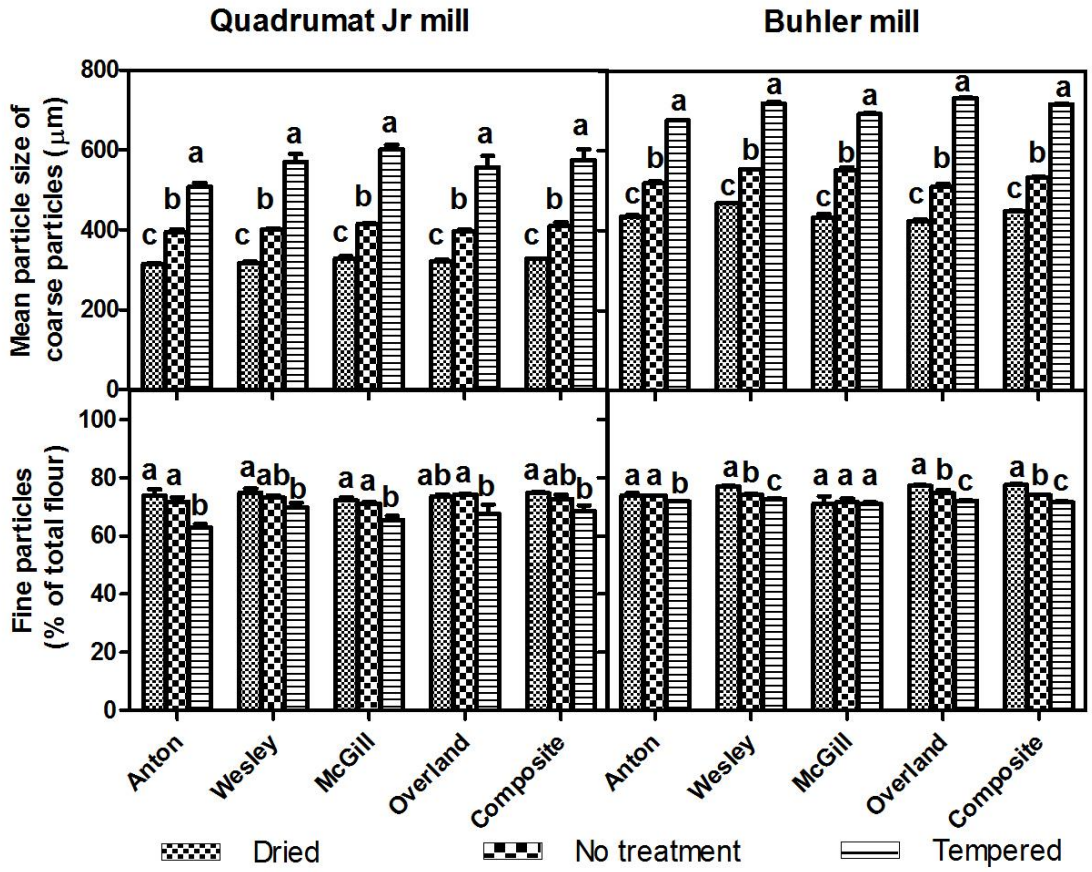

Figure 1. Mean particle size (µm) of coarse particles (>212 µm) and total fraction of fine particles (<212 µm; % of total weight). Means with the same letter within cultivar and mill type are not significantly different ($p>0.05$).

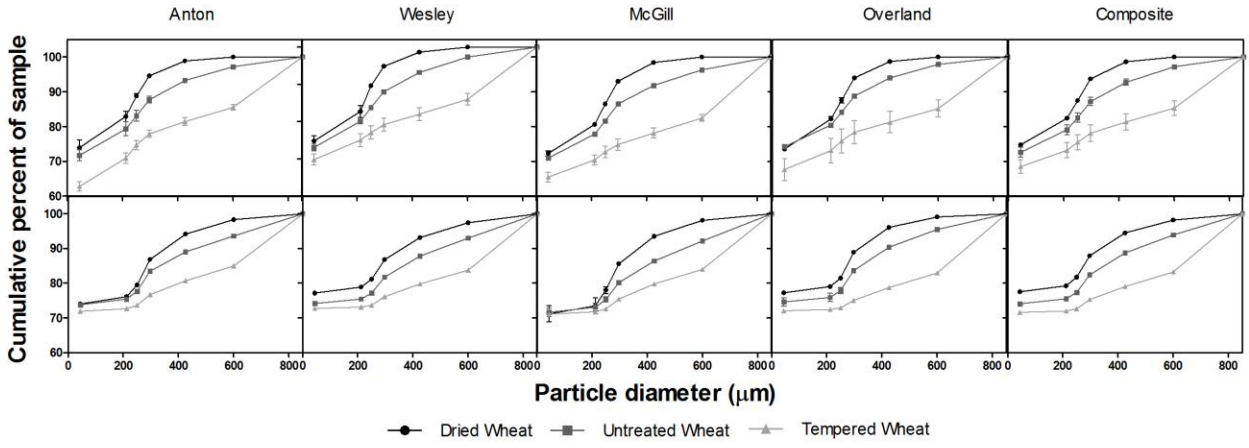

Figure 2. Differences in particle size distribution of five whole wheat flours from five different wheat cultivars. Upper row shows distribution of wheat milled with a Quadrumat Jr. mill whereas lower row represents results from a Buhler mill.

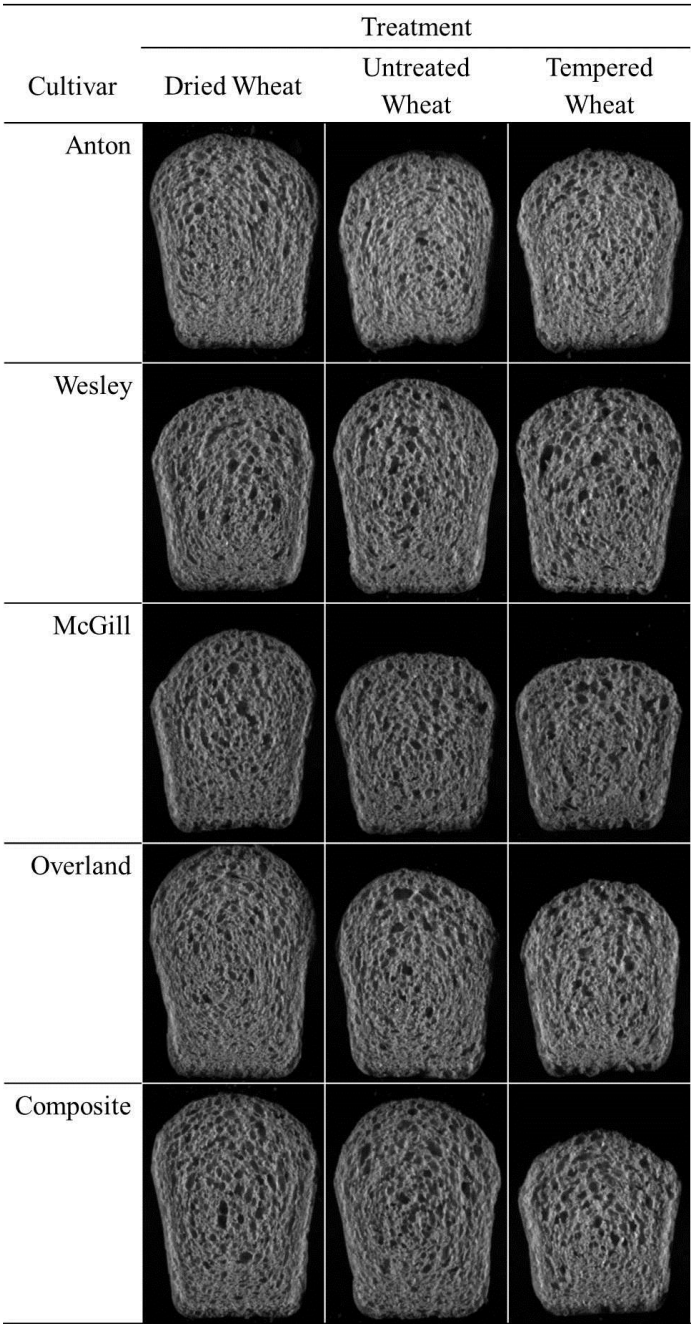

Figure 3. Representative pictures of bread from each treatment*cultivar combination.

Table 1. Classification, protein and moisture content of wheat kernels prior to milling[a]

Cultivar	Classification[b]	Protein (%; 14% moisture basis)	Moisture (% wb)					
			Quadrumat Jr. Mill			Buhler Mill		
			Dried	No treatment	Tempered[c]	Dried	No treatment	Tempered
Anton	Good	13.0 ± 0.2	6.90 ± 0.09	9.00 ± 0.10	15.6	7.29 ± 0.03	9.95 ± 0.19	15.6
Wesley	Good	13.9 ± 0.0	6.94 ± 0.07	9.22 ± 0.04	15.6	7.83 ± 0.10	9.25 ± 0.05	15.6
McGill	Intermediate	13.3 ± 0.1	7.01 ± 0.06	9.05 ± 0.33	15.6	7.29 ± 0.07	10.6 ± 0.0	15.6
Overland	Poor	11.3 ± 0.0	7.00 ± 0.06	9.74 ± 0.27	15.6	7.98 ± 0.04	9.41 ± 0.08	15.6
Composite[d]	—	12.8 ± 0.1	6.91 ± 0.04	9.08 ± 0.08	15.6	7.78 ± 0.10	9.10 ± 0.05	15.6

[a]Means of triplicates ± standard deviation.

[b]Breeder classification based on breadmaking quality using white flour: good, intermediate, poor.

[c]By calculation (approved method 26-95.01; AACC International 2012).

[d]Equal mixture (by weight) of the red cultivars (Wesley, McGill, and Overland).

Table 2. Starch damage (% db) in whole wheat flours[a]

Cultivar	Quadrumat Jr. Mill			Buhler Mill		
	Dried Wheat	Untreated Wheat	Tempered Wheat	Dried Wheat	Untreated Wheat	Tempered Wheat
Anton	3.97 ± 0.21 b	4.80 ± 0.14 a	4.94 ± 0.37 a	7.74 ± 0.18 b	8.27 ± 0.42 ab	8.93 ± 0.34 a
Wesley	2.80 ± 0.23 c	3.63 ± 0.23 b	4.18 ± 0.17 a	7.62 ± 0.37 b	8.40 ± 0.26 a	7.85 ± 0.31 ab
McGill	2.85 ± 0.43 c	3.95 ± 0.18 b	4.81 ± 0.32 a	6.64 ± 0.27 b	7.02 ± 0.17 ab	7.38 ± 0.33 a
Overland	3.00 ± 0.43 c	3.30 ± 0.18 b	4.51 ± 0.32 a	7.52 ± 0.27 a	8.24 ± 0.24 a	6.67 ± 0.42 b
Composite[b]	2.58 ± 0.07 c	3.60 ± 0.09 b	4.21 ± 0.38 a	7.50 ± 0.46 a	7.89 ± 0.42 a	7.45 ± 0.25 a

[a]Means of triplicates ± standard deviation; means with the same letter within row and mill type are not significantly different ($p>0.05$).

[b]Equal mixture (by weight) of the red cultivars (Wesley, McGill, and Overland).

Table 1. Mixing properties of whole wheat flours[a]

Property	Cultivar	Treatment		
		Dried	Untreated	Tempered
Peak time (min)	Anton	3.63 ± 0.13a	3.41 ± 0.27a	3.33 ± 0.21a
	Wesley	4.00 ± 0.22a	4.17 ± 0.02a	4.02 ± 0.08a
	McGill	3.94 ± 0.19a	3.99 ± 0.08a	4.19 ± 0.06a
	Overland	3.76 ± 0.10ab	4.15 ± 0.14a	3.40 ± 0.25b
	Composite[b]	3.83 ± 0.05a	4.01 ± 0.12a	3.88 ± 0.14a
Peak Height (%)	Anton	49.3 ± 0.4a	49.1 ± 1.0a	49.3 ± 0.7a
	Wesley	49.9 ± 0.3a	49.9 ± 0.6a	48.6 ± 0.7a
	McGill	47.5 ± 0.7a	46.3 ± 0.1ab	45.7 ± 0.5b
	Overland	44.9 ± 0.1a	43.0 ± 0.1b	42.4 ± 0.4c
	Composite	46.6 ± 0.5a	46.5 ± 0.7a	46.7 ± 0.4a
Right of Peak Slope (%/min)	Anton	-3.58 ± 0.37a	-3.97 ± 0.43ab	-5.08 ± 0.73b
	Wesley	-2.37 ± 0.96a	-4.67 ± 0.39b	-4.02 ± 0.50b
	McGill	-2.99 ± 0.36a	-3.21 ± 0.78a	-2.57 ± 0.63a
	Overland	-3.10 ± 0.15a	-2.23 ± 1.40a	-2.33 ± 0.66a
	Composite	-2.99 ± 0.27a	-3.24 ± 0.51a	-3.20 ± 0.35a
Predicted Water Absorption (%)	Anton	73.9 ± 1.5a	73.9 ± 1.2a	72.9 ± 0.9a
	Wesley	75.3 ± 0.6b	77.3 ± 1.0a	75.1 ± 0.8b
	McGill	74.3 ± 0.8a	73.5 ± 1.5a	73.5 ± 0.4a
	Overland	74.8 ± 1.9a	74.2 ± 1.9a	71.9 ± 1.6a
	Composite	74.5 ± 1.1a	74.9 ± 0.4a	73.7 ± 0.1a

[a]Means of triplicates ± standard deviation; means in the same row with the same letter are not significantly different ($p>0.05$).

[b]Equal mixture (by weight) of the red cultivars (Wesley, McGill, and Overland).

Table 2. Whole wheat bread loaf volume, firmness, and image analysis[a]

Bread Analysis Parameter	Cultivar	Treatment		
		Dried	Untreated	Tempered
Loaf volume (cc)	Anton	520± 0a	518± 13a	472 ± 21b
	Wesley	545 ± 0a	558 ± 6a	487 ± 31b
	McGill	502 ± 16a	485 ± 15a	430 ± 0b
	Overland	535 ± 29a	495 ± 35ab	443 ± 6b
	Composite[b]	550 ± 0a	522 ± 10b	443 ± 3c
Bread firmness (N)	Anton	4.05 ± 0.88ab	3.50± 0.60b	4.45 ± 1.03a
	Wesley	3.01 ± 0.54a	3.65 ± 0.87a	3.72 ± 1.02a
	McGill	2.69 ± 0.31b	3.00 ± 0.98b	3.75 ± 0.48a
	Overland	3.61 ± 0.77b	3.71 ± 1.01b	4.77 ± 0.92a
	Composite	2.65 ± 0.65a	2.97 ± 0.69a	3.18 ± 0.53a
Slice brightness	Anton	98.5 ± 3.8a	101.0± 3.6a	98.3 ± 1.7a
	Wesley	82.2 ± 2.1b	85.2± 2.6a	86.2 ± 2.4a
	McGill	76.4 ± 2.5b	77.0 ± 1.4b	80.2 ± 2.8a
	Overland	85.0 ± 2.9b	85.0 ± 1.4b	89.5 ± 1.1a
	Composite	83.2 ± 2.5b	83.6 ± 1.5ab	85.6 ± 2.9a
Number of cells	Anton	1683 ± 132a	1548 ± 101b	1615 ± 79ab
	Wesley	1580 ± 59a	1573 ± 88a	1505 ± 82a
	McGill	1466± 81a	1467 ± 101a	1467 ± 65a
	Overland	1809 ± 163a	1688 ± 119a	1514 ± 102b
	Composite	1604 ± 86a	1492 ± 175ab	1439 ± 185b
Average cell elongation (mm)	Anton	1.55 ± 0.04a	1.55 ± 0.02a	1.53 ± 0.02a
	Wesley	1.51 ± 0.03a	1.50 ± 0.02a	1.51 ± 0.03a
	McGill	1.54 ± 0.04a	1.53 ± 0.03ab	1.50 ± 0.02b
	Overland	1.61 ± 0.03a	1.57 ± 0.01b	1.53 ± 0.02c
	Composite	1.54 ± 0.03a	1.54 ± 0.04a	1.55 ± 0.03a

[a]Means of triplicates ± standard deviation; means in the same row with the same letters are not significantly different ($p>0.05$).

[b]Equal mixture (by weight) of the red cultivars (Wesley, McGill, and Overland).

CHAPTER 4. INHIBITION OF LIPASE FOR THE STABILIZATION OF WHOLE WHEAT FLOUR DURING STORAGE USING SALTS COMMONLY FOUND IN BAKING FORMULATIONS

1. ABSTRACT

Lipolytic activity in whole wheat flour (WWF) is largely responsible for the loss in baking quality during storage. Metal ions affect the activity of seed lipases; however, no previous studies have applied this information to WWF in a way that reduces lipase activity, is practical for commercial manufacture, and uses common food ingredients. NaCl, KCl, Ca-propionate, or FeNa-ethylenediaminetetraacetic acid (FeNa-EDTA) were applied to hard red winter (HRW) and hard white spring (HWS) wheats during conditioning as aqueous solutions at concentrations that would be acceptable in baked goods. All salts except Ca-propionate inhibited lipase; NaCl reduced activity by up to 76.7±6.8%. Inhibition was greater in HRW compared with HWS WWF, probably due to higher lipase activity in HRW wheat. In HRW WWF, salt treatments reduced hydrolytic and oxidative rancidity during storage, and the 1% NaCl treatment resulted in higher loaf volume and lower firmness than untreated WWF after 24 weeks of storage.

Key words: Enzymes; shelf-life; stability; functionality

2. INTRODUCTION

Dietary guidelines suggest that at least half of the grain products in the diet should come from whole grain foods [US Department of Agriculture (USDA) & US Department of Health and Human Services (USDHHS), 2010]. Therefore, consumers have demanded more whole grain options (Vocke, Buzby & Wells, 2008), which the food industry is providing in the form of new partial to 100% whole grain food products (i.e., breads, tortillas, pasta, crackers, and snacks). Unfortunately, research has not kept abreast of processing and product stability issues surrounding whole wheat flour (WWF; Doblado-Maldonado, Pike, Sweley & Rose, 2012).

When WWF is stored, lipase hydrolyzes lipids into non-esterified fatty acids (NEFA). NEFA are oxidized non-enzymatically during storage (Warwick & Shearer, 1980) or enzymatically by lipoxygenase when the flour is mixed with water (Galliard, 1986). These processes result in a decrease in the nutritional value and functional and sensory characteristics of WWF (Galliard & Gallagher, 1988). Thus, more stable WWF would be desirable because it would not require such careful control of storage time and conditions and may result in higher quality bakery products.

Researchers have focused on the inhibition of lipase to stabilize WWF (Molterberg, Vogt, Nilsson & Frolich, 1995; Rose, Ogden, Dunn & Pike, 2008). Heat treatments have been explored; however, this technology would require elevated costs in a large-scale operation, and the exposure to high temperature can initiate autooxidation and lead to non-enzymatic spoilage (Cuendet, Larson, Norris & Geddes, 1954; Molterberg et al., 1995).

Another strategy to stabilize WWF may involve the addition of lipase inhibitors. Metal ions are known to affect the activity of lipases from cereals and oilseeds (Barros, Fleuri & Macedo, 2010). Nevertheless, the practical applicability of this approach is limited, due to manufacturing limitations and regulations. Therefore, the objective of this research was to inhibit lipolytic activity in WWF through the inclusion of salts that could be found in baked good formulations at levels that fit within food manufacturing guidelines.

3. MATERIALS AND METHODS

3.1 Materials

Hard red winter (HRW) and hard white spring (HWS) wheats, NaCl, and pure olive oil were purchased from a local market in Lincoln, Nebraska, USA. KCl was obtained from Sigma Chemical Company (St. Louis, MO, USA). FeNa-ethylenediamine tetraacetic acid (FeNa-EDTA) was obtained from MP Biomedicals (Solon, OH, USA). Ca-propionate was obtained from Acros Organics (Fair Lawn, NJ, USA).

3.2 Whole wheat flour milling

Moisture content of wheat was determined in triplicate after approved method 44-19.01 (AACC International, 2012). In preparation for milling, wheat was tempered in duplicate to 15.2% moisture according to approved method 26-10.02 (AACC International, 2012), except in the treated samples water was replaced with different salt solutions.

Salt solutions were applied in duplicate such that the final wheat would contain doses that could be reasonably found in baking formulations (all reported as % of flour

weight on a 14% moisture basis). For NaCl, approved methods 10-10.02 and 10-13.03 suggest 1.5% flour basis in bread (AACC International, 2012). Heidolph et al. (2011) noted that baked goods commonly contain 0.5-2% NaCl flour basis. Due to the current recommendations to reduce salt intake (USDA & USDHHS, 2010), we limited the NaCl treatments to ≤1% flour basis. KCl treatment contained 0.45% KCl because Braschi, Gill and Naismith (2009) reported that 30% replacement of NaCl with KCl produced breads that were not significantly different from control bread containing only NaCl. FeNa-EDTA was added at 0.03% to contain 20 mg of iron per pound of flour, as outlined in the US Code of Federal Regulations [Food and Drug Administration (FDA), 2012]. FeNa-EDTA was used as the iron source to limit iron-catalyzed lipid oxidation (Huma, Rehman, Awan, Murtaza & Arshad, 2007) and because it is the only iron salt recommended for fortification of high extraction flours (>0.8% ash; Hurrell et al. 2010). The World Health Organization (WHO) recommends FeNa-EDTA levels of 15-40 ppm in the fortification of flour, which are equivalent to 7-18 mg of iron per pound of flour (WHO, 2009). Ca-propionate was added at 0.19% as suggested in Grundy (1996). Controls were conditioned with only water.

The duplicate samples of treated wheat and controls were milled using an experimental mill (Buhler, Minneapolis, MN) after approved method 26-21.02 (AACC International, 2012). Fractions (i.e., flour, bran, and shorts) were collected, and the bran fraction was further milled to pass through a 1 mm screen using a cyclone sample mill (Udy Corporation, Fort Collins, CO). All fractions were then recombined in a tumbling mixer to obtain WWF.

3.3 Determination of lipase activity

Lipase activity was determined on a portion of flour immediately following milling. Samples were defatted with hexane and evaluated for lipolytic activity after Rose and Pike (2006), except a 0.2 g sample size was used and the reagents were reduced proportionally. Sample weight was corrected for the weight of salt added during treatment. The lipase reaction proceeded with 30 µl of water and 0.12 ml of pure olive oil (from a local market) in a water bath at 40 ºC for 16 h prior to colorimetric quantification of NEFA. A zero time reading was analyzed by omitting the incubation step in a separately prepared sample to determine NEFA released by the enzyme. Each duplicate WWF sample was analyzed twice.

3.4 Whole wheat flour storage

Wheat lipase has an optimum incubation temperature of about 40 ºC (Rose and Pike, 2006; Barros et al., 2010); thus, flours were packaged in paper bags and stored in an incubator that ranged from 37-43 ºC. Relative humidity was not controlled and ranged from 15-26% during the storage period (temperature and relative humidity were recorded daily, data not shown). Samples were collected at 0, 4, 8, 12, and 24 weeks of storage and stored in zipper-sealed plastic bags in a freezer at -80 ºC prior to analysis (between 1 and 4 weeks).

3.5 Determination of lipid degradation during storage

NEFAs and conjugated dienes (CDs) at each time point during storage were determined according to Rose, Ogden, Dunn and Pike (2008). Each duplicate flour sample was analyzed twice.

Hexanal was quantified in duplicate, at 0, 12, and 24 weeks of storage, using the extraction method of Kaseleht, Leitner and Paalme (2011) and the gas chromatographic

method from Cramer Mattinson, Fellman and Baik (2005). The limited hexanal measurements were due to financial constraints and limited sample remaining.

3.6 Evaluation of baking quality

Baking quality of control flours and flours treated to contain 1% NaCl (14% moisture basis) were determined after 0, 12, and 24 weeks of storage using approved method 10-13.02 (AACC International, 2012), except water absorption and mixing time were estimated from Mixograph data (National Manufacturing, Lincoln, NE, USA; approved method 50-40-02; AACC International, 2012) and by making test doughs. NaCl and flour additions in the formulations were adjusted to maintain equivalent levels of salt and flour in the dough of the treatments and the control. Loaf volume was determined according to approved method 10-05.01 (AACC International, 2012) following cooling for about 1 h. Loaves were sliced 12.5 mm thick per slice using an electric knife and bread slicing guide (Black & Decker Corporation, Towson, MD). Bread firmness was determined according to approved method 74-10.02 (AACC International, 2012), and image analysis of the crumb was performed using a C-Cell imaging system (Calibre Control International Ltd., UK) following the manufacturer's instructions.

3.7 Experimental design and data analysis

Data were analyzed with SAS software (version 9.2, SAS Institute, Cary, NC, USA) using a generalized linear mixed model analysis of variance. For the storage experiment a repeated measures analysis with a factorial design was used to fit the responses for lipid degradation or baking quality with the type of salt and storage time as factors. To determine significant differences among samples, Fisher's least significant difference test was used. Significance was defined as $P<0.05$.

4. RESULTS AND DISCUSSION

4.1 Effect of salt solutions on lipase activity

In the present study, salt solutions were applied to wheat during conditioning. It was hypothesized that adding the salts in this fashion would allow the metal ions to diffuse into the kernel (along with the water) , interacting and ultimately inhibiting the lipase enzyme more readily and more practically than spraying the solutions on the bran after milling (e.g., Munshi, Bhatia, Sekhon & Sukhija, 1993).

The five salt solutions applied during wheat conditioning influenced lipase activity in both HRW and HWS WWF (Figure 1). FeNa-EDTA, KCl, and NaCl reduced activity, while Ca-propionate either did not affect lipase activity (HRW) or enhanced lipase activity (HWS).

O'Connor and Harwood (1992) reported an inhibition of purified wheat lipase in 100 mM NaCl. Matsuda and Hirayama (1979) found that lipase from rice bran could be inhibited by FeNa-EDTA and NaCl, but its activity increased in the presence of $ClCl_2$. Similar results were found for lipase from pigeon pea (Khan, Dahot & Noomrio, 1991).

While others have shown that salts can inhibit seed lipases (Barros et al., 2010), this is the first report showing an inhibition of lipase activity in wheat through the application of salt solutions during flour conditioning. Previous studies have not made this strategy practical. For instance, some salts have been tested at doses that are not acceptable for food applications (O'Connor & Harwood, 1992), and salts that are not suitable for food application have been used (e.g., $NiCl_2$; Munshi et al., 1993). Moreover, application of the salt treatment has been by spraying over the bran (Prabhakar &

Venkatesh, 1986; Munshi et al. 1993). This would require an extra processing step and require complete physical separation of the bran from the endosperm.

The HRW WWF control sample showed noticeably higher lipase activity compared with the HWS sample. Differences in lipase activity between the HRW and HWS wheat were expected, since others have shown that lipase activity in wheat differs among kernel fractions (i.e., botanical parts) and cultivars (O'Connor, Perry & Harwood, 1992; Rose & Pike, 2006). Since the HRW and HWS wheats used in this study were commercial blends, we cannot say whether low lipase activity is an inherent property of red wheats compared with white wheats or spring wheats compared with winter wheats. Determining differences in lipase activity among wheat classes and cultivars may help identify low lipase activity varieties that could be used to produce WWF with inherently low lipase activity, which could lead to enhanced stability without further treatment.

Because NaCl had the greatest inhibitory effect on lipase, HRW and HWS WWF were evaluated for a NaCl dose response (Figure 2). In the HRW WWF, all doses of NaCl significantly decreased lipase activity, with reduction rates ranging between 23-55% of the original lipase activity. It appeared that a maximum inhibition was achieved, since treatment beyond 0.75% NaCl did not further decrease lipase activity. In the HWS WWF, lipase was not further inhibited by treatments higher than 0.25% NaCl. Differences in the inhibitory effects of NaCl on lipase activity between HRW and HWS WWF may be attributed to the differences in original lipase activity, since the enzyme was considerably more active in the HRW wheat.

4.2 Hydrolytic rancidity during storage

NEFA appearance in both HRW and HWS WWF during storage was evaluated. In the case of HRW WWF, all treatments showed reduced NEFA compared with the control after 4 weeks of storage (Figure 3A). Between 12 and 24 weeks of storage, a decline in NEFA in the control was evident, presumably due to NEFA oxidation, which was not observed in the treatment samples. In contrast to HRW WWF, the release of NEFA in WWF from HWS wheat was much lower and the effectiveness of the salts on lipase inhibition was diminished (Figure 3B).

Among the different NaCl dosages in HRW WWF, 1% NaCl showed the greatest inhibition in NEFA release after 24 weeks of storage (Figure 4A). The reduction in NEFA release in stored HWS WWF flours was slight, although significant, at all treatment levels except 0.5% (Figure 4B).

The release of NEFA over the storage time is related to the lipase activity (Rose & Pike, 2006). In our study, NaCl and KCl treatments on HRW WWF had the greatest inhibitory effect on lipase activity (Figure 1A) and the development of NEFA during storage (Figure 3A). Strangely, Ca-propionate showed a trend toward enhancing lipase activity (Figure 1A, 1B), but inhibited NEFA release during storage (Figure 3A). Since NEFA content can be used as an indicator of WWF deterioration (Galliard, 1986), these results suggested that a longer shelf life may be obtained by the salt treatments.

4.3 Oxidative rancidity during storage

Galliard (1986) found that products from hydrolytic rancidity (i.e., NEFA) prompt oxidative rancidity; therefore, the concentration of CD and hexanal were evaluated as measures of primary and secondary lipid oxidation, respectively. In the HRW WWF between 12 and 24 weeks of storage, a noticeable increase in CD was observed in the

control sample (Figure 5A), which may explain the decline in NEFA during this time period (Figure 3A). All treatments inhibited CD formation compared with the control, with few meaningful significant differences among them (Figure 5A). Different doses of NaCl differed only slightly in their inhibition of CD formation (Figure 6A). In the HWS WWF, an increase in CD development was evident in all samples during storage; none of the salt treatments had an important effect on CD formation (Figures 5B and 6B).

Hexanal in HRW WWF rose from 11.0±1.3 ng/g to 157±54 ng/g in the control flour and from 11.6±0.8 ng/g to 131±20 ng/g in the 1% NaCl treatment after 24 weeks of storage. Although the NaCl treated HRW WWF had a numerically lower hexanal content at the end of storage, the difference from the control was not significant ($p=0.59$). Similar results were seen with the HWS WWF samples: the control rose from 8.33±0.80 ng/g to 269±34 ng/g and the NaCl treated WWF from 16.0±0.90 ng/g to 233±44 ng/g (p for difference at 24 weeks=0.37).

Although few differences in lipid oxidation among salt treatments were evident, they were nevertheless partially effective in preventing oxidative rancidity in the HRW WWF. This is remarkable, since our treatments were designed to inhibit hydrolytic rancidity and not necessarily oxidative rancidity. Enhanced oxidative stability may lead to greater sensory acceptability as less off flavors and undesirable odors may be perceived during tasting (Galliard & Gallagher, 1988; Heinio, Lehtinen, Oksman-Caldentey & Poutanen, 2002).

4.4 Effect on baking quality

The effect of the 1% NaCl treatment on baking properties was evaluated (Table 1). At time zero in HRW WWF, the treatment slightly reduced loaf volume compared

with the control; however, after 12 weeks of storage no significant difference was observed, and after 24 weeks loaf volume was substantially higher in the treated sample compared with the control. Higher loaf volume in the NaCl samples after 24 weeks of storage was accompanied by reduced firmness compared with the control. In loaves made from HWS WWF, no significant differences in volume or firmness between 1% NaCl and the control were found at any storage time point.

Air cell parameters (e.g., slice area, height, brightness and cell diameter) presented some differences between the treatments. Treated HRW WWF resulted in a greater number of cells with a higher diameter and lower elongation after 12 weeks of storage. Therefore, during baking, the dough made from treated flour was able to change into a sponge structure that exhibited better gas holding capacity and a brighter product.

Higher contents of NEFA and CD in the HRW WWF control may have contributed to poor baking quality after 24 weeks of storage, taking into account that NEFA in WWF reduce dough mixing properties and rheological properties (Carr, Daniels & Frazier, 1992; Goesaert, Brijs, Veraverbeke, Courtin, Gebruers & Delcour, 2005).

5. CONCLUSIONS

While no salt treatments completely inhibited WWF lipid degradation, salt solutions applied during wheat conditioning significantly influenced lipase activity, with NaCl showing the greatest inhibition. These effects were most clear in the HRW WWF, which had a higher lipase activity than the HWS WWF used in this study. This translated into enhanced lipid stability in the HRW WWF during storage, which was reflected in improved baking properties in the flour after 24 weeks of storage. Thus, these treatments

may be effective in partially stabilizing WWF. Future research using single wheat lines, additional salts, or combinations of salts may be beneficial.

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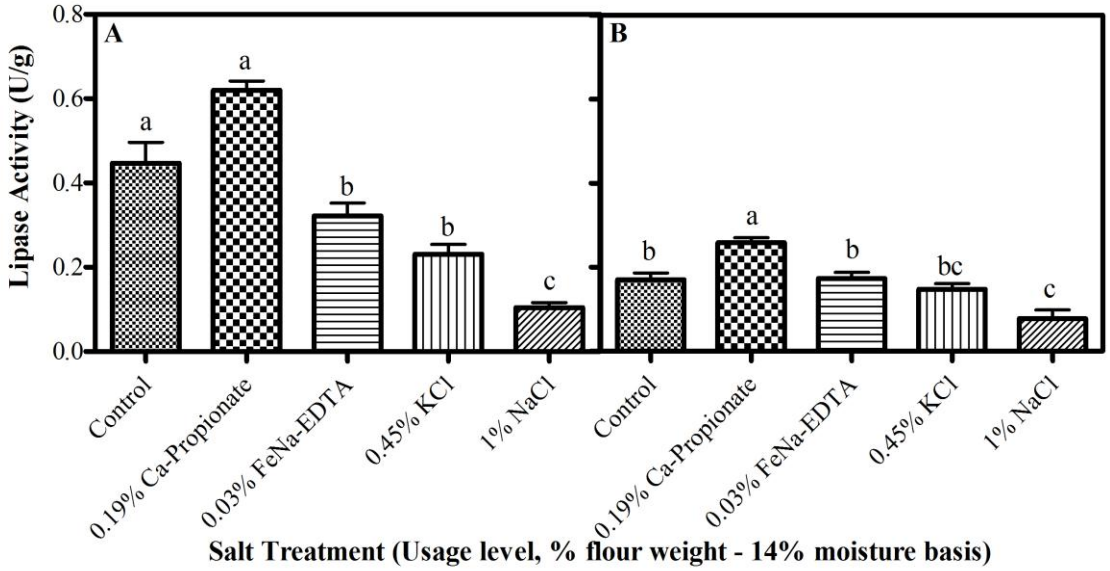

Figure 1. Lipase activity of whole wheat flours milled from hard red winter (A) and hard white spring (B) wheats treated with different salts; U= µmol equivalents of oleic acid liberated/h; error bars represent standard error; significant differences are shown as different letters ($P<0.05$); n=2.

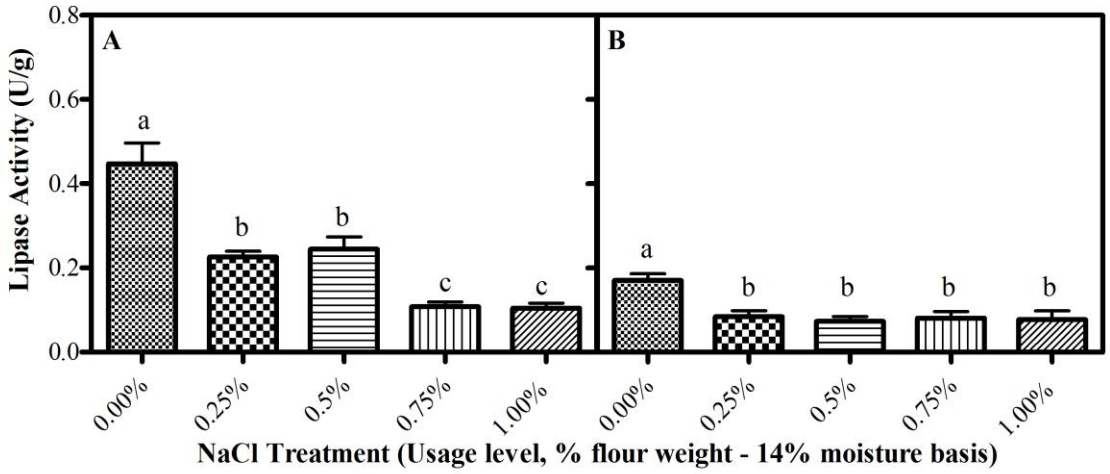

Figure 2. Lipase activity of whole wheat flours milled from hard red winter (A) and hard white spring (B) wheats treated with NaCl at different concentrations; U= µmol equivalents of oleic acid liberated/h; error bars represent standard error; significant differences are shown as different letters ($P<0.05$); n=2.

Description	Symbol	HRW WWF				HWS WWF			
		4w	8w	12w	24w	4w	8w	12w	24w
Control		a	a	a	a	ab	a	ab	b
0.19%Ca-Propionate		b	b	b	a	b	b	bc	bc
0.03% FeNa-EDTA		b	b	b	a	a	a	a	a
0.45% KCl		c	c	c	b	b	b	cd	d
1.00% NaCl		d	d	d	c	c	c	d	cd

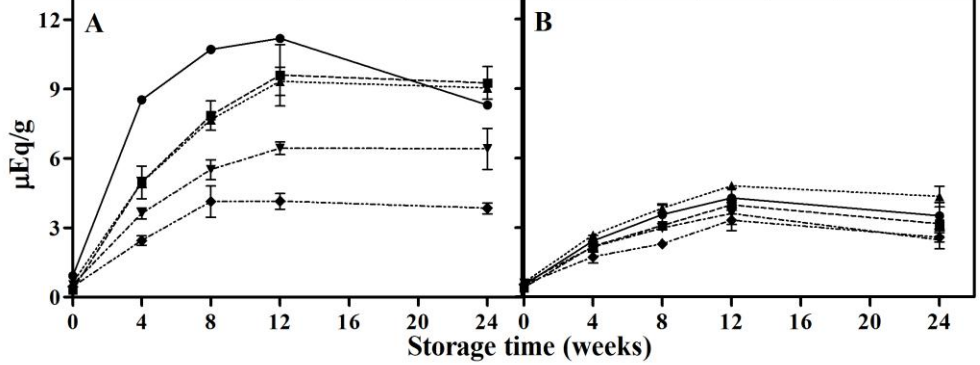

Figure 3. Appearance of non-esterified fatty acids (NEFA) whole wheat flours (WWF) treated milled from hard red winter (HRW; A) and hard white spring (HWS; B) wheats with different salts and stored for 24 weeks (w); values are reported as molar equivalents of oleic acid; error bars represent standard error; significant differences at each time point are shown as different letters within column ($P<0.05$); n=2.

Description	Symbol	HRW WWF				HWS WWF			
		4w	8w	12w	24w	4w	8w	12w	24w
0.00% NaCl		a	a	a	a	a	a	a	a
0.25% NaCl		bc	b	b	b	ab	b	b	c
0.50% NaCl		b	bc	bc	b	ab	ab	b	ab
0.75% NaCl		bc	d	cd	b	bc	c	c	bc
1.00% NaCl		c	cd	d	c	c	c	bc	c

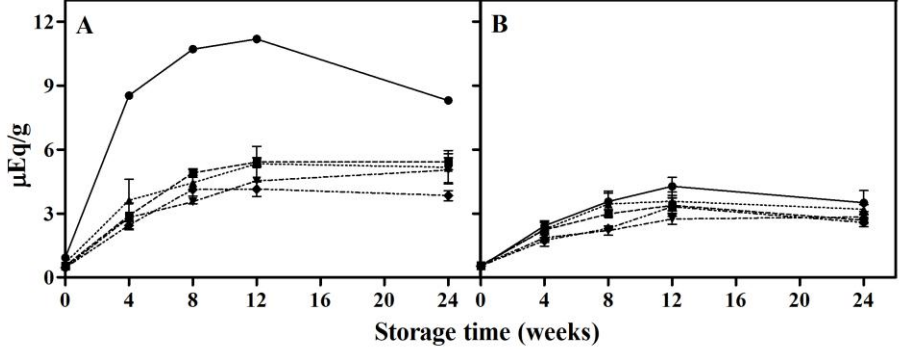

Figure 4. Appearance of non-esterified fatty acids (NEFA) in whole wheat flours (WWF) milled from hard red winter (HRW; A) and hard white spring (HWS; B) wheats treated with NaCl at different levels and stored for 24 weeks (w); values are reported as molar equivalents of oleic acid; error bars represent standard error; significant differences at each time point are shown as different letters within column ($P<0.05$); n=2.

Description	Symbol	HRW WWF				HWS WWF			
		4w	8w	12w	24w	4w	8w	12w	24w
Control		a	a	a	a	ab	c	b	a
0.19%Ca-Propionate		a	b	b	bc	a	a	b	a
0.03% FeNa-EDTA		a	c	c	c	b	b	c	a
0.45% KCl		b	b	c	c	ab	a	ab	a
1.00% NaCl		b	bc	c	b	b	b	a	a

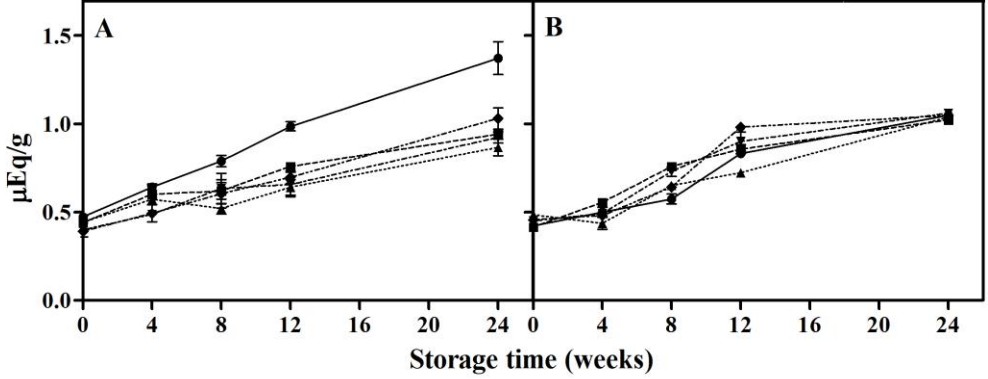

Figure 5. Appearance of conjugated dienes in whole wheat flours (WWF) milled from hard red winter (HRW; A) and hard white spring (HWS; B) wheats treated with different salts and stored for 24 weeks (w); values are reported as molar equivalents of linoleic acid hydroperoxide; error bars represent standard error; significant differences at each time point are shown as different letters within column ($P<0.05$); n=2.

Description	Symbol	HRW WWF				HWS WWF			
		4w	8w	12w	24w	4w	8w	12w	24w
0.00% NaCl		a	a	a	a	a	b	c	b
0.25% NaCl		b	b	c	b	a	ab	bc	ab
0.50% NaCl		b	b	bc	c	a	a	c	a
0.75% NaCl		a	b	b	bc	a	a	b	a
1.00% NaCl		b	b	c	bc	a	a	a	b

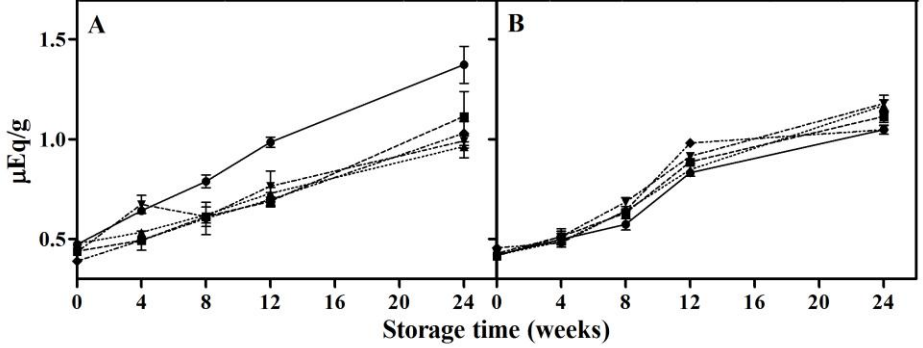

Figure 6. Appearance of conjugated dienes in whole wheat flours (WWF) milled from hard red winter (HRW; A) and hard white spring (HWS; B) wheats treated with NaCl at different levels and stored for 24 weeks; values are reported as molar equivalents of linoleic acid hydroperoxide; error bars represent standard error; significant differences at each time point are shown as different letters within column ($P<0.05$); n=2.

Table 1 Bread loaf volume, firmness, and image analysis of whole wheat bread loaves made with flour stored for up to 24 weeks.[a]

Bread Analysis Parameter	Storage time (weeks)	HRW WWF		HWS WWF	
		0% NaCl	1% NaCl	0% NaCl	1% NaCl
Loaf volume (cc)	0	623 ± 5	592 ± 7*	570 ± 12	575 ± 6
	12	503 ± 11	497 ± 7	514 ± 10	506 ± 12
	24	439 ± 9	485 ± 13*	512 ± 6	506 ± 11
Bread firmness (N)	0	2.23 ± 0.11	2.22 ± 0.13	1.83 ± 0.07	1.72 ± 0.08
	12	3.78 ± 0.20	3.70 ± 0.26	2.80 ± 0.23	2.84 ± 0.27
	24	5.16 ± 0.37	4.14 ± 0.37*	2.79 ± 0.09	3.00 ± 0.19
Slice area (mm^2)	0	3651 ± 24	3567 ± 30*	3456 ± 39	3549 ± 23
	12	3186 ± 30	3183 ± 35	3375 ± 32	3314 ± 63
	24	2892 ± 56	3182 ± 45*	3453 ± 25	3307 ± 43*
Slice height (mm)	0	65.8 ± 0.5	64.6 ± 0.5	61.6 ± 0.7	62.6 ± 0.3
	12	61.2 ± 0.5	60.0 ± 0.6	63.2 ± 0.4	62.0 ± 1.0
	24	55.8 ± 0.8	60.4 ± 0.7*	63.4 ± 0.3	61.7 ± 0.6*
Slice brightness	0	86.5 ± 0.6	87.1 ± 0.6	108 ± 1	111 ± 1
	12	91.0 ± 0.5	90.8 ± 0.3	115 ± 1	117 ± 1
	24	89.2 ± 0.4	91.0 ± 0.4*	114 ± 1	115 ± 1
Number of cells	0	2034 ± 41	1948 ± 39	1805 ± 35	1836 ± 24
	12	2333 ± 39	2122 ± 43*	2363 ± 21	2278 ± 41
	24	2241 ± 51	2341 ± 34	2406 ± 34	2306 ± 23*
Cell diameter (mm)	0	2.16 ± 0.05	2.20 ± 0.03	2.28 ± 0.04	2.27 ± 0.03
	12	1.64 ± 0.02	1.80 ± 0.03*	1.69 ± 0.02	1.76 ± 0.02*
	24	1.55 ± 0.03	1.65 ± 0.04	1.70 ± 0.02	1.73 ± 0.02
Average cell elongation (mm)	0	1.62 ± 0.01	1.58 ± 0.01*	1.56 ± 0.01	1.54 ± 0.01
	12	1.72 ± 0.01	1.66 ± 0.01*	1.72 ± 0.01	1.67 ± 0.01*
	24	1.76 ± 0.02	1.72 ± 0.01*	1.73 ± 0.01	1.74 ± 0.01

[a] Values represent mean ± standard error; *significantly different from the corresponding control at the same time point for the same bread analysis parameter ($P<0.05$); HRW=hard red winter; HWS=hard white spring; WWF=whole wheat flour.

GENERAL CONCLUSIONS

The present thesis has reported new strategies to overcome current issues in the milling industry related to milling and storage of whole wheat flour.

The first hypothesis was that the production of whole wheat flour is not a standard process in the milling industry (Kihlberg et al., 2004; Doblado-Maldonado et al., 2012), and therefore there is a wide variation in particle size distribution among retail flour samples. After the evaluation of three lots of four commercial brands of whole wheat flour different particle size distributions were observed, not just between brands, but also within some of them. In addition, after compositional analysis, it was determined the bran fraction of the kernel was milled to different degrees. Thus, not only are whole wheat flours different among milling companies with respect the particle size distribution, flours purchased from the same company are different. This would be expected to impact functional properties.

For the second hypothesis, it was expected that milling wheat kernels with low moisture content would provide a smaller mean bran particle size and more functional flour. When wheat was milled at low moisture contents (i.e., 7-8%), mean bran particle size ranged between 314-469 μm and better breadmaking quality was observed compared to traditionally tempered wheat (at 15.6% moisture). Thus this method for milling avoided the negative effects of very coarse bran on bread quality. Furthermore, it also avoided the negative effects of very fine bran on bread quality. It is hoped that this will provide a practical way to produce functional whole wheat flour in the laboratory.

Finally, the third hypothesis stated that the addition of different salts at normal usage levels would reduce lipase activity and prolong shelf life of whole wheat flour by reducing hydrolytic rancidity and improving functionality after long term storage. A significant inhibition of lipase was observed when wheat kernels were tempered in NaCl solutions. Inhibition was dependent on initial lipase activity, which varied between wheat samples. The NaCl treatment lead to better breadmaking properties after 24 weeks of storage compared to a control flour.

Further studies are suggested to optimize the effectiveness of the proposed methods and its applicability in the milling industry and cereal science research facilities. Regarding milling of whole wheat flour, a comparison between ultra-fine whole wheat flours and flours from low moisture content wheat might be beneficial. It may help to understand the importance of an optimum mean particle size in terms of its functional and nutritional properties. Regarding shelf-life, new studies should aim to find the variability of lipase activity between single lines of wheat cultivars. Finding cultivars with low lipase activity may provide flours with longer shelf life. Also, the characterization of the inhibition mechanism of the enzyme may serve as a basis to propose new and /or more optimum strategies in the inhibition of wheat lipases.

APPENDICES

APPENDIX A. Lipase activity in wheat products - microscale assay

1. Accurately weigh 200 mg whole wheat flour (dry, salt-free basis) into each of three 2.0 ml microfuge tubes (standard, blank, and sample)
2. To each tube add 1 ml of hexane; close tube and mix well; make sure all of the flour has been suspended in the solvent
3. Mix/shake for 20 min
4. Centrifuge at 14,000 rpm for 5 min
5. Remove the hexane layer with a Pasteur pipette and discard
6. Repeat steps 2-5 once more
7. Use a toothpick to break up and dislodge the pellet from the bottom of the microfuge tube
8. To one of the tubes, add 50 µl of a standard solution containing ~20 mg oleic acid/ml in hexane (1 mg oleic acid per standard tube); designate this tube as the **standard**
9. Allow the pellets to dry completely by laying the tubes on their side in the hood; leave toothpicks in each tube; continue on to step 9 when the pellets appear dry and no hexane odor can be detected; mixing the tubes occasionally during drying will speed up the drying
10. Set aside one of the tubes; designate this tube as a **blank**
11. Add 0.12 ml of pure olive oil and 30 µl of water to the standard and sample tubes; mix well with the toothpick to create a uniform paste
12. Keeping the toothpick in the tube, place tubes in a water bath at 40 °C for 16 h

13. Immediately before removing the tubes from the water bath, add 0.12 ml of pure olive oil and 30 µl of water to the reserved blank tube
14. Add 1.2 ml of isooctane to all tubes and mix well with the toothpick; carefully remove the toothpick, scraping the toothpick along the side of the tube to dislodge any flour adhering to the toothpick
15. Cap the tube and mix well by vortexing
16. Centrifuge the slurry at 14,000 rpm for 5 min
17. Pour the supernatant into a fresh microfuge tube and add 0.2 ml of cupric acetate pyridine reagent (dissolve 5 g cupric acetate in 60 ml water overnight; adjust the pH to 6.1 with pyridine; adjust the final volume of the solution to 100 ml; store in an amber bottle)
18. Cap and shake tubes vigorously by hand for 1 min
19. Centrifuge for 1 min at 14,000 rpm
20. Transfer the organic (top) layer to a low volume cuvette and read the absorbance at 715 nm
21. Use the absorbance of the blank and standard to calculate the µmol of oleic acid liberated/g flour/h by the lipase enzyme (formula weight of oleic acid is 282.46 g/mol)

APPENDIX B. Evaluation of baking quality of whole wheat flour

1. Mixing properties of dough

1. Measure protein (LECO system, N factor: 5.85) and moisture content of whole wheat flour.
2. Adjust to 14% moisture content basis.
3. Determine the preliminary water absorption following AACCI Approved method 54-40A, using the equation:

$$Y = 1.5X + 43.6$$

   Where X is the percent of flour protein content (14% moisture basis) and Y is the water absorption.
4. Add 1.8 to the obtained "preliminary" water absorption (Bruinsma, 1978).
5. Use this water absorption as the one to be tested in the mixograph for 10g of whole wheat flour adjusted to 14% moisture content.
6. Run each sample in triplicate and the average of the peak time will be used as the mixing time during baking.
7. For the water absorption of flour, the values that include the 1.8% adjustment should be used.

2. Breadmaking

1. Formulation for one hundred g loaves: 100g whole wheat flour (14% moisture content basis),1g yeast, 3g unemulsified shortening, 1.5g salt, 6g sucrose, 0.2g malt syrup, 4g whey, 0.1g ammonium phosphate, 20 ppm ascorbic acid (Note 1), water according to water absorption. (AACC Approved Method 10-13A).

2. Weigh all dry ingredients (i.e., flour, yeast, salt, sucrose, malt syrup, whey, ammonium phosphate) and mix them well. If you are preparing many loaves on the same day, it is recommendable to weigh all dry ingredients the day before and mix them in a well-sealed plastic bag.
3. Add dry ingredients to the mixer bowl.
4. Add water, shortening and ascorbic acid solution.
5. Mix dough to peak time as measured on a mixograph.
6. Ferment dough for 180 min at 30°C (86°F) in a covered container, previously greased with shortening.
7. Sheet dough at gaps of 0.87, 0.47, and 0.32 cm (11/32, 3/16, and 1/8 in).
8. Mold and pan in a greased pan.
9. Proof at 37.5°C and 85% rh for about 1h.
10. Bake in 204°C (400°F) oven for 25 min.
11. Cool loaves in a rack for at least one hour.

3. Evaluation of baking quality

1. Loaf (Note 2):
    a. Volume: Use a rapeseed displacement test, and record volume for each bread loaf.
    b. Record weight of each loaf.
2. Slice (Note 2):

    Slice bread using an electric knife and a cutting guide to obtain slices of about 12.5 mm thick. Use three slices per loaf located in the center of it.

    a. Appearance: Run an image analysis using a C-Cell instrument.

b. Texture: Measure bread firmness following AACCI Approved method 74-09.

Note 1: Prepare a solution of 2mg/ml of ascorbic acid in water and add 1 ml of it to the dry ingredients. Subtract that 1 ml from the total amount of water that is going to be added according to the water absorption experiments.

Note 2: Quality tests of bread loaves must be run right after the cooling process is finished, except for c-cell measurements. Slices can be stored in plastic bags until being analyzed for appearance.

APPENDIX C. Mean particle size of whole wheat flour

1. Prepare the following set of sieves: #20, #30, #40, #50, #60, #70, and a pan.

2. Weigh all sieves and the pan.

3. Weigh 100g of whole wheat flour.

4. Place the sample on the top sieve of the set of sieves on a sieve shaker.

5. Sieve for 10 min.

6. Weigh and record the retained material on all sieves.

7. Calculate mean particle size using the equation:

$$d_{gw} = log^{-1}\left[\frac{\sum(w_i \log \bar{d}_i)}{\sum w_i}\right]$$

Where:

$\bar{d}_i = \sqrt{(d_i \times d_{i+1})}$

d_i=diameter of the sieve openings of the i'th sieve. The value for the opening size of the pan is 44 μm.

d_{i+1}= diameter of openings in next larger than i'th sieve (just above in a set). In the case of sieve #20 use "1000" as the opening in the next larger sieve.

w_i= weight fraction on i'th sieve.

d_{gw}=geometric mean diameter.

8. Calculate the geometric standard deviation using the equation:

$$S_{gw} = log^{-1}\left[\frac{\sum w_i \left(\log \bar{d}_i - \log d_{gw}\right)^2}{\sum w}\right]^{\frac{1}{2}}$$

Where:

S_{gw}= geometric standard deviation.