

**Quality, Phytonutrient and Antioxidant Properties of
Wholegrain Bread Baked with Different Methods**

by

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ABSTRACT

QUALITY, PHYTONUTRIENT AND ANTIOXIDANT PROPERTIES OF BREAD BAKED WITH DIFFERENT METHODS

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Wholegrain foods are recognized sources of dietary fiber and antioxidants. This study investigated the effect of using different bread-making methods and subsequent storage on the quality, phytonutrient contents and antioxidant properties of wholegrain bread. The wholegrain breads were prepared by three methods, straight dough, sponge dough, and sourdough (15%–35% starter) and stored at room temperature for 7 days. Quality of wholegrain bread was significantly influenced by the bread-making method with the highest loaf volume and better crumb softness was obtained in bread made by sourdough method with 15% starter. In addition, 15% sourdough breads exhibited the least changes during storage as compared to straight and sponge dough breads (yeast-leavened). Significant increases were found in free ferulic acid for all the bread products, whereas slight increases were observed in the bound form particularly in sourdough breads. Sourdough fermentation also increased total carotenoid content but reduced total flavonoid content. All wholegrain bread products had significant increases in antioxidant properties as measured by the DPPH, ABTS and ORAC assays, compared with the wholegrain flour. During storage, the sponge dough and sourdough methods were more effective in preserving phytonutrients compared to straight dough method. The results suggest that the sourdough method would be a useful tool in producing high-quality wholegrain breads rich in phytonutrients that would satisfy consumer needs and boost health benefits.

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**I lovingly dedicate this thesis to my father Dr. Ali Sahli,
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Hamdi

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CHAPTER 1

INTRODUCTION

Wholegrain bakery products are gaining increasing interest from the food industry and consumers alike due to their high content of nutrients, antioxidants, dietary fiber and phytonutrients. Phytonutrients, also known as bioactive compounds, are physiologically active compounds found in fruits, vegetables and grains exhibiting diverse structures and functions. They have been associated with reduced risk of major chronic diseases such as cardiovascular disease, diabetes and some types of cancer (Slavin 2003; Jones et al 2004; Liu 2003; 2004).

Although wholegrain baked products are considered as healthy food choices, the quality of these products could impede their consumption. The quality of food products baked from wholegrain flours is not comparable to those made from refined flours. In the baking industry, different bread-making methods are being used to produce breads with different qualities and palatability. Straight dough and sourdough are the main bread-making methods that have been traditionally practiced. The straight dough bread-making method involves fermentation by baker's yeast, whereas the sourdough method is primarily based on lactic acid bacteria fermentation. The sourdough method substantially affects rye bread quality, especially improving flavor and loaf volume (De Vuyst and Vancanneyt 2007). Rye bread is mainly consumed in central and northern Europe while wheat bread is consumed across the world.

Sourdough fermentation has been studied for its effect on the quality (bread flavor and texture) and shelf life of wheat and rye leavened baked products (Gobbetti et al 2014). Many studies have reported that sourdough improves loaf volume and crumb firmness (Corsetti et al 2000; Clarke et al 2002) as well as shelf life (Clarke and Arendt 2005; Dal Bello et al 2007).

Besides the positive effects of sourdough fermentation on bread quality and shelf life, it also contributes to the nutritional value. Sourdough fermentation may improve dietary fiber properties and increase the uptake of vitamins, minerals and phytonutrients (Gobbetti et al 2014).

Baking also affects content and composition of phytonutrients in wholegrain products. Abdel-Aal and Rabalski (2013) reported that baking increased free ferulic acid of wholegrain bread. Similarly, Gelinas and McKinnon (2006) reported an increase in total phenol content of wholegrain bread regardless of baking time (10, 20 or 35 min). Sourdough fermentation during wheat and rye bread-making method showed an increase in phenolic acids (Liukkonen et al 2003; Katina et al 2007; Banu et al 2010; Konopka et al 2014). Increasing free phenolic compounds would result in boosting their bioavailability since the bioavailability of bound phenolic acids is too low (Anson et al 2009). Thus, baking could be a good approach to improve bioavailability of phenolic acids (the dominant compounds in wheat) in wholegrain products (Abdel-Aal and Rabalski 2013). Baking formula could also impact bioavailability of phytonutrients in wholegrain baked products. Read et al (2015) studied the bioavailability of lutein in the wholegrain bread, cookie and muffin using a fasted and fed digestion model. The fed model resulted in much higher estimates of bioavailability of lutein and the highest fat products (cookie and muffin) resulted in higher overall bioavailability.

The wholegrain products should be considered good sources of phenolic antioxidants. Currently, there are a few studies in the literature about the effect of different bread-making methods such as straight, sponge and sourdough on the phytonutrients of wholegrain products and their contribution to antioxidant properties. Moore et al (2009) reported that long fermentation and increased baking times or higher temperatures can increase the availability of antioxidants in whole wheat pizza crust and that increasing the baking temperature from 204 to

288C increased the antioxidant properties by as much as 82%. Yu and Nanguet (2013) also reported that baking method decreased the DPPH scavenging capacity, but increased the ORAC values of wholegrain bread. The authors found that wholegrain flour resulted in bread with higher antioxidant properties compared to the refined flour. The aim of this study was to investigate the effect of different bread-making methods on phytonutrients and antioxidant capacity of wholegrain bread products. Since wholegrain wheat flours are a good source of phytonutrients such as phenolic acids and flavonoids, it was hypothesized that the different fermentation types (e.g. yeast versus sourdough) and the bread-making methods (e.g. straight dough, sponge dough, and sourdough) might affect phytonutrients content and antioxidant properties of wholegrain bread products. Different replacement levels of sourdough were also investigated as they could also influence quality, phytonutrients, and antioxidant capacity of wholegrain bread. Specifically the following objectives were studied:

- 1) To investigate the impact of various bread-making methods on wholegrain bread quality and shelf life.
- 2) To study the effect of various bread-making methods on composition of phenolic acids and contents of flavonoids and carotenoids in wholegrain bread.
- 3) To investigate the impact of various bread-making methods on antioxidant properties of wholegrain bread.
- 4) To investigate changes of phytonutrient contents and antioxidant properties of wholegrain bread during storage.

The study results help better understand the effect of different bread-making methods on phytonutrients and antioxidants in wholegrain bread products. Moreover, it would help identify preferred bread-making method(s) that would preserve nutritional and bio-functional properties

of wholegrain bread. Such information is useful for the baking industry and wheat researchers to help make informed decisions with regard to the production of improved wholegrain bread products.

CHAPTER 2

LITERATURE REVIEW

2.1. Wholegrain and Wholegrain Products

The American Association of Cereal Chemists International (AACCI) defines wholegrain as “wholegrains shall consist of the intact, ground, cracked, or flaked caryopsis (kernel or seed), whose principal anatomical components—the starchy endosperm, germ, and bran—are present in the same relative proportion as they exist in the intact caryopsis” (AACCI International 2000). The grain or kernel contains three main parts: endosperm (80 - 85% of the grain), germ (2-3%) and outer layers or bran (13-17%). The endosperm is composed of cells having starch granules embedded in a protein matrix. The germ is rich in oil and fat-soluble vitamins, and the bran contains high concentrations of minerals, cellulose and hemi-celluloses. In addition, the outer layers are rich in bioactive components such as phenol compounds, anthocyanins, β -glucan and dietary fiber (Abdel-Aal et al 2006; Ragaei et al 2014).

AACCI defined wholegrain food as “A wholegrain food must contain 8 grams or more of wholegrain per 30 grams of product” (AACCI 2013). According to the 2005 Dietary Guidelines for Americans recommendations, a person’s daily intake of grain products should be at least three servings with at least half of it being wholegrain to reach the recommended daily intake of fiber (USDA 2005). The 2010 Dietary Guidelines for Americans supports this recommendation (USDA 2010). According to Canada’s Food Guide, the daily intake of grain products should be 6-8 servings and the recommended daily intake of fiber is “38 g/d for men 19-50, 30 g/d for men 51 and older, 25 g/d for women 19-50, and 21 g/d for women 51 and older” (Health Canada 2012). Wholegrain products such as wholegrain bread and pasta are a unique source of dietary

fiber (Liu 2003) and bioactive components such as phenolic acids (Abdel-Aal and Rabalski 2008). In general, wholegrain products are considered a good source of phytonutrients such as phenolic compounds, tocopherols, tocotrienols, carotenoids, plant sterols, and lignans (Slavin et al 2000; Jones et al 2004; Okarter and Liu 2010; Borneo and Leon 2012). These compounds play significant roles in human health and disease prevention such as cardiovascular disease, cancer and chronic diseases (Slavin et al 2000). As consumer became more health alert, baking industry is facing new challenges (Huttner et al 2010) to obtain an acceptable wholegrain bread quality with improved nutritional properties (Katina et al 2006).

2.2. Bread-Making

Wheat bread is a widely consumed baked product in the world. The bread-making method is the most common practice used in the baking industry. Several baking methods are used in the production of bread including straight dough, sponge and dough, and Chorleywood method (Giannou et al 2003; Mondal and Datta 2008) and sourdough method. The basic ingredients used in making bread are flour (refined or wholegrain), water, yeast, and salt. The sourdough method is primarily used in making rye bread.

In the straight dough method, all the ingredients are mixed in one step. Dough ingredients may differ according to manufacturer's choice (Giannou et al 2003). In Sponge dough method, the dough ingredients are mixed in two steps. In the first step, leavening agent is prepared by mixing part of the total flour (70%) with water, yeast, and yeast food. The mixture is left to develop for few hours. In the second step, the sponge is incorporated with the rest of the flour, water, and other ingredients to make the dough. (Giannou et al 2003; AACCI Approved Method 10-11.01). In Chorleywood bread method (CBP) or "no-time dough" method all the ingredients are mixed in a high-speed mixer for few minutes (Giannou et al 2003). The CBP has been mainly

used in the baking industry in the United Kingdom and some other countries. The principal of CBP is the use of mechanical energy to develop dough in the mixer, so fermentation time will be eliminated (Tronsmo et al 2003). Although it is no-time dough, some bakers may apply short period of fermentation (20-30 min) to support the dough development after mixing (Cauvain and Young 2007).

The sourdough method has been used for thousands of years (Paramithiotis et al 2007), where fermentation occurs by yeast and lactic acid bacteria (LAB) (Katina et al 2009). Sourdough usually used for rye bread production (Corsetti and Settanni 2007). Sourdough method is similar to sponge dough method where the mixing of the ingredient performed in two steps. Initially part of the flour is mixed with the yeast and sufficient amount of water to make “sponge” that is fermented in the normal atmosphere for a long time, typically overnight. Afterward, the sponge is mixed with the rest of the flour, water, fat and salt (Giannou et al 2003; Mondal and Datta 2007).

According to De Vuyst and Neysens (2005), there are three types of sourdough depending on the method and technology used by industry. These methods include the following: Type I or traditional sourdough; Type II or accelerated sourdough; Type III or dried sourdough.

Type I sourdough is performed at 20-30°C for 3-48h and the pH is about 4.0. Traditional sourdough produced using traditional technique (back slopping) by continuing refreshments daily to keep the microorganisms active (De Vuyst and Neysens 2005). Examples of products produced by type I sourdough are sourdough rye bread, San Francisco sourdough, French bread, Toscanon and Altamura bread, and Panettone (De Vuyst and Neysens 2005).

Type II sourdough is a semi-fluid silo preparation that is fermented at >30°C to speed up the fermentation methods to 24h and the pH is about >3.5. Due to the low pH of type II

sourdough, it has been used very often as acidification supplements during bread-making (Hammes and Ganzle 1997). Type II sourdough can be last for 2-5 days which assist industries to use it for controllable, fast, efficient, and large-scale fermentation method (De Vuyst and Neysens 2005).

Type III sourdough is a dried sourdough in a powder form that is initiated by “defined starter cultures” (De Vuyst and Neysens 2005). Stolz and Böcker (1996) reported that drum drying or spray drying can be applied to extend shelf life of sourdough and turn it into a standard product for further use. Thus, dried sourdough is a standardized end product, simple in use and convenient (Stolz and Böcker 1996).

Sourdough does not only contain yeast, but also contains different types of lactic acid bacteria, that give the final product a sour taste by producing acetic acid and lactic acid (Ravyts and De Vuyst 2011). The ratio of lactic acid bacteria to yeast in sourdough fermentation is generally 100:1 (Ottogalli et al 1996). However, yeast preparations often contain Lactic acid bacteria such as lactobacillus spp (Corsetti and Settanni 2007). The microflora in sourdough, represented by yeast and lactic acid bacteria, is responsible for the dough characteristics (Corsetti and Settanni 2007).

2.3. Bread Quality

Loaf Volume (LV), specific loaf volume (SLV) and crumb firmness are the main quality characteristic of bread (Katina et al 2006). The most common method of assessing whole product volume is by the rapeseed displacement method (AACCI 2011). In addition, image analysis methods have been applied for product volume measuring. The loaf specific volume, the ratio of bread volume to bread weight, is commonly used to assess bread quality (Belz et al 2012). Moreover, bread texture is a critical factor for consumer acceptance (Belz et al 2012). Crumb

firmness is important in bread texture assessment because it is associated with the perception of bread freshness (Cauvain 2003). This important parameter can be evaluated by texture profile analyses (TPA) as well as the sensory evolution test. The compression test is usually used to measure firmness, which is “defined as the force required to compress the crumb a fixed distance, or to evaluate freshness, defined as the distance a fixed force will compress a crumb” (AACCI 2011). The external appearance of the bread product is a major factor in attracting consumers. Thus, other external bread quality parameters should be assessed such as color, weight, height, shape, and flavor.

Bread made from wholegrain wheat flour often has lower loaf volume, firmer dense crumb, and darker crumb and crust comparing with bread made from refined wheat flour (Cai et al 2014). Incorporating wheat bran/fiber to enhance the nutritional properties could result in low bread quality. For example, it is difficult to obtain an acceptable loaf volume with high fiber flour (Cai et al 2014) since the addition of wheat bran decreases loaf volume (Katina et al 2006). The reduction in wholegrain or fiber-rich bread loaf volume is due to several factors including: 1) dilution of wheat gluten by the added fiber; 2) mechanical interference of insoluble fiber with the formation of gluten network causing rupture of gas cells (Gill et al 2002; Courtin and Delcour 2002); and 3) the higher water binding ability of fiber (both soluble and insoluble) causing less water available for the development of gluten network and less steam production (Gill et al 2002). Bread improvers (Gomez et al 2003) or commercial enzyme mixtures (Katina et al 2006) can be used to improve the quality of wholegrain or high-fiber bread. However, Pre-fermentation of wheat bran with lactic acid bacteria and yeast improved loaf volume and crumb softness (Katina et al 2006; Pountanenn et al 2009). Clarke et al (2002) reported that adding sourdough during wheat bread-making method increased the loaf volume more than chemical

acidification alone.

Sourdough is a key element in wholegrain bread-making (especially, rye) to contribute flavor and texture to the bread product (Pountanenn et al 2009). Sourdough can be used for improving quality of bread products without using other improvement additives (Rieder et al 2012). Sourdough bread-making method produced improved bread with good quality such as improved texture, flavor, and shelf life (Katina et al 2006), besides preserving some bioactive compounds such as phytate, folates, tocopherols, and phenolics (Liukkonen et al 2003; Michalska et al 2007; Hansen et al 2002; Katina et al 2007; Banu et al 2010). Using sourdough also increases the anti-mold activity level to 112 h, which is 115% higher than anti-mold activity of the control bread prepared without sourdough (Najafi et al 2012). The study suggested that the anti-molding activity is depending on the microorganisms presented in the sourdough. *Lactobacillus plantarum*, *L. acidophilus*, and *Leu.mesenteroides* showed high anti-mold activities (Najafi et al 2012).

2.4. Phytonutrients in Wholegrain

In general, phytonutrients are natural compounds produced by plants as protective substances against external stress and/or pathogenic attack (Chew et al 2009). They are secondary metabolites that play significant roles in plant defense and enable plants to overcome temporary or continuous threats integral to their environment. The main classes of phytonutrient s in wholegrains are phenolics (e.g., phenolic acids, flavonoids, and alkylresorcinols), carotenoids, tocols (vitamin E) and lignans (Liu 2007, Okarter and Liu 2010). Phenolics are the most studied phytonutrient s in wholegrains (Okarter and Liu, 2010). They exist in soluble free compounds, soluble conjugated esterifies to sugars and other low molecular weight compounds, and insoluble bound compounds (Zilic et al 2012). The most common phenolics in wholegrain

are phenolic acids and flavonoids (Zilic et al 2012; Liu 2007). The type and concentration of phytonutrients vary among grains and genotypes (Adom et al 2003). Abdel-Aal and Rabalski (2008) determined phytonutrients including phenolic acids, carotenoids and tocopherols in seven wheat species. Total phenolic content significantly varied between wheat species and cultivars ranging from 881 to 2382 $\mu\text{g/g}$. Ferulic acid concentration ranged broadly (220-574 $\mu\text{g/g}$) in the wheat species due to their environmental and genetic diversity of wheat used in the study.

2.3.1. Phenolic acids:

Phenolic acids are hydroxybenzoic acid or hydroxycinnamic acid derivatives (Figure 2.1). The common derivatives of hydroxybenzoic acid in grains are *p*-hydroxybenzoic, protocatechuic, vanillic, syringic, and gallic acids. The common derivatives of hydroxycinnamic acid are *p*-coumaric, caffeic, ferulic, and sinapic acids. The foremost phenolic acid found in grains is ferulic acid which is primarily found in a bound form, linked to cellulose, or hemicellulose through ester links in the cell wall (Liu 2007).

Ferulic acid is the major phenolic acid found in grains (Liu 2007; Zilic et al 2012). In wheat, ferulic acid is the main phenolic acid, which accounts for 90% of the total phenolic acids present in the wheat grain (Abdel-Aal and Rabalski 2008). Other studies found that ferulic acid present in free and bound forms in cereal grains (Manach et al 2004; Liu 2007). Ferulic acid is mostly concentrated in the aleurone and pericarp of the grains, while minor quantity is present in the starchy endosperm (Liu 2007). Manach et al (2004) reported that the wheat grain aleurone layer and pericarp hold 98% of the total ferulic acid, which make up approximately 90% of the total polyphenols.

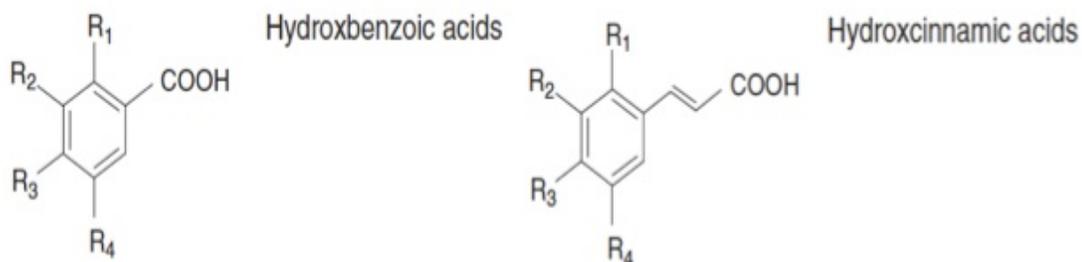


Figure 1.1 Chemical structures of phenolic acids (Ragaee et al 2014).

2.3.2. Carotenoids:

Carotenoids, in general, are a collection of tetraterpenoid compounds, with the basic carotenoid structural backbone consisting of isoprenoid units formed either by head-to-tail or by tail-to-tail biosynthesis. Carotenoids are compounds with a forty-carbon skeleton and they commonly exist in nature in the all-trans form (Figure 2.2). The primary groups of carotenoids are carotenes and xanthophylls. Carotenes are carotenoids that contain only hydrocarbon made up from isoprene units, such as α -, β -, and γ -carotene and lycopene. Xanthophylls are carotenoids that contain oxygen as hydroxyl, keto, carboxyl, methoxyl, and epoxy group, such as lutein and zeaxanthin (Abdel-Aal et al 2007). Lutein is the main yellow pigment in wheat (Abdel-Aal et al 2007). The concentration of carotenoids in cereal grains exhibited a wide range from very low in white and red wheat to relatively high in einkorn and durum wheat (Abdel-Aal et al 2002, 2007).

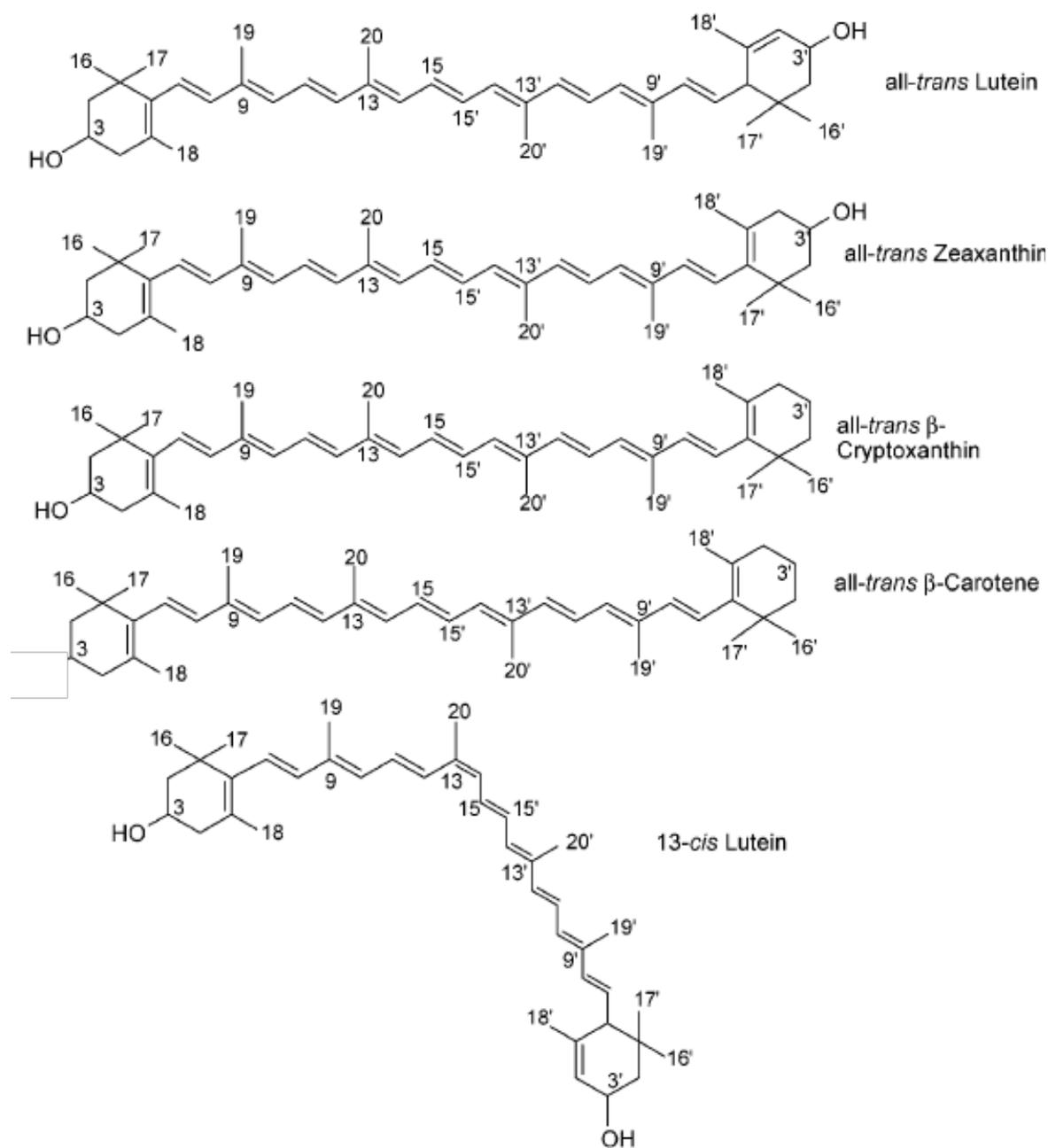


Figure 2.2 Chemical structures of carotenoids present in wheat (Abdel-Aal et al 2007).

2.3.3. Flavonoids:

Flavonoids are phenolic compounds containing two aromatic rings linked by a three-carbon structure and exist in an oxygenated heterocyclic ring (Figure 2.3) (Okarter and Liu 2010). Flavonoids include flavonols, flavanones, flavones, isoflavones, flavans, and anthocyanins (Duodu 2011). Flavonols such as quercetin, kaempferol and myricetin are very common flavonoids in foods (Martel et al 2010). Tricin or 5,7,4'-trihydroxy 3',5'-dimethoxyflavone is known as the dominant flavone pigment in wheat. In addition, two C-glycosylflavones, 6-C-pentosyl-8-C-hexosylapigenin and 6-C-hexosyl-8-C-pentosylapigenin, were found in wheat bran (Feng and McDonald 1989). Total flavonoid content of 11 diverse wheat varieties ranged from $122 \pm 10 \mu\text{mol/g}$ (catechin equivalents) to $149 \pm 17 \mu\text{mol/g}$ (catechin equivalents) (Adom et al 2003). In cereal grains, flavonoids are located in the pericarp (Dykes and Rooney 2007). Most of the total flavonoids (79%) found in wheat present in the bran and germ fraction (Okarter and Liu 2010). Anthocyanins, a group of water-soluble flavonoids impart red, purple and blue colors in plants (Abdel-Aal et al 2006; Dykes and Rooney 2007; Martel et al 2010).

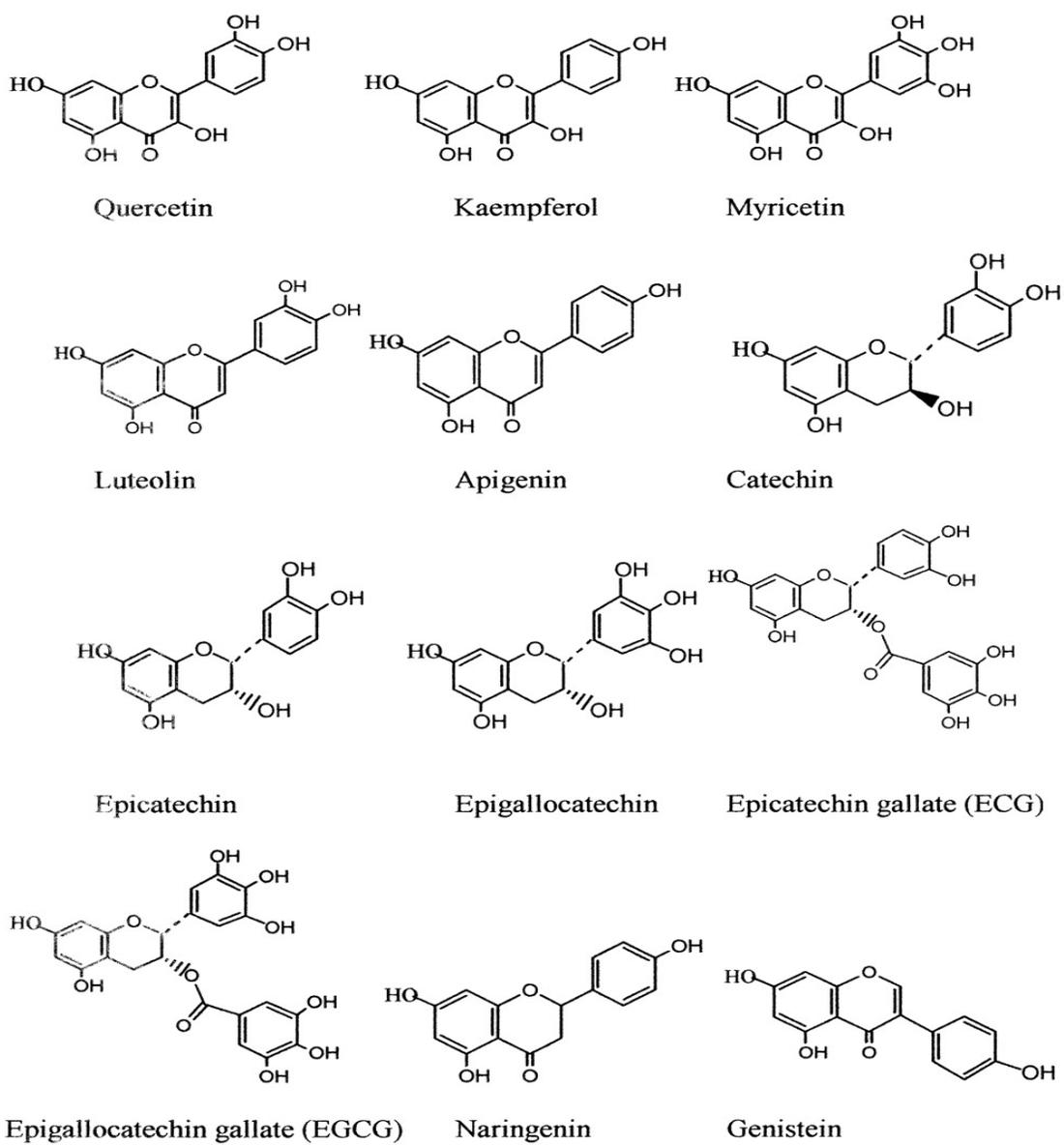


Figure 2.3 Chemical structures of common flavonoids in foods (Liu 2004).

2.3.4. Tocopherols and tocotrienols

Tocopherols and tocotrienols or vitamin E precursors are fat-soluble compounds that include a 6-hydroxychroman group and a phytyl side chain made of isoprenoid units (Figure 2.4) (Liu 2007). Tocopherols (α , β , γ , and δ -tocopherol) and tocotrienols (α , β , γ , and δ -tocotrienol) have almost the same structure but tocopherols contain a fully saturated phytyl side chain while tocotrienols contain a polyunsaturated phytyl side chain (Okarter and Liu 2010). Tocopherols and Tocotrienols can be found in different types of foods and wholegrains, especially in the germ fraction (Liu 2007).

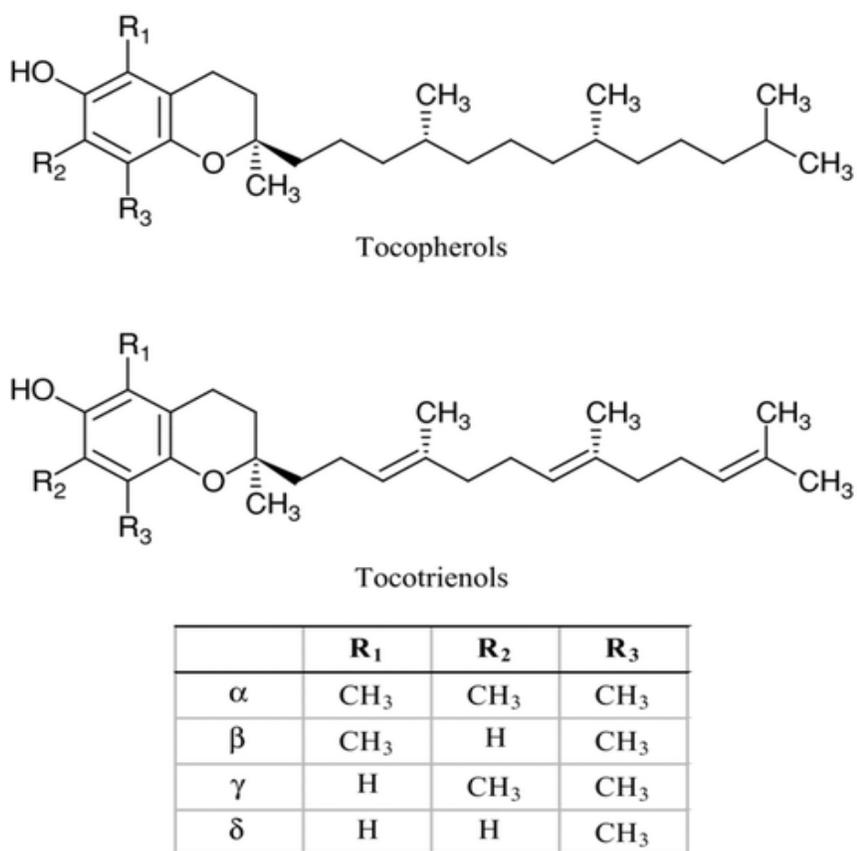
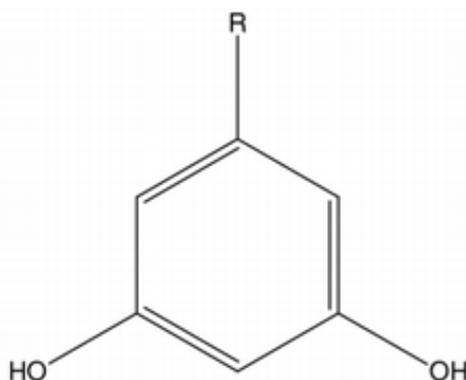


Figure 2.2 Chemical structures of vitamin E: tocopherols and tocotrienols (Okarter& Liu 2010).

2.3.5. Alkylresorcinols:

Alkylresorcinols and alkenylresorcinols are amphiphilic derivatives that are derived from 1,3-dihydroxybenzene, and contain an odd-numbered alkyl or alkenyl chain at position 5 of the benzene ring (Figure 2.5). Alkylresorcinols are mostly found in the grain bran which explains why they are not found in refined wheat products (Ross et al 2003). Rye contains the highest total alkylresorcinol content ($734 \pm 23 \mu\text{g/g}$) followed by wheat ($583 \pm 82 \mu\text{g/g}$) and barley ($45 \pm 5 \mu\text{g/g}$) on a dry matter basis (Ross et al 2003). However, in corn, millet, oats, rice, and sorghum no alkylresorcinols were detected (Okarter and Liu 2010).



Name	R	Structural Formula
5-n-pentadecylresorcinol	C ₁₅ H ₃₁	CH ₃ -(CH ₂) ₃ -CH=CH-(CH ₂) ₈ -CH ₂ -
5-n-heptadecylresorcinol	C ₁₇ H ₃₅	CH ₃ -(CH ₂) ₃ -CH=CH-(CH ₂) ₁₀ -CH ₂ -
5-n-nonadecylresorcinol	C ₁₉ H ₃₉	CH ₃ -(CH ₂) ₃ -CH=CH-(CH ₂) ₁₂ -CH ₂ -
5-n-heneicosylresorcinol	C ₂₁ H ₄₃	CH ₃ -(CH ₂) ₃ -CH=CH-(CH ₂) ₁₄ -CH ₂ -
5-n-tricosylresorcinol	C ₂₃ H ₄₇	CH ₃ -(CH ₂) ₃ -CH=CH-(CH ₂) ₁₆ -CH ₂ -

Figure 2.3 Chemical structures of Alkylresorcinols (Okarter & Liu 2010).

2.3.6. Lignans and lignin:

Lignans are compounds that contain two coupled C₆C₃ units and they are considered as a kind of dietary phytoestrogen (Figure 2.6). Secoisolariciresinol, pinoresinol, matairesinol,

lariciresinol, and syringaresinol are the common plant lignans. Lignans can be found in different plant foods such as flaxseeds, wholegrains, including corn, oats, wheat, and rye, legumes, fruits, and vegetables (Liu 2007). Lignin is an aromatic polymer derived from hydrocinnamic alcohols in plants (Figure 2.7) (Peterson et al 2010; Liu 2010). It is considered as insoluble dietary fiber in cereal bran (Peterson et al 2010).

Lignans

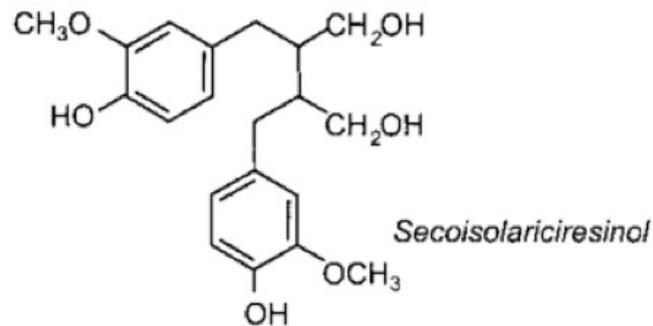


Figure 2.4 Chemical structures of Lignans (Manach et al 2004).

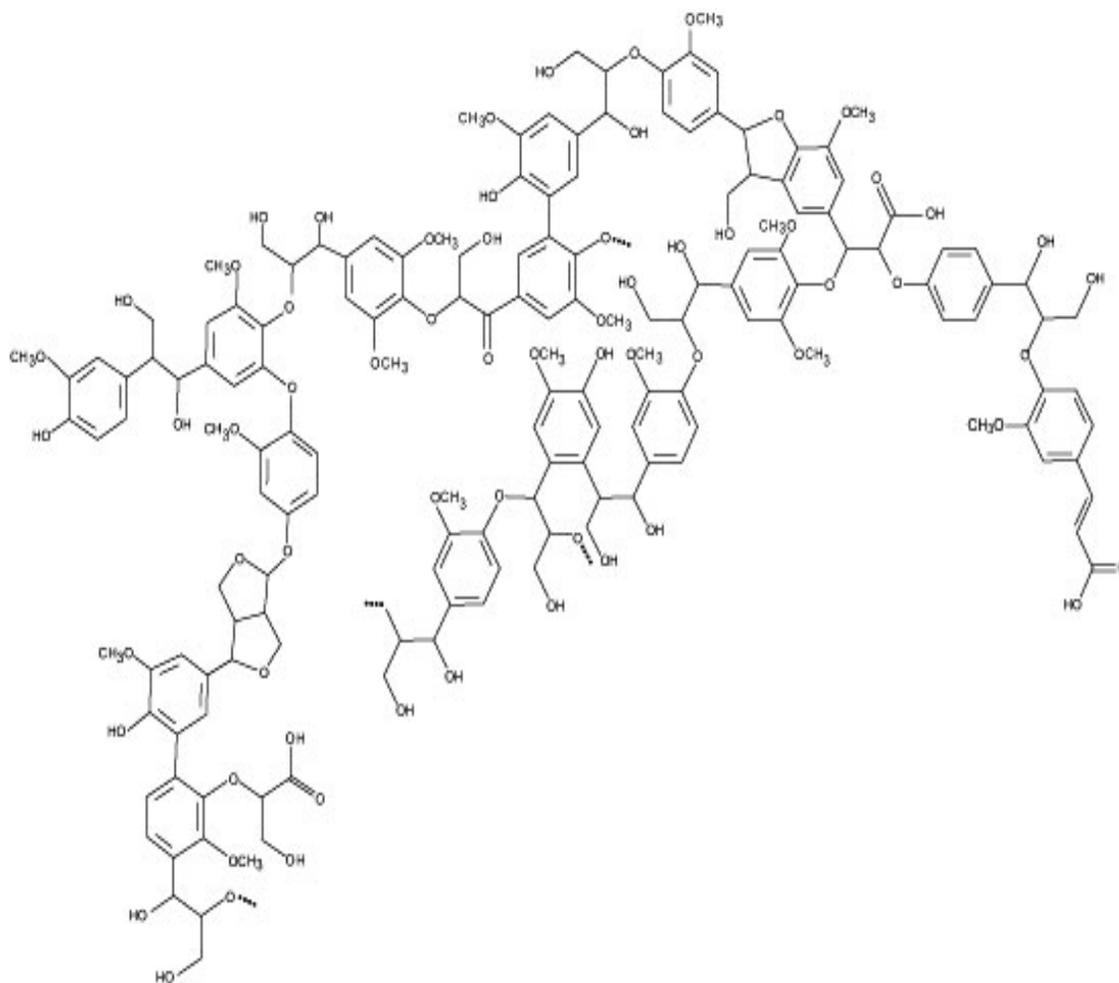


Figure 2.7 Chemical structures of Lignin polymers

2.5. Antioxidant Properties of Wholegrain

Research on dietary antioxidants has received much attention since the free radical theory of aging introduced in 1956 (Harman 2009). Antioxidants are substances that at low concentration can delay or inhibit the oxidation of a substance by free radicals, reactive oxygen species or other reactive species (Halliwell 1994). According to Halliwell (1994) free radicals are atoms, ions or molecules with unpaired electrons on an open shell configuration. It has been well documented that the free radicals and reactive oxygen species generated during peroxidation of lipids and other biological molecules or cellular metabolism play significant roles in the pathogenesis of chronic diseases such as coronary heart disease and cancer. Dietary antioxidants combat free radicals and reactive oxygen species to help in reducing the risk of chronic diseases (Abdel-Aal and Rabalski 2008).

Phenolic compounds especially phenolic acids are the main antioxidant contributors in whole-grain products (Abdel-Aal et al 2012, 2013). Generally, hydroxycinnamic acids and in particular ferulic acid are the main phytonutrient s in wholegrain. The antioxidant potential of ferulic acid is mainly attribute to the electron donation and hydrogen atom transfer to free radicals (Liu 2007). Beside ferulic acid, wheat grain contains other hydroxycinnamic acids with antioxidant activity such as coumaric, sinapic and caffeic acid (Shahidi and Chandrasekara 2009). Additionally, vitamin E or tocopherols are strong antioxidants because of their ability to scavenge lipid peroxyl radicals and reactive nitrogen and oxygen species (Zilic et al 2012).

Ragae et al (2006) reported that hard wheat flours had slightly higher radical scavenging capacity, as measured by DPPH and ABTS, than soft wheat flour. Moore et al (2009) found a correlation between the levels of free phenolic acids of wheat bran and antioxidant capacity. The study showed that the greater antioxidant capacity, the greater the release of bound phenolic

acids. Additionally, Adom et al (2003) discovered the highest phenolic content and radical scavenging capacity occurred with the white spring durum cultivar. Iqbal et al (2007) measured the radical scavenging capacity of wheat grown in Pakistan by ABTS, DPPH, and ORAC. The authors indicated that the antioxidant capacity was related to total phenol and anthocyanin content.

2.5. Wholegrain Phytonutrients and Human Health

Wholegrain contain unique phytonutrients that are not found in fruits and vegetables such as ferulic acid and diferulates (Adom and Liu 2002). Several studies reported that phytonutrients in wholegrain could exhibit bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties (Okarter and Liu 2010; Lillioja et al 2013; Jideani et al 2014).

The antioxidant properties of wholegrain phytonutrients play a significant role in cancer prevention (Rastmanesh 2011). For example, wholegrain flavonoids have been demonstrated to act as antioxidant and anticancer compounds (Adom and Liu 2002). The antioxidant ability of flavonoids depends on the number and position of hydroxyl groups in the molecule (Zilic et al 2012). The authors suggested that eating foods rich in anthocyanin reduced the risk of colon cancer by inhibiting cancer cell production in the colon (Zilic et al 2012). Moreover, most of the phytonutrients in wholegrain are in bound form and these bound phytonutrients are not easy to digest in the gastrointestinal tract. However, after surviving digestion in the gastrointestinal tract, they reach the colon, which, in turn, causes this bound phytonutrient to prevent colon cancer (Adom and Liu 2002). Ferulic and diferulic acids in cereal bran can be released by gastrointestinal esterase particularly microflora and intestinal mucosa (Liu 2007). This provides an explanation for the relationship between wholegrain consumption and the reduced risk of colon cancer

(Adom and Liu 2002). There was an inverse association between serum concentrations of α -carotene, β -carotene, lutein, lycopene and β -cryptoxanthin and the risk of lung cancer (Woggon and Kundu 2004). A study by Freudenheim et al (1996) proved that the intake of carotenoids, especially lutein and zeaxanthin, is correlated with reduced risk of premenopausal breast cancer.

Borneo and Leon (2012) suggested that the antioxidants in wholegrains are connected with the cardiovascular system's health. For instance, phenolics in grains reduce LDL cholesterol oxidations and prevent platelet aggregation, which are the two most common causes of cardiovascular disease. Moreover, tocotrienols and tocopherols inhibit the oxidative damage of cell membranes (Borneo and Leon 2012). Naderi et al (2003) found that increasing flavonoids in dietary intake can help to decrease coronary artery disease.

2.7. Effect of Bread-Making on Phytonutrients and Antioxidants

2.6.2. Mixing:

Mixing is the first step in bread-making method that starts with blending the ingredients together with water to hydrate the flour components and to develop dough. The incorporation of water during mixing initiates oxidative enzymes (lipoxygenase) present in wheat flour, which affect phytonutrients such as carotenoids, tocols, phenols (Maraschin et al 2008; Ktenioudaki et al 2014). Vogrincic et al (2010) studied the effect of bread-making methods on flavonoids content (rutin and quercetin) using tartary buckwheat flour. They found that rutin decreased during bread-making methods (mixing and proofing) and was not detectable after baking in most samples. Moreover, a significant decrease in ferulic acid in rye wholegrain (from 1079 to 1022 $\mu\text{g/g}$) was observed after mixing (Hansen et al 2002). Leenhardt et al (2006) reported a decrease in carotenoid content after mixing during the bread-making method of French bread using wholegrain and white flour. Wholegrain showed the greatest loss of carotenoids (66%). In the

same study, there was a strong correlation between lipoxygenase activity and carotenoid losses ($r^2 = 0.97$).

2.6.3. Fermentation:

Fermentation is the next step that follows the formation of dough, in which the dough is left to rise before baking. Moore et al (2009) studied the effect of proofing on the antioxidant properties of pizza dough made with wholegrain wheat flour. They found that soluble free ferulic acid content significantly increased after fermentation for 18 or 48 hours under refrigerated condition at 4°C. On the other hand, the insoluble bound ferulic acid significantly decreased by about 61% after 48 hours of fermentation. However, no changes were observed in antioxidant capacity measured by the ORAC assay, the ABTS or DPPH scavenging capacity (Moore et al 2009).

The content of bioactive compounds including phenolic acids, alkyresorcinols, tocols, sterols, folate, lignans, and thiamin could be modified by sourdough fermentation (Banu et al 2010). There can be an increase or decrease of these compounds level depending on the sourdough method or on the compounds nature itself (Katina et al 2005). Wheat and rye sourdough fermentation increases total content of phenolics (Coda et al 2010), free ferulic acid content and antioxidant properties (Katina et al 2007, Konopka et al 2014).

Katina et al (2007) found that in general yeast fermentation (straight dough) or lactic acid bacteria fermentation (sourdough) increased the levels of lignans, free ferulic acids, and stabilization of alkylresorcinols, native and germinated rye. Similarly, Liukkonen et al (2003) examined the effect of sourdough fermentation on several bioactive compounds such as phenolic acids, sterols, folates, tocopherols and tocotrienols, alkylresorcinols, and lignans. Sourdough fermentation increased the content of phenolic compounds and folates, whereas reduced the

content of tocopherols and tocotrienols with no significant changes in the other bioactive compounds that were examined. There was an increase in the antioxidant capacity reported during fermentation due to the increase of methanol extracted phenolic compounds (Liukkonen et al 2003).

2.6.4. Baking:

In bread-making methods baking temperature and time can vary, which in turn affect bioactive compounds and antioxidant properties in a different way due to their dissimilar sensitivity to heat. Maillard reaction plays an important role in increasing the antioxidant capacity of baked products, particularly in the crust as compared to bread crumb because of its exposure to a higher temperature in the baking oven (Lindenmeier and Hofmann 2004; Vogrincic et al 2010; Ktenioudaki et al 2013).

Abdel-Aal and Rabalski (2013) investigated the changes in free and bound phenolic acids that occurred during baking in wholegrain breads, cookies, and muffins. The products were also fortified with lutein. The authors showed that baking increased free phenolic acids in the three products (breads, cookies, and muffins). Bound phenolic acids, on the other hand decreased in bread and were slightly changed in cookies and muffins. Gelinias and McKinnon (2006) showed that baking increased the concentration of phenolic compounds in bread when compared with the flour, regardless of baking time. They found that the phenolic compounds were significantly increased after baking, perhaps as a result of the Maillard reaction. However, the increase of phenolic compounds was observed only in white bread, which is bran-free, and not in wholegrain bread (Gelinias and McKinnon 2006). Ragaee et al (2011) also observed slight differences in the levels of phenolic compounds contained in flours and the levels in the resultant breads: there were few changes in phenolic content due to baking. Moore et al (2009) studied the

effect of baking on phenolic acids in whole wheat pizza dough. The study reported an increase in the level of extractable free ferulic acid in one of the two wheat varieties studied, whereas the soluble conjugated ferulic acid content decreased for both varieties after baking. They also found that increasing the time or the temperature during baking increased antioxidant capacity (Moore et al 2009). Increasing thermal treatment, by increasing the baking time from 7 to 14 minutes at 204° C or by increasing the temperature from 204 to 288° C while maintaining a 7-minute baking time, resulted in a significant increase in ABTS scavenging properties and RDSC for both wheat varieties compared with the unbaked dough. ORAC values were affected only by increasing the temperature to 288° C for 7 minutes (Moore et al 2009). Another study by Vogrincic et al (2010) found that polyphenol content decreased during baking for breads containing tartary buckwheat. The authors reported that rutin was degraded during mixing and about 85% was transformed to quercetin, while quercetin did not change during baking. Alvarez-Jubete et al (2010) studied the stability of phenolic acids and flavonoid compounds in amaranth, quinoa, and buckwheat during the bread-making process. The authors reported a significant reduction in phenolic acid content in the bread when compared to the flour. Furthermore, the contents of flavonoid compounds such as quercetin and kaempferol glycosides in 100% quinoa breads decreased. Abdel-Aal et al (2010) studied the stability of lutein and zeaxanthin in unfortified and fortified baked products (pan bread, flat bread, cookies, and muffins) using different baking recipes and baking conditions. Baking of flat bread resulted in a significant reduction in all-trans-lutein: losses of about 37-41% for unfortified breads and 29-33% for fortified breads. Leenhardt et al (2006) reported losses ranging from 35% to 45%, depending on the wheat species used. Hidalgo et al (2010) also showed carotenoid loss during processing. Bread crumbs lost 21% of their carotenoid content, while 47% of the carotenoids were lost in bread crusts due to manufacturing. The highest losses

were observed in the crust, which is exposed to higher temperatures than is the crumb. Wennermark and Jagerstad (1992) reported vitamin E losses of between 24% and 47% in white breads and between 10% and 15% in wheat and rye breads because of baking. They found that baking losses occur due to the extractability changes in vitamin E.

Most phenolic substances are concentrated mainly in the outer layer of cereal grains; using wholegrain flour during bread making therefore reduces the loss of phytonutrients and increases health benefits for consumers. Most of the studies reviewed have shown that the bread-making process produces various effects on phytonutrient and antioxidant capacity. As a result, the choice of bread-making method and baking ingredients will help in producing healthful bread.

CHAPTER 3

Effect of Bread-Making Methods on Quality and Shelf Life of Wholegrain Bread

3.1. ABSTRACT

Wholegrain breads are good sources of dietary fiber and phenolic antioxidants that could deliver health benefits in addition to basic nutrition. However, it is a challenge to produce high quality bread from fiber-rich or wholegrain flour. This study was conducted to investigate quality and shelf life of wholegrain bread made by three bread-making methods, namely straight dough, sponge dough and sourdough. Sourdough breads were made with three levels of sourdough starter 15, 25 and 35%. Bread quality was assessed for freshly baked breads and during storage for 7 days at room temperature. Significant differences ($P \leq 0.05$) in loaf volume (LV), specific loaf volume (SLV) and crumb firmness were found among bread made by straight, sponge and sourdough methods. Breads made by sourdough bread-making method improved LV and SLV values and crumb firmness. Use of 15% sourdough starter produced bread having highest LV (600 cm^3) and SLV ($4.01 \text{ cm}^3/\text{g}$) and lowest firmness (207g). Storage of breads at room temperature caused changes in crumb firmness and moisture content with the lowest effect observed in breads made from sourdough. The results suggest that wholegrain breads made from sourdough containing 15% starter showed better quality over straight or sponge dough method.

Keywords: wholegrain, sourdough, straight dough, sponge dough, bread quality.

3.2. INTRODUCTION

Bread is an essential part in human diets around the world for millennia. Since consumers today are interested in healthy foods, producing bread with wholegrain flour is one approach for making healthier breads as opposed to that made from refined flours (Slavin 2004). The health benefits associated with the consumption of wholegrain food have been well documented (Liu 2007; Slavin 2010; Okarter and Liu 2010; Anderson 2014). Wholegrain foods have been linked with reduced risk of coronary heart disease, diabetes, and cancer (Fardet 2010; Borneo and Leon 2012; Gani et al 2012). Nutritionally, wholegrain wheat flour contains higher concentrations of dietary fiber, vitamins, minerals, phytic acid, and phenolic compounds than refined wheat flour (Liu 2007; Jaekel et al 2012). Along with the perceived health benefits of wholegrain bread, the baking industry and consumers are also concerned with bread quality.

The relatively high content of fiber in wholegrain flours as opposed to refined flours significantly affects the quality of the end product resulting in breads with low loaf volume, dense crumb, dark crust and crumb, and reduced crumb softness (Gellynck et al 2009). Similarly, the incorporation of fiber into white flour negatively affects bread quality (Hager et al 2010). Even though the quality of fiber-rich bread can be improved by adding some bread improvers, there are still noticeable differences in quality compared to white bread (Gomez et al 2003). The shelf life of bread made from wholegrain or fiber-enriched flours could also be influenced. After baking, bread has a short shelf life as determined by crust crispiness and crumb softness, which change during storage at room temperature for about 7 days (Katina et al 2006; Yazar and Tavman 2012). During storage, there is an increase in crumb hardness and loss of bread freshness known as staling that reduces palatability (Hebeda et al 1990). To keep the high quality

of bread during storage a number of different techniques could be applied such as various formulations, packaging technologies, and/or processing methods (Corsetti et al 1998).

The method of bread making can impact the quality and shelf life of baked bread. Several methods such as straight dough, sponge and dough, and the Chorleywood method are now available (Giannou et al 2003; Mondal and Datta 2007). Additionally, the sourdough method is traditionally used in making rye bread to improve both taste and nutritional quality (Bondia-Pons et al 2009). Sourdough, a mixture of flour and water fermented together with lactic acid bacteria (LAB), can change the properties of bread (De Vuyst and Neysens 2005; Ravyt and De Vuyst 2011). A number of studies have been carried out to determine the potential of using the sourdough method to achieve higher quality bread with extended shelf life (Arendt et al 2007; Dal Bello et al 2007; Rieder et al 2012; Flander et al 2011). The present study was designed to investigate effects of three bread-making methods including straight dough, sponge dough, and sourdough on the quality and shelf life of wholegrain bread.

3.3. MATERIAL AND METHODS

3.3.1. Materials

Wholegrain flour was obtained from (Parrish and Heimbecker Milling Group) Dover Flour, Cambridge. The wholegrain flour contains protein, ash, and total dietary fiber at an average content of 13.7%, 1.4% and 11.5%, respectively. A freeze-dried sourdough starter culture, Florapan® LA6, *Lactobacillus brevis*, was purchased from Lallemand Baking Solutions Ltd, Mississauga, ON, Canada. Dry yeast (Fleischmann's Traditional Active Dry Yeast, product of Canada) and other ingredients were purchased from a local grocery in Guelph, ON, Canada.

3.3.2. Water Absorption and Gluten Strength of Wholegrain Flour

Flour's water absorption (%) was determined according to the AACCI approved method

54-21-02 (AACCI 2011) using a Brabender Farinograph-E (Brabender GmbH & Co. KG., Duisburg, Germany) equipped with a 50 g bowl. Flour's gluten strength was measured in accordance with Kaur and Seetharaman (2012) using a gluten peak test (GPT) (Brabender GmbH and Co. KG., Duisburg, Germany). Solvent (9.5 g, 0.5 M CaCl₂) was weighed in the GPT stainless steel mixing cylinder and 8.5 g flour was added to the solvent, and the test began immediately at 1,900 rpm for 10 min. Test temperature was adjusted to 34° C using a Brabender water bath connected to the GPT. The torque (Brabender equivalents, BE) and peak maximum time (PMT) resulting due to formation of a gluten network were calculated using GPT software (version 1, Brabender GmbH and Co. KG., Duisburg, Germany).

3.3.2. Bread-Making Methods

Three bread-making methods including straight dough, sponge dough, and sourdough were used to prepare wholegrain breads. Straight dough loaves were prepared according to the AACCI approved method 10.10.03 (AACCI 2011). The 60 min fermentation time was applied. The baking ingredients are presented in Table 3.1. Sponge dough loaves were prepared based on the AACCI approved method 10.11.01 (AACCI 2011). The baking ingredients are presented in Table 3.1.

Sourdough starters were prepared by mixing 0.1 % (based on flour weight) of Florapan LA6 starter culture with the flour on low speed for 30 seconds. Lukewarm water was added in a ratio of 1:1 flour to water and the sourdough was mixed in a KitchenAid (Professional 600 Series) stand mixer (Mississauga, ON, Canada) for 6 min on low speed. The sourdough was placed in a beaker, covered, and incubated at 30° C for 20 h. After 20 h of incubation, the sourdough was used for bread making. Sourdough breads were made with three levels of sourdough starter (15, 25, and 35%).

Preliminary experiments were carried out to adjust fermentation and proofing time and to determine the effect of incorporating gluten to improve quality of sourdough. The sourdough loaves were prepared in accordance with Flander et al (2011). The amount of wholegrain flour and water used in the prepared sourdough starter was taken into account and the corresponding amount of wholegrain flour and water was omitted from the bread formula to achieve consistent flour and water content in the bread dough. Sourdough breads were made with 15%, 25%, and 35% sourdough levels by adding 30 g, 50 g, and 70 g of sourdough starter, respectively, to the bread dough mixture. The amounts of other ingredients used in the formula are presented in Table 3.1. (3.2 g) was suspended in part of the water (15-30 mL) (24 °C). In a spiral mixer, half of the flour was thoroughly blended with the sugar. The yeast suspension was added to the mixture along with the shortening, salt, and the rest of the water. The dough was mixed at a high speed (200 rpm) for 6 min. Sourdough starter and the rest of the flour were added to the dough and mixed again at a low speed (100 rpm) for 6 min. The dough was rested at room temperature for 12 min, divided into two pieces, rounded and molded, and placed in a pan sprayed with grease. After proofing for 45 min at 39° C and 60% RH in a Hobart proofer (Hobart, USA) the breads were baked at 210° C for 30 min (Figure 3.1). Ten baking trials were carried out for each method. Loaves were allowed to cool down and stored in a polyethylene Zip-lock bag at room temperature for 7 days for storage experiment.

3.3.3. pH of Sourdough Starter, Bread Dough and Baked Bread

The sourdough starters were thoroughly mixed with a stirring bar and pH was measured directly using a pH meter. For bread dough and baked bread, 5 g was suspended in 10 mL of distilled water, and the suspension was thoroughly mixed with a stirring bar and measured with a pH meter.

3.3.4. Loaf Volume and Specific Loaf Volume

Bread loaves were cooled to room temperature (approximately 2 h after removal from the oven). Loaf weight was measured using an analytical balance, and loaf volume was measured by the rapeseed displacement technique as outlined in AACCI approved method 10-05-01 (AACCI 2011). Specific Loaf volume was calculated as cm^3/g by dividing the loaf volume by its weight. Four loaves of each type were placed in airtight plastic Ziploc bags and stored at room temperature for 1, 3, 5, and 7 days after baking.

3.3.5. Crumb Firmness

Crumb firmness was measured on the baking day and during storage time (1, 3, 5, and 7 days after baking) at room temperature. Crumb firmness was measured using Texture Analyzer (TA.XT2. Plus, Texture Technologies Corp., Scarsdale, NY, USA) provided with the software Texture Exponent 32 using a modified AACCI 74-09 method (2011). Bread slices (25 mm) from the middle of each loaf were used in the measurement. The test was conducted with a 5g-trigger force and a test speed of 1.7 mm/sec. The results were calculated using Texture Exponent 32 software (Texture Technologies Corp Scarsdale, NY, USA) and presented by hardness force (g). Means and standard deviations for hardness were reported.

3.3.6. Crumb Moisture Content and Water Activity

The moisture content and water activity of the crumb were determined on the baking day and during storage time. Moisture content was measured using the Ohaus Halogen Moisture Analyzer MB45 (Ohaus, Switzerland). Water activity was measured using the Water Activity Analyzer (Aqua Lab 4TE, Decagon Devices, USA).

3.3.7. Crust and Crumb Color Determination

The color of crust and crumb was analyzed using a Konica Minolta CM-3500d Spectrophotometer (Konica Minolta Sensing, Inc., New Jersey, USA) equipped with SpectraMagic NX CM-S100 software. The spectral attributes were based on CIE system, such as lightness (L^*), greenness/redness (a^*), and blueness/yellowness (b^*). The Browning index (BI) of crumb and crust were calculated according to the following equation outlined by Ragae et al (2010):

$$BI = \frac{(a^* + 1.75 \times L^*)}{(5.645 \times L^* + a^* - 3.012 \times b^*)}$$

3.3.8. Statistical Analysis

All analyses were performed at least in duplicate, and the data were reported as mean \pm standard deviation (SD). Analysis of variance was performed using IBM SPSS Statistics 21 software for Mac (Armonk, New York, USA). Significant difference ($p \leq 0.05$) among means were detected using a Tukey's multiple range test at a fixed level of $\alpha = 0.05$.

3.4. RESULTS AND DISCUSSION

3.4.1. Quality of Wholegrain Flour

Flour quality affects bread quality when making bread. In the current study, flour quality was assessed using a farinograph and gluten peak tester (GPT). The amount of water required to fully dehydrate flour and form developed gluten is key in making quality bread. Since wholegrain flours contain soluble fibre, it is crucial to efficiently determine its water absorption. The farinograph water absorption of wholegrain flour used in this study was 71%, which is

higher than refined hard wheat flour (63%). Other studies (Rieder et al 2012; Schmiele et al 2012) reported similar observations when 10–40% of wheat flour was replaced by wheat or oat bran. Water absorption increased from 63% for hard wheat refined flour to 72% for wheat flour containing 40% wheat bran (Schmiele et al 2012). The increase in the water absorption of wholegrain or bran-rich flour was probably due to the high fibre content. The gluten quality of wholegrain flour was measured with the GPT. GPT is a rapid shear-based method for identifying gluten quality and the functionality of flour in an aqueous solution. The maximum torque (MT) and peak maximum time (PMT), also known as gluten aggregation time, are obtained because of the gluten network formation. The GPT of the wholegrain flour results showed a MT 56.4 ± 1.03 (BE) and PMT 0.80 ± 0.05 (min). High torque response and short PMT are indications of high-quality gluten (Chandi and Seetharaman 2012). A previous study showed that the addition of wheat bran resulted in higher MT and shorter PMT compared to the refined hard wheat flour (Adams et al 2015).

3.4.2. Adjustments of Fermentation Time in Sourdough Method

Baking wholegrain bread improves nutritional properties but in order to produce an acceptable bread quality, a few adjustments in dough formation are needed. Sourdough bread was prepared according to Flander et al (2011) study that conducted in Finland where sourdough bread is commonly consumed. Authors optimized the method to improve oat–wheat bread quality. Besides, they used the same dried sourdough starter culture that obtained for the current study. All the details about incorporation of sourdough starter in bread dough and process steps were very clear. However, a long proofing time of 65 min, used in the adapted Flander method (Flander et al 2011), resulted in wholegrain breads with an unacceptable loaf volume. The bread collapsed in the oven resulting in low loaf volume (Table 3.2). However, the method of Flander

and others was used to bake refined wheat flour supplemented with oat flour. In the current study, several preliminary experiments were conducted (using 15% sourdough starter only) to determine the optimal fermentation/proofing time in the presence or absence of 2% gluten added to the sourdough bread formula (Table 3.2). To prevent the collapse during baking, the proofing time (65 min) was divided into two steps: fermentation (30–35 min) and proofing (30–45 min). Results demonstrated that 30 min of fermentation and 45 min of proofing time, in the absence of gluten, produced a bread of good appearance and loaf volume. The addition of 2% gluten slightly increased loaf volume and improved bread quality among all experimental trails. Flander et al (2007) showed that optimizing proofing time and temperature play a significant role in final bread quality.

3.4.3. pH of Dough and Baked Bread

Dough acidity can affect the final bread product. The pH values of the sourdough samples were lower than that of the straight and sponge dough (Table 3.3). In sourdough lactic acid bacteria in the starter produce more organic acids compared with yeast in straight or sponge dough (Gamel et al 2014). The pH value of breads varied from 5.0 to 6.1 depending on bread type and the amount of sourdough starter in sourdough bread (Table 3.3). As expected, the lowest bread pH was attained when 35% sourdough starter was used. Moderate bread acidity (pH > 5.5) was attained with 15 grams of sourdough starter. Similar studies (Crowley et al 2002; Flander et al 2011) indicated that increasing the level of sourdough (10–40%) increased the acidity of the bread and similar pH values were reported. The acidity may have positive or negative effects on the volume of sourdough bread depending on the gluten network and acidity profile (Gänzle et al 2008).

3.4.4. Effect of Bread-Making Methods on Bread Quality

Loaf Volume (LV), specific loaf volume (SLV) and crumb firmness are the main quality characteristic of bread (Katina et al 2006). The appearance of breads made by the three bread-making methods is presented in Figure 3.2 and their loaf volume and specific loaf volume in Figure 3.3. Wholegrain breads made by sponge dough or 15% starter sourdough method had better looking and loaf shape as compared to straight dough, 25% starter sourdough or 35% starter sourdough method (Figure 3.2). Among sourdough bread samples, an inverse relationship between the amount of sourdough added and bread volume was observed based on LV and SLV values. The highest LV (600 cm^3) and SLV ($4.01 \text{ cm}^3/\text{g}$) was attained with a low amount (15%) of sourdough starter (Figure 3.3). Increasing the sourdough level to 25% or 35% resulted in lower LV and SLV values. There were significant differences ($P \leq 0.05$) between bread made from 15% sourdough compared to straight dough and sponge dough breads. Previous studies have shown that high SLV ($3.40 \text{ cm}^3/\text{g}$) was observed with a lower level (10-15%) of wheat sourdough starter (Crowley et al 2002) and oat-wheat sourdough starter (Flander et al 2011). Moreover, increasing sourdough level to 40% resulted in lower loaf volume compared to straight dough breads (Katina et al 2006). Gamel et al (2015) reported that sponge dough and straight dough breads had LV about 15% and 10% greater than sourdough bread. However, the negative effect of sourdough due to the high levels of sourdough starter (40%–80%) applied. Meanwhile, Zhao et al (2015) indicated no changes observed in bread loaf volume or crumb texture between sourdough and straight dough breads made from whole wheat flour with 3 or 6% replacement level of dried rye malt gluten (1:1) sourdough. The use of sourdough at high levels could affect bread quality due to acidity-induced activation of proteolytic enzymes in the flour (Clarke et al 2002). The current results indicate that high levels of sourdough starter produced breads with

lower LV perhaps due to the intense acidification, which affects gluten development and formation. The use of 15% sourdough starter was superior in terms of bread loaf volume as compared to higher levels (25 and 35%).

Crumb firmness is a key quality attribute to the consumer acceptance of wholegrain bread. In this study, the crumb firmness of fresh bread made with 15% sourdough was significantly ($p \leq 0.05$) lower than that of other bread samples, whereas the straight dough bread had the highest crumb firmness values (Figure 3.4). Nevertheless, the firmness increased as the level of sourdough starter increased. Several studies have shown that the sourdough bread-making process improves the quality of fibre-rich wheat, barley, sorghum, oat, and oat-wheat bread in terms of crumb firmness and shelf life (Arendt et al 2007; Dal Bello et al 2007; Flander et al 2011; Galle et al 2011; Rieder et al 2012; Rizzello et al 2014), but its influence on bread staling has not been fully understood. Moreover, a positive impact of the increase in loaf volume was reflected in the sourdough breads' lower crumb firmness values. According to Maleki et al. (1980), loaf volume was positively correlated with softness of bread crumb. The data on increasing loaf volume and reducing crumb firmness of wholegrain sourdough bread were in strong agreement with those found in other previous studies on wheat (Clarke et al 2002; Corsetti et al 2000) and oat-wheat (Flander et al 2011) sourdough bread. The sourdough fermentation increased acidity in the dough, thus improving protein solubility and enhancing proteolysis. The proteolytic enzymes present in wheat flour (Clarke et al 2002) or originated by lactic acid bacteria (LAB) (Corsetti et al 1998) modify the gluten network, resulting in changes in the physical properties of gluten and a decrease in the firmness of the bread (Armero and Collar 1997; Corsetti et al 1998; Clarke et al 2002). The intensity of these modifications depends on the acidity levels obtained.

Moisture content and water activity are important factors that affect bread quality, consumer acceptance and shelf life. Use of different bread-making methods was found to significantly affect moisture content but not water activity of wholegrain bread (Figure 3.5). The moisture content of wholegrain bread products ranged from approximately 40% to 42% with bread made by 15% sourdough having the highest moisture content (Figure 3.5A). On the other hand, straight dough bread had the lowest moisture content. No significant differences were observed in the water activity of all bread products (Figure 3.5B). Differences in moisture content between yeast-leavened, sourdough, and yeast/sourdough bread products were previously reported being 44%, 43%, and 42%, respectively (Abdel-Aal and Rabalski 2008). On the contrary, Corsetti et al (2000) reported no changes in the moisture content of all wheat bread studied using bakers' yeast, chemical acidification, or various LAB strains. Additionally, the authors showed differences in water activity values of yeast-leavened or chemically acidified bread (0.992) and sourdough bread (0.986).

Besides the loaf volume and crumb firmness, color of bread crumb and crust also determines overall bread quality. Color of bread products depends on baking conditions and dough characteristics (Koletta et al 2014). In this study the effect of bread-making method on color of crust and crumb was measured and reported as the browning index (BI) (Figure 3.6). Breads made with sourdough had darker crust and lighter crumb than that baked from straight dough or sponge dough. The BI of crust and crumb of sourdough breads was significantly ($p \leq 0.05$) higher than other breads products. Among sourdough breads, increasing the sourdough level used in bread formula resulted in a higher crust BI value. Coda et al (2010) reported similar observation; the study showed that crust lightness of wheat sourdough breads was lower than that of control breads. During baking, crust coloring results from the Maillard reactions between

reducing sugars and amino acids. In the current study, bread formulations, especially the amount of salt and baker's yeast, had the most effect on Maillard reactions. The salt amount (1.5%) present in the straight dough bread formulation was lower than sourdough (2.2%), which leads to lower Maillard reaction. In the absence of salt, yeast activity increases resulting in a reduction in the amount of free reducing sugars remaining for Maillard reaction (Belz et al 2012). In addition to the reduced salt, straight dough bread contains more yeast (8.6%) compared to sourdough (3.2) and sponge dough bread (3.5%), which may result in greater yeast activity and lower Maillard reaction.

3.4.5. Effect of Bread-Making Method on Bread Shelf Life

In this study, the crumb firmness of wholegrain wheat bread improved by using the sourdough bread-making method compared to the yeast-fermented dough bread-making methods. Significant increases ($p \leq 0.05$) were observed during storage at room temperature in crumb firmness of breads made by sourdough and sponge dough methods, whereas slight changes were noticed with bread made by the straight dough method (Figure 3.7). The most notable increase in crumb firmness was at days 5 and 7 for all bread samples. During storage for 7 days, the 15% sourdough bread-making method showed a positive effect in delaying crumb hardening as compared to 25 and 35% sourdough breads (Figure 3.7). At day 5 of storage, bread produced using the sponge and dough method had signs of external molds, whereas straight dough bread was mold-free until day 7. Thus storage experiment of sponge dough breads was stopped at Day 5. It was noticeable that breads produced using the sourdough method had a longer shelf life where molds were seen at day 10 (data not shown). The use of sourdough fermentation has been reported to increase shelf-life of wheat bread (Corsetti et al 2000; Crowley et al 2002). Crowley et al (2002) studied the crumb firmness changes during storage and found

that sourdough bread crumb were softer than wheat straight dough bread. Moreover, wheat sourdough bread containing 20% starter maintained superior texture characteristics during the storage time while increasing the starter level to 40% had a negative effect (Crowley et al 2002). Moore et al (2007) showed that sourdough breads were softer than the chemically acidified gluten-free breads after 5 days of storage.

During storage time, the moisture content of all bread products significantly decreased, while there were no significant differences in water activity among all bread products during the 7-day storage period (Figure 3.8). A significant drop in moisture content was observed at day 3, and this behaviour was found for all the bread-making methods studied. The reduction of moisture content occurred in bread crumb during storage could be due to the redistribution of moisture from crumb to crust. Corsetti et al (2000) reported that bread products maintained constant moisture content of approximately 42% during storage because they were wrapped in polyethylene bags. However, the authors showed a decrease in water activity values of yeast-leavened or chemically acidified bread (0.992) and sourdough bread (0.986), with a decrease during the 144 h of storage. Changes in starch retrogradation and water migration from crumb to crust during storage contribute to bread staling (Cai et al 2014). Thus it is critical to minimize these changes during storage of breads. Regarding bread color, small reductions in BI values of bread crust were observed during storage particularly for straight dough bread at day 5 and 15% sourdough bread at day 3 (Table 3.4).

3.5. CONCLUSION

The yeast-fermented and sourdough methods showed different effects on bread quality and shelf life. The 15% sourdough bread-making method has the most positive effect on loaf volume and firmness of wholegrain bread. However, this study also indicated that the level of

sourdough starter might have a positive or negative impact on bread quality. To eliminate negative effects, it is essential to adjust the amount of sourdough starter. Traditionally sourdough is used to bake rye flour, but it can also be used to produce breads from other cereal flours such as wheat, barley, oat, etc. But, it is important to optimize the amount of starter and fermentation and proofing conditions. Storage of breads at room temperature for 7 days resulted in no signs of spoilage except for breads made from sponge dough, which lasted for 5 days. Changes in crumb firmness and moisture content were observed during storage with 15% sourdough method holds a promise to maintain bread quality during storage up to 7 days. More research to investigate the relationships between sourdough fermentation and bread dough rheology to understand the mechanism of bread staling delay is needed. In general sourdough bread-making method with low levels of sourdough starter (e.g. $\leq 15\%$) could be promising for the production of high-quality wholegrain bread with an extended shelf life.

Table 3.1 Bread Formulations Based on 100 g Flour

Ingredients (100%)	Straight dough	Sponge dough	Sourdough		
			15% starter	25% starter	35% starter
Flour	100.0	30.0	85.0	75.0	65.0
Water	71.0	20.0	56.0	46.0	36.0
Sugar	6.0	-	3.2	3.2	3.2
Salt	1.5	2.0	2.2	2.2	2.2
Yeast	8.6	3.5	3.2	3.2	3.2
Shortening	3.0	3.0	3.2	3.2	3.2
Corn syrup	-	9.0	-	-	-
Sponge ^a	-	121	-	-	-
Sourdough starter ^b	-	-	30.0	50.0	70.0
Total water	71.0	71.0	71.0	71.0	71.0
Total flour	100.0	100.0	100.0	100.0	100.0

^aSponge = 70g flour + 51 water and fermented for 2.5h

^bStarter= 50:50 of flour:water and fermented with 0.01% culture for 20h.

Table 3.2 Bread Loaf Volume (LV) and Specific Loaf Volume (SLV) at Various Fermentation and Proofing Times

Method	Factors			Bread quality		
	Gluten (%)	Fermentation (min)	Proofing (min)	Loaf weight (g)	Loaf volume (cm ³ /g)	Specific loaf volume (cm ³ /g)
A	-	0	65	148 ± 0.5	585 ± 21.0	3.9 ± 0.1
B	2	0	65	152 ± 0.4	575 ± 7.1	3.8 ± 0.1
C	-	35	30	152 ± 0.6	575 ± 3.5	3.8 ± 0.1
D	2	35	30	155 ± 0.1	600 ± 0.0	3.8 ± 0.0
E	-	30	45	148 ± 0.8	635 ± 14.1	4.3 ± 0.1
F	2	30	45	151 ± 0.6	670 ± 35.4	4.5 ± 0.2
G	-	40	20	149 ± 0.0	565 ± 14.1	3.8 ± 0.1
H	2	40	20	153 ± 0.4	545 ± 7.1	3.5 ± 0.1

A-F: Flander method at different fermentation and proofing times with or without gluten.

G-H: AACCI approved method 10-10.03 without or with gluten.

Table 3.3 pH Value of Sourdough Starter, Dough and Wholegrain Bread Products

Method	Sourdough starter	Dough	Bread
Straight dough	–	5.95 ± 0.2 ^a	6.11 ± 0.2 ^a
Sponge dough	–	5.72 ± 0.1 ^a	5.77 ± 0.0 ^b
Sourdough			
15%	4.01 ± 0.0 ^a	5.41 ± 0.0 ^{ab}	5.58 ± 0.0 ^c
25%	4.02 ± 0.0 ^a	5.10 ± 0.0 ^b	5.21 ± 0.0 ^d
35%	4.01 ± 0.0 ^a	4.83 ± 0.2 ^b	5.01 ± 0.0 ^d

Means in a column with the same letter are not significantly different at $p \leq 0.05$.

Table 3.4 Browning Index (BI) of Wholegrain Breads during storage at room temperature.

Method	BI				
	Day0	Day1	Day3	Day5	Day7
<i>Bread Crust</i>					
Straight dough	0.4356 ^b	0.4403 ^a	0.4403 ^a	0.4356 ^b	0.4343 ^b
Sponge dough	0.4405 ^a	0.4355 ^a	0.4355 ^a	0.4383 ^a	-
Sourdough					
15%	0.4587 ^a	0.4587 ^a	0.4551 ^b	0.4587 ^a	0.4587 ^a
25%	0.4830 ^a	0.4830 ^a	0.4807 ^a	0.4830 ^a	0.4830 ^a
35%	0.4837 ^a	0.4814 ^a	0.4837 ^a	0.4837 ^a	0.4837 ^a
<i>Bread Crumb</i>					
Straight dough	0.4089 ^a	0.4110 ^a	0.3920 ^c	0.4013 ^b	0.3997 ^b
Sponge dough	0.4089 ^a	0.4110 ^a	0.4070 ^b	0.4142 ^a	-
Sourdough					
15%	0.4007 ^a	0.4143 ^a	0.4038 ^a	0.4130 ^a	0.4085 ^a
25%	0.3995 ^a	0.3928 ^b	0.3909 ^c	0.4032 ^a	0.4062 ^a
35%	0.3954 ^a	0.4018 ^a	0.3955 ^a	0.3980 ^a	0.4016 ^a

Means in a row with the same letter are not significantly different at $p \leq 0.05$.

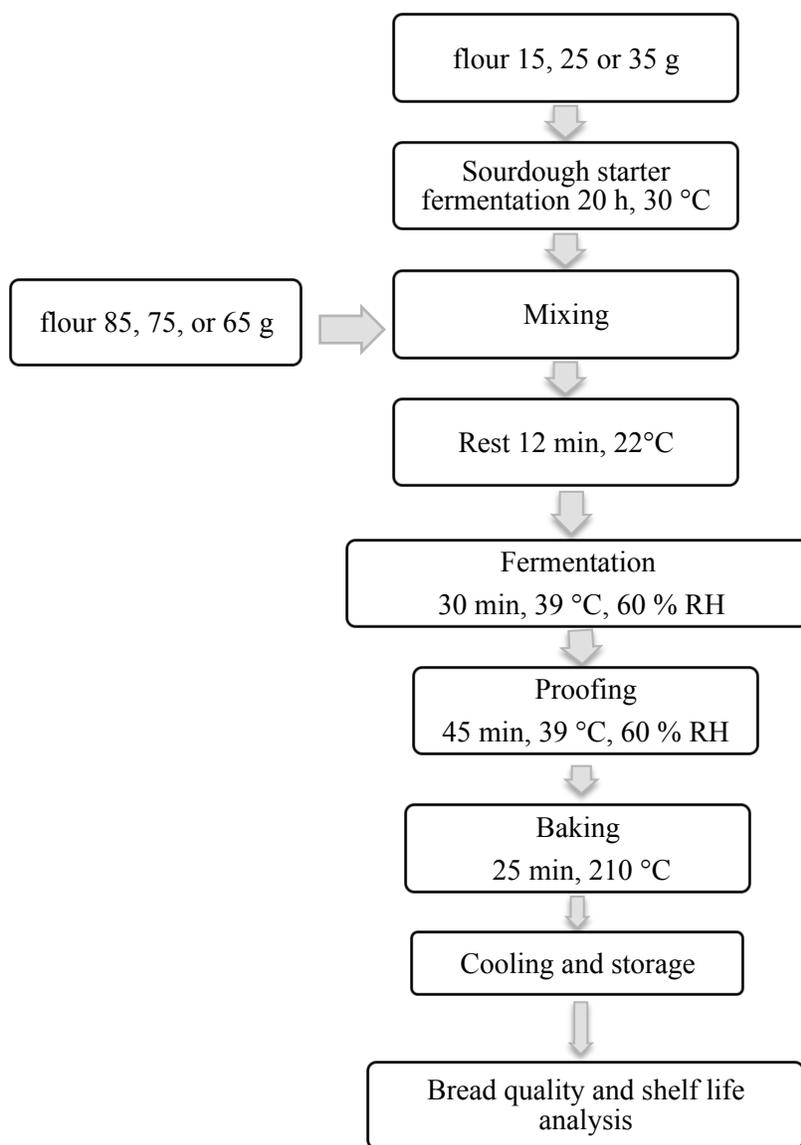


Figure 3.1 Sourdough bread making process

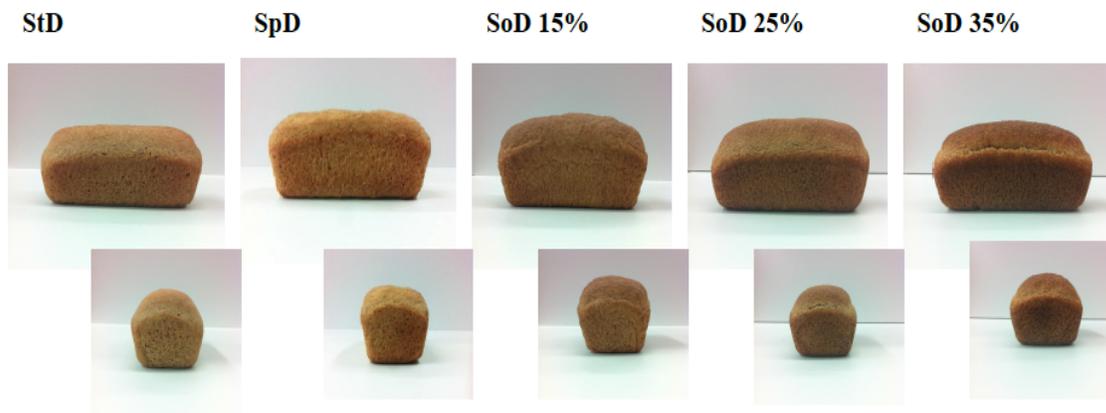


Figure 3.2 Appearance of Wholegrain breads made by different bread-making method. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough

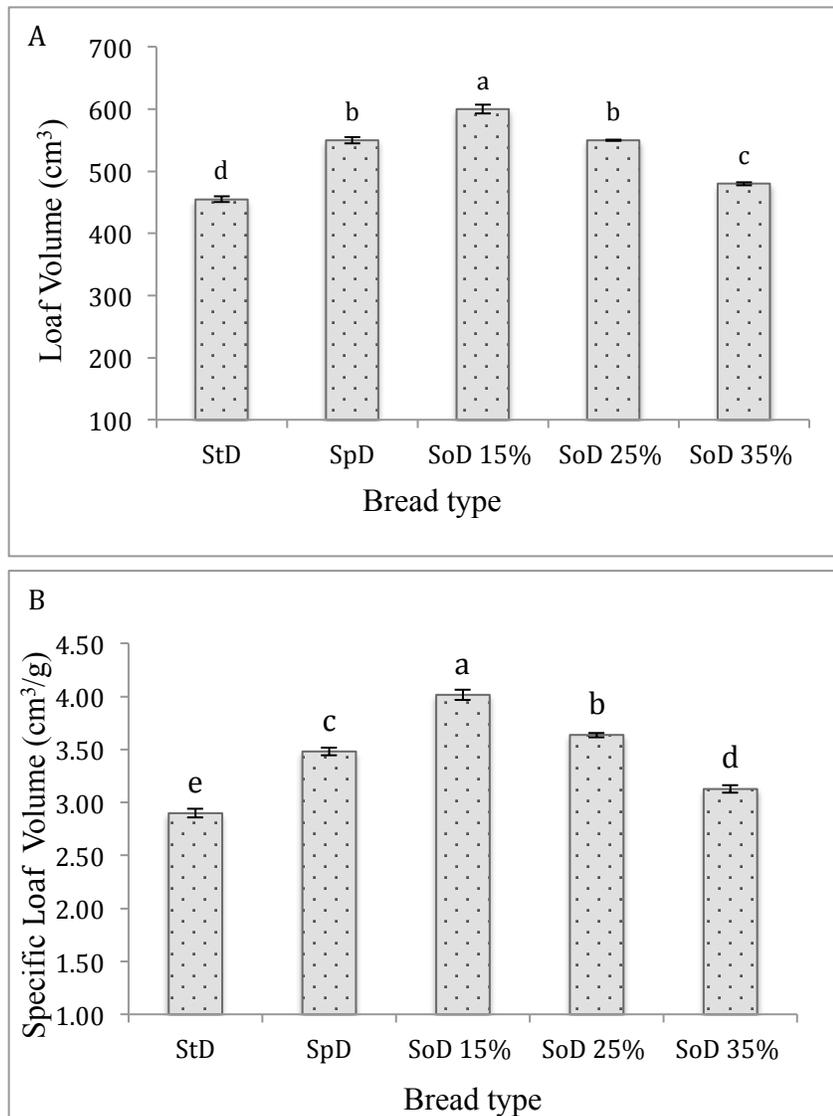


Figure 3.3 Effects of Bread-Making Methods on Loaf Volume (A) and Specific Loaf Volume (B) of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

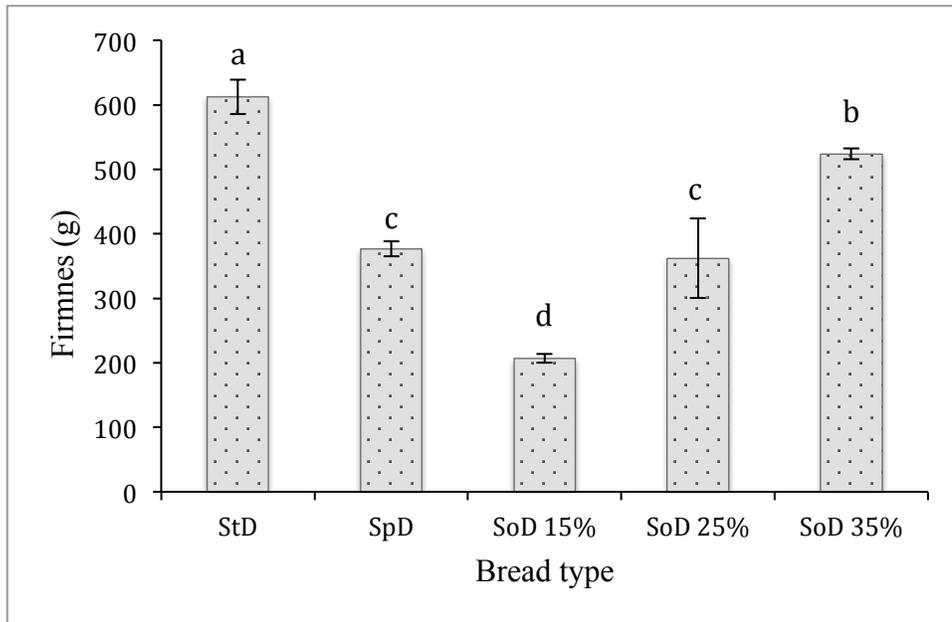


Figure 3.4 Effects of Bread-Making Methods on Crumb Firmness of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

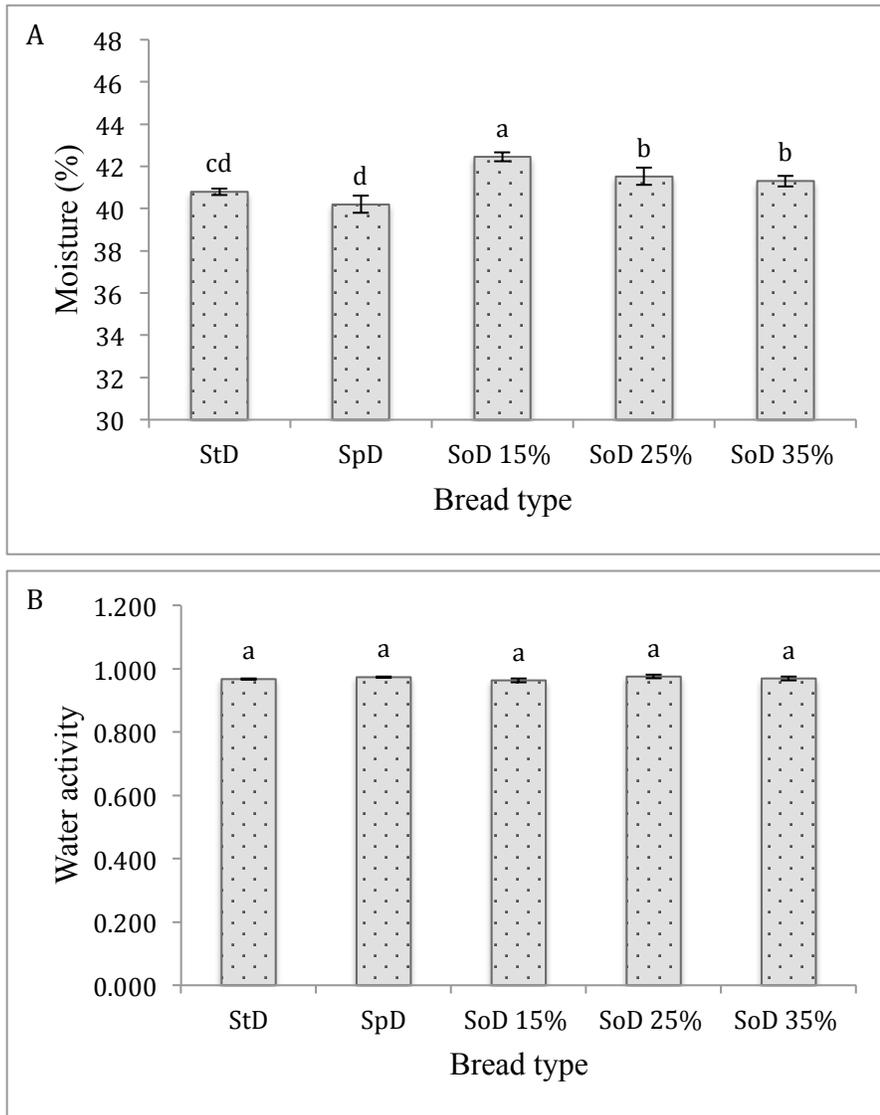


Figure 3.5 Effects of Bread-Making Methods on Moisture Content (A) and Water Activity (B) of wholegrain bread products. Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

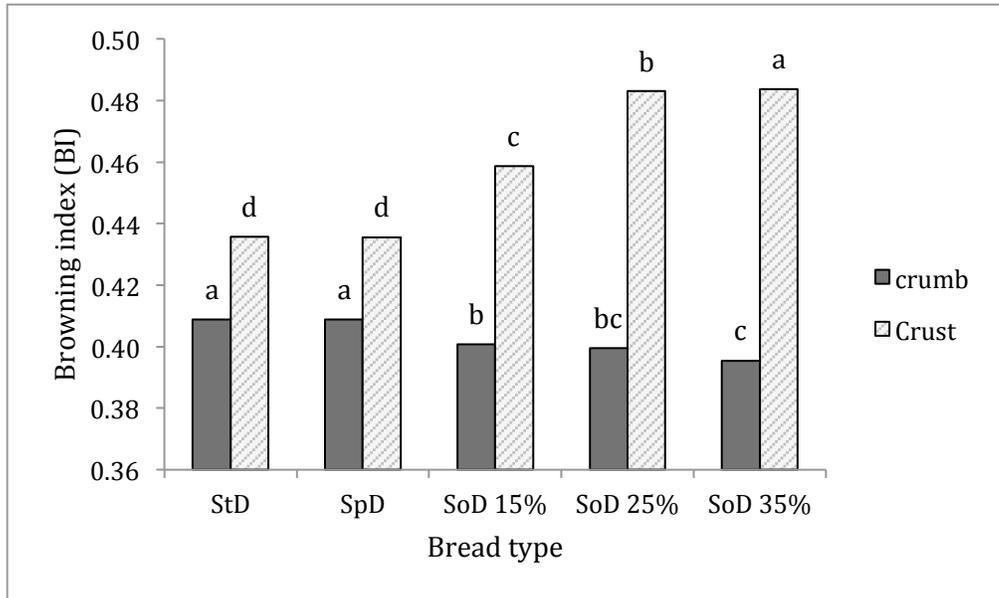


Figure 3.6 Effects of Bread-Making Methods on Crust and Crumb Color of wholegrain bread products. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

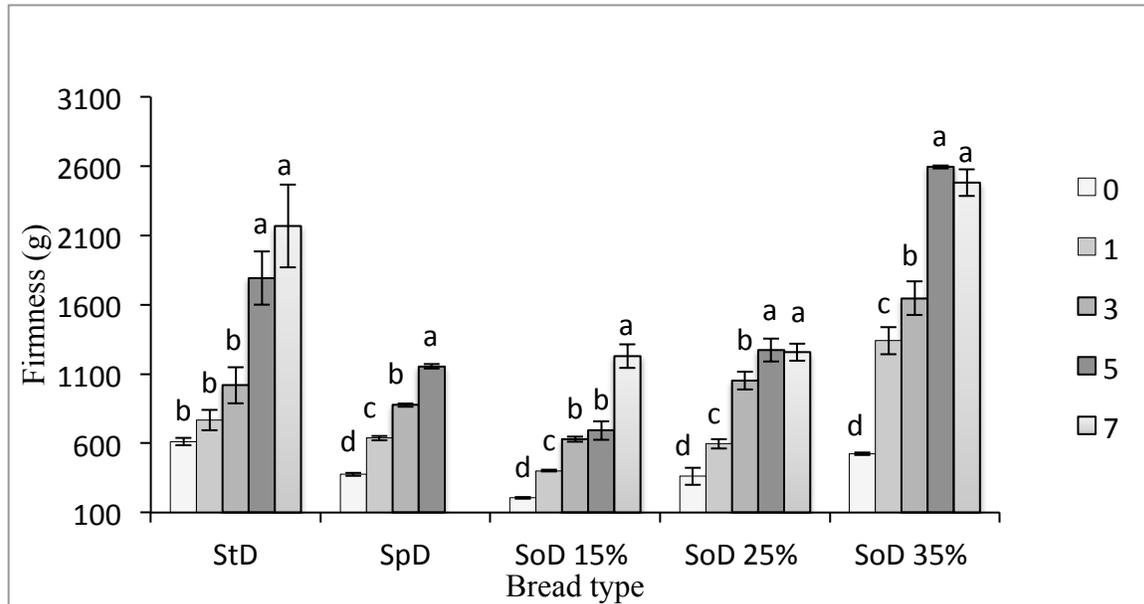


Figure 3.7 Effect of Storage (7days) on Crumb Firmness of wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters within each product group indicate significant differences between bread samples at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

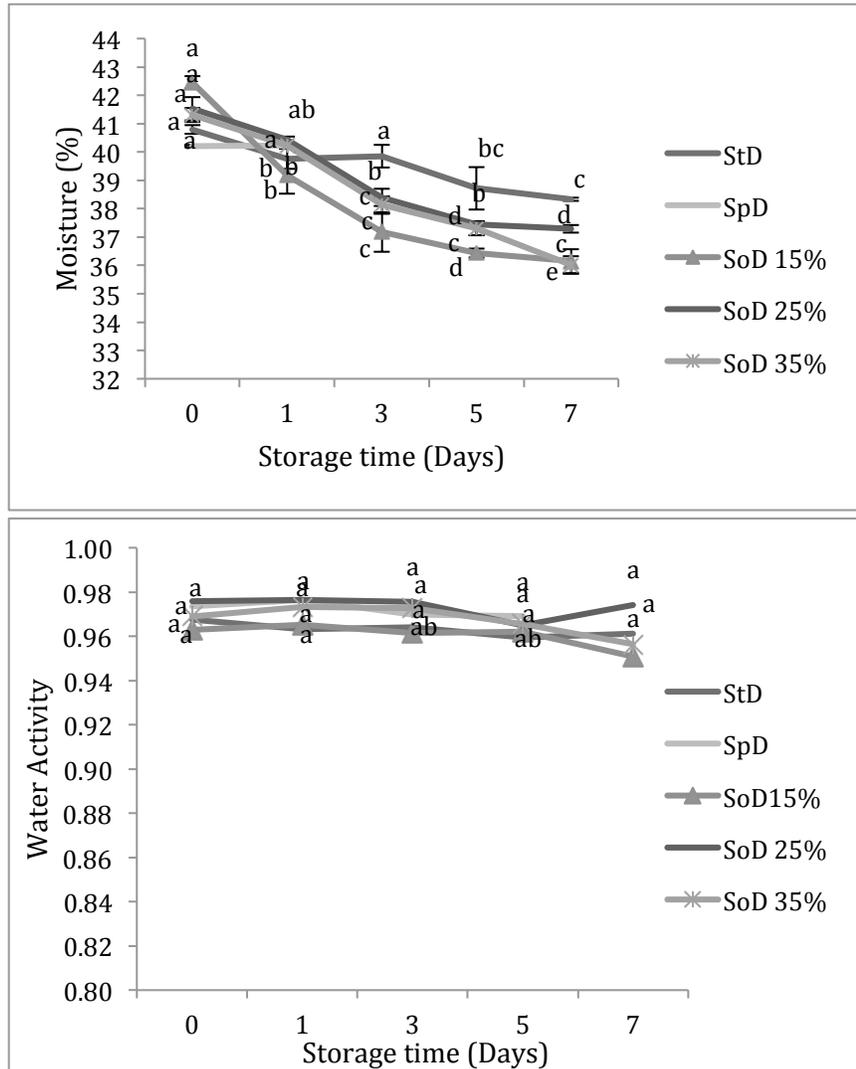


Figure 3.8 Effect of Storage Time (7days) on (A) Moisture content and (B) Water Activity of wholegrain Bread products. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

CHAPTER 4

Effect of Various Baking Methods on Phytonutrient and Antioxidant Properties of Wholegrain Bread

4.1. ABSTRACT

Wholegrain breads are good sources of dietary fiber and antioxidants. This study aimed to investigate the effect of different bread-making processes (straight dough, sponge dough and sourdough) on the content of free and bound phenolic acids, total flavonoid, total carotenoid and antioxidant capacity measured by three assays, ABTS, DPPH and ORAC. Free ferulic acid content in all the three bread products significantly ($P < 0.05$) increased by about 215-424% due to baking process. Slight increases were also observed in bound ferulic acid (16 – 27%) particularly in breads made with sourdough method. Total flavonoid content influenced differently by the baking processes, with the straight dough method having the lowest impact. Significant increases in total carotenoid content were found in all breads with sourdough breads having the highest content. Antioxidant assays showed that sourdough method significantly increased DPPH and ORAC scavenging capacity. In general, the results suggest that phytonutrients in wholegrain bread could be manipulated via baking process in particular ferulic acid, the predominant antioxidant in wheat, to increase its free form, which could improve its bioavailability.

Keywords: wholegrain, bread-making, phenolic, antioxidants.

4.2. INTRODUCTION

Growing evidence suggests that wholegrain consumption is associated with reducing the risk of chronic diseases such as cardiovascular disease, cancer and diabetes (Borneo and León 2012; Gani et al 2012). It is believed that dietary fiber and antioxidants in wholegrain, are responsible for the associated health benefits (Slavin 2004; Borneo and León 2012; Anderson 2014). Dietary antioxidants could prevent or mitigate oxidative damage to cellular components through their abilities to scavenge reactive oxygen species and other free radicals (Abdel-Aal and Rabalski 2013).

Wholegrain antioxidants include a large group of compounds that have numerous chemical structures, which can be classified as lipophilic and hydrophilic antioxidants (Konopka et al 2014). The main lipophilic antioxidants found in wholegrain are carotenoids, tocopherols, and tocotrienols (Konopka et al 2014). Phenolic acids are the main hydrophilic antioxidants in wholegrain products (Abdel-Aal and Rabalski 2013). The phenolic compounds are present in the free or bound form in which would affect their antioxidant properties and bioavailability, and eventually their beneficial health effects, but their composition and content could be altered during wholegrain processing (Abdel-Aal and Rabalski 2013).

It has been reported that processing of cereals or pulses may positively or negatively affect the content of phenolic compounds, which possibly affects their bioactive properties and health benefits (Duodu 2011). Milling and bread making are the main cereal processes that could alter the phenolic antioxidant properties of wholegrain bread (Ktenioudaki et al 2014). Dough fermentation using yeast or bacteria “sourdough” is an essential step during bread making. Sourdough, a mixture of flour and water fermented together with lactic acid bacteria (LAB), can change the properties of bread (De Vuyst and Neysens 2005; Ravyt and De Vuyst 2011). Rye

and refined wheat flours are the two most common flours used for sourdough bread production (Flander et al 2011). Rye and wheat sourdough fermentation can affect phytonutrient levels (phenolic acids, tocopherol and tocotrienol, alkylresorcinols, lignans); however, the increase or decrease of phenolic compounds levels depends on the compound nature and the type of sourdough bread process (Katina et al 2005; Katina et al 2007; Banu et al 2010).

Previous studies on yeast-leavened wheat bread have shown that fermentation, kneading, and baking might decrease carotenoid and tocopherol contents (Leenhardt et al 2006). Only a small effect on carotenoids was evident as a result of the bread leavening (Hidalgo et al 2010), whereas significant losses occurred during baking (Abdel-Aal et al 2010). In bread leavening, insignificant carotenoid losses of only 3% were observed (Hidalgo et al 2010). Moreover, some studies have indicated that wheat bread baking increases the content of free phenolic acids, particularly ferulic acid, and improve the antioxidant properties of bread (Moore et al 2009; Ragaei et al 2011; Abdel-Aal and Rabalski 2013 ; Konopka et al 2014). This study aimed to investigate the effect of different bread-making methods on the content of free and bound phenolic acids, total flavonoids, total carotenoids, and antioxidant capacity. Three commonly used antioxidant assays, ABTS, DPPH and ORAC, were employed to investigate antioxidant properties of wholegrain breads.

4.3. MATERIAL AND METHODS

4.3.1. Materials

Wholegrain flour was obtained from (Parrish and Heimbecker Milling Group) Dover Flour, Cambridge. The wholegrain flour contains protein, ash, and total dietary fiber at average of 13.7%, 1.4% and 11.5%, respectively. A freeze-dried sourdough starter culture, Florapan® LA6, *Lactobacillus brevis*, was purchased from Lallemand Baking Solutions Ltd, Mississauga,

ON, Canada. Dry yeast (Fleischmann's Traditional Active Dry Yeast, product of Canada) and all other ingredients were purchased from a local grocery in Guelph, ON, Canada.

4.3.2. Bread-Making Methods

Three bread-making methods including straight dough, sponge dough, and sourdough were used to prepare wholegrain bread. Straight dough loaves were prepared according to the AACCI approved method 10.10.03 (AACCI 2011). The 60 min fermentation time was applied. Sponge dough loaves were prepared based on the AACCI approved method 10.11.01 (AACCI 2011).

Sourdough starters were prepared by mixing 0.1 % (based on flour weight) of Florapan LA6 starter culture with the flour on low speed for 30 seconds. Lukewarm water was added in a ratio of 1:1 flour to water and the sourdough was mixed in a KitchenAid (Professional 600 Series) stand mixer (Mississauga, ON, Canada) for 6 min on low speed. The sourdough was placed in a beaker, covered, and incubated at 30° C for 20 h. After 20 h of incubation, the sourdough was used for bread making. Sourdough breads were made with three levels of sourdough starter (15, 25, and 35%).

The sourdough loaves were prepared in accordance with Flander et al (2011) with some modifications. The amount of wholegrain flour and water used in the prepared sourdough starter was taken into account and the corresponding amount of wholegrain flour and water was omitted from the bread formula to achieve consistent flour and water content in the bread dough. Sourdough breads were made with 15%, 25%, and 35% sourdough levels by adding 30 g, 50 g, and 70 g of sourdough starter, respectively, to the bread dough mixture. The amounts of other ingredients used in the formula are presented in Table 3.1. The yeast (3.2 g) was suspended in part of the water (15-30 mL) (24 °C). In a spiral mixer, half of the flour was thoroughly blended

with the sugar. The yeast suspension was added to the mixture along with the shortening, salt, and the rest of the water. The dough was mixed at a high speed (200 rpm) for 6 min. Sourdough starter and the rest of the flour were added to the dough and mixed again at a low speed (100 rpm) for 6 min. The dough was rested at room temperature for 12 min, divided into two pieces, rounded and molded, and placed in a pan sprayed with grease. After proofing for 45 min at 39° C and 60% RH in a Hobart proofer (Hobart, USA) the breads were baked at 210° C for 30 min (Figure 3.1). Ten baking trials were carried out for each method. Loaves were allowed to cool down and stored in a polyethylene Zip-lock bag at room temperature for 7 days for storage experiment. Bread samples were dried at room temperature according to the AACCI approved method 62-05.01 (AACCI 2011). The dried samples were passed through a sieve with 0.5 mm opening and kept at -20 °C freezer until extraction and analyses.

4.3.3. Analytical Tests

4.3.3.1. Phenolic acids extraction and HPLC analysis

Free and bound phenolic acids in wholegrain bread products were extracted as previously described by Abdel-Aal and Rabalski (2013). Free extracts were prepared from a 0.5 g sample in 5 mL 80% methanol using platform shaker for 30 min. The tube content was centrifuged at 10,000 g for 10 min, and the extraction was repeated on the residual pellet. Both extracts were pooled together, purged with nitrogen and kept in the refrigerator until further processing and analysis. The residual pellet obtained after removing free phenolic compounds was processed immediately for quantifying the bound phenolic acids. First the left over pellet was washed with hexane, and then centrifuged at 10,000 g for 15 min. The hexane supernatant was discarded. A 5 mL of 2 M sodium hydroxide was added to the pellet and the content was purged with nitrogen. The sealed tube was placed in a water bath on a laboratory heater stirrer plate for 1 h at 70 °C.

Then the mixture was cooled and acidified to pH 2 with 2 M hydrochloric acid and centrifuged at 10,000 g for 15 min. The acidic supernatant was transferred into a clean tube. The residual pellet was washed with 10 mL of nano-pure water, and then centrifuged at 10,000 g and the water supernatant was combined with the acidic supernatant. The combined mixture was extracted three times with 10 mL of ethyl acetate and ethyl ether 1:1 ratio (v/v), then centrifuged and pooled together and eventually dried under nitrogen stream until dryness. The extracted residue was re-dissolved in 5 mL of nano-pure water, filtered through 0.45 mm Acrodisc syringe filter, and stored in a freezer prior to HPLC analysis.

Free and bound phenolic acids were analyzed by high performance liquid chromatography (HPLC) using a 1100 Series chromatography system (Agilent, Mississauga, ON) equipped with Supelcosil LC C18 column 58298) as outlined by Abdel-Aal and Rabalski (2013). The phenolic acids were eluted at 26 °C using a gradient elution starting with 100% of 6% formic acid and 0% of 6% acidified acetonitrile. The gradient was gradually changed over 35 min to 82% of formic acid and 18% acidified acetonitrile and then kept for 5 min plus 2 min to return to the starting conditions. Separation of phenolic acids was monitored at 5 different channels: 260, 275, 300, 320 and 330 nm. Typical HPLC chromatograms showing the separation of bound phenolic acids from the bread products are presented in Figure 4.1. A mixture of 12 authentic phenolic acid standards including gallic, protocatechuic, p-hydroxybenzoic, gentisic, 3-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, sinapinic and o-coumaric acids was used for calibration, identification and quantification. The concentrations of standard phenolic acids ranged from 2.9 to 36.1 µg/mL. Five injection volumes were used (1, 5, 10, 25, 50 µL) to prepare the standard curve for each acid. The following regression equations and determination coefficients were obtained from the curves: protocatechuic ($y = 3545.9x - 4.9619$,

$R^2 = 0.9998$); *p*-hydroxybenzoic ($y = 5695.1x - 3.3635$, $R^2 = 0.99979$); vanillic ($y = 3441.5x + 12.161$, $R^2 = 0.99871$); gallic ($y = 2557.7x - 2.5527$, $R^2 = 0.99485$); syringic ($y = 2978x - 7.9183$, $R^2 = 0.99881$); 3-hydroxybenzoic ($y = 846.41x - 3.698$, $R^2 = 0.99734$); *p*-coumaric ($y = 8848.6x + 4.1718$, $R^2 = 0.99975$); caffeic ($y = 5560.8x - 14.831$, $R^2 = 0.99963$); ferulic ($y = 6335.8x + 50.054$, $R^2 = 0.99964$); sinapinic ($y = 4104.9x - 3.5684$, $R^2 = 0.99854$); and gentisic ($y = 1363.7x - 2.0627$, $R^2 = 0.9997$). Where y is the sample area and x is the concentration of phenolic acid ($\mu\text{g/mL}$)

4.3.3.2. Total Phenol Content (TPC)

Free and bound extracts were used for determining the TPC based on a Folin-Ciocalteu method (Singleton and Rossi 1965) with some modifications. A 0.25 mL extract was oxidized with Folin-Ciocalteu (1.5 mL of freshly diluted 10-fold Folin Ciocalteu reagent) and allowed to equilibrate for 5 min. Then, the reaction mixture was neutralized with 1.5 mL of sodium carbonate (60 g/L). The mixture was incubated at room temperature for 90 min before measuring the absorbance at 725 nm against 80% methanol/water used as the blank. Ferulic acid was used as a standard and the results were expressed as ferulic acid equivalents ($\mu\text{g/g}$ sample). The concentration of the standard ferulic acid was (50-300 $\mu\text{g/mL}$). The regression equation for quantification is : $y = 0.0043x + 0.0088$ and determination coefficient (R^2) = 0.98957. Where y is the sample absorbance at 725 and x is the concentration of ferulic acid ($\mu\text{g/mL}$)

4.3.3.3. Total Carotenoid Content (TCC)

Total carotenoid content was determined according to the AACC Intl. approved method 14-60-01 (AACCI 2011).

4.3.3.4. Total Flavonoid Content (TFC)

Total Flavonoid content was extracted and measured using the spectrophotometric

method as described by Michalska et al (2007). Briefly, 0.5 g sample was extracted with 5 mL of 80% methanol by shaking for 2 h at 37°C. Then the sample was centrifuged at $10,000 \times g$ for 10 min in a Sorval RT1 Centrifuge (Thermo Scientific Inc.). The flavonoid extract (0.25 mL) was diluted with 1.25 mL water, mixed with 75 μL of 5% NaNO_2 and equilibrate for 6 min before mixing in 150 μL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. After letting the mixture equilibrate for 5 min, a 0.5 mL of 1 M NaOH solution was added and well mixed. The mixture was centrifuged at $10000 \times g$ for 5 min using a Sorval RT1 Centrifuge (Thermo-Fisher, Ottawa, ON) and the absorbance was read at 510 nm with UV-visible spectrophotometer (Varian Inc, Pao Alto, CA) against methanol as blank. Rutin and catechin were used separately as standards and the results were expressed as catechin or rutin equivalents ($\mu\text{g/g}$ sample). The concentration of rutin and catechin was (0-80 $\mu\text{g/mL}$) and the regression equations for quantification are: rutin ($y = 0.0017x - 0.0066$, $R^2 = 0.98656$) and catechin ($y = 0.0044x - 0.0043$, $R^2 = 0.99602$) where y is the sample absorbance at 510 and x is the concentration of catechin or rutin ($\mu\text{g/mL}$)

Changes in the content of phytonutrients in wholegrain bread products were calculated as % difference from their corresponding original flours. The phytonutrients in bread products were adjusted to 100% flour prior to the calculation of % difference. The following equation as outlined by (Abdel-Aal and Rabalski, 2013) was used to calculate % difference:

$$\% \text{ difference} = \frac{[\text{pytonutrient in flour (original)} - \text{pytonutrient in product (adjusted)}]}{[\text{pytonutrient in flour}]} \times 100$$

4.4.4. Antioxidant properties Tests

4.4.4.1. DPPH radical scavenging capacity assay

Direct QUENCHER method was used in this study (Serpen et al 2012). Ten mg \pm 1.0 mg of ground bread samples was weighed into a centrifuge tube. The antioxidant reaction

was initiated by adding 10 mL of the working DPPH solution. A stock solution of 2, 2-diphenyl-1 picrylhydrazyl (DPPH) was prepared daily by dissolving 40 mg of DPPH in 100 mL of ethanol, and then diluted with 100 mL of deionized water. A working DPPH solution was prepared by diluting 200 mL of stock solution with approximately 800 mL of 50% ethanol to obtain a solution having an absorbance value of 0.75–0.80 at 525 nm (Brand-Williams et al 1995; Serpen et al 2012). The tube was shaken rigorously for 1 min and placed on an orbital shaker in the dark. The mixture was shaken at 300–400 rpm at room temperature to facilitate the surface reaction between the solid bread particles and the reagent. Centrifugation was performed at 9,200 g for 2 min. The clear supernatant (2 mL) was transferred into a cuvette and the absorbance was measured at 525 nm at room temperature. The inflection points (where the curve changes from convex to concave or vice versa) of DPPH was calculated by Hockey Stick Regression analysis (SAS software) and Polynomials regression analysis to determine reading times (Appendix A). Three different times (10, 20 and 60 min) were chosen for the DPPH depletion measurement. The DPPH scavenge capacity of wholegrain bread products was measured and calculated as $\mu\text{mole trolox equivalents/g sample}$. A standard trolox solution was prepared in ethanol at a concentration range between 0 and 600 $\mu\text{g/mL}$. Exactly 0.1 mL of each trolox concentration was added to 9.9 mL of DPPH radical solution. After 30 min of incubation at room temperature, 2 mL of the reaction solution was transferred into a cuvette and the absorbance was measured at 525 nm. A reagent blank was prepared using deionized water instead of trolox solution. The determination coefficient (R^2) of the method is 0.9743 and regression equation is $y=0.0096x + 0.0609$.

4.4.4.2. ABTS cation radical scavenging capacity assay

Direct QUENCHER method was prepared as described above in DPPH assay (Serpen et

al 2012). Three different times (15, 25 and 60 min) were chosen for the ABTS depletion measurement. A solution of 7 mmol/L (2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ABTS was prepared by adding 5 mL of deionized water to 38.41 mg of ABTS. A 2.45 mmol/L potassium persulfate solution was prepared by adding 5 mL of deionized water to 6.615 mg potassium persulfate. Five mL from each solution were mixed to make a stock solution of ABTS, which is kept in the dark at room temperature for 12–16 h before use (Re et al 1999). A working ABTS solution was prepared daily by diluting the 10 mL of stock solution with approximately 800 mL of 50% ethanol to obtain a solution having an absorbance value of 0.75–0.80 at 734 nm (Serpen et al 2012). The ABTS scavenge capacity of wholegrain bread products was measured and calculated as $\mu\text{mole trolox equivalents/g sample}$. A standard trolox solution was prepared as explained above in DPPH assay. The determination coefficient (R^2) of the method is 0.9784 and regression equation is $y=0.0103x + 0.1005$.

4.4.4.3. Oxygen Radical Absorbance Capacity (ORAC) assay

The ORAC assay is based on the method of Ou et al (2001). Ten mg of ground samples was weighed into eppendorf tube with 1 mL of 75 mM phosphate buffer pH 7.5. The mixture was vortexed for 1 min and centrifuged at 9,000g for 45 sec. The sample extracts were diluted several times (1:1-1:4) until an appropriate curve was obtained over the test period. Twenty-five μL sample extract, trolox standard solution or nano pure water (blank) were mixed with 150 μL of fluorescein in each of the 96 micro-plate well. The mixture was conditioned at 37°C for 30 min, then 25 μL of 2,2'-Azobis (2-methylpropion-amidine) dihydrochloride (AAPH) as a peroxy radical generator was added to start the decaying of fluorescein. The degradation of fluorescein progressed for 60 min in the heated chamber of BioTech FLX800TBI with the following settings: the fluorescence excitation 485 nm, the emission wavelength 528 nm, and reading was

taken every min for 1 hr. The micro-plate fluorescent reader was operated by Gen 5 software version 1.11.5 (BioTek). In this assay, the scavenge capacity of wholegrain bread extracts was also calculated as $\mu\text{mole trolox equivalents/g sample}$. The determination coefficient (R^2) of the method is 0.9915 and regression equation is $y=0.2568x + 5.867$.

4.4.5. Statistical Analysis

All analyses were performed at least in duplicate, and the data are reported as mean \pm standard deviation (SD). Analysis of variance was performed using IBM SPSS Statistics 21 software for Mac (Armonk, New York, USA). Significant difference ($p \leq 0.05$) among means were detected using a Tukey's multiple range test at a fixed level of $\alpha = 0.05$.

4.4. RESULTS AND DISCUSSION

4.4.1. Effect of Bread-Making Methods on Free and Bound Phenolic Acids

Phenolic acids are the main antioxidant compounds in wheat and other commonly consumed cereal grains such as corn, rye, and barley. They are known for their ability to scavenge free radicals (Abdel-Aal et al 2012), inhibit oxidation of human LDL cholesterol (Abdel-Aal and Gamel 2008), and impede singlet oxygen or chelate pro-oxidant metals (Larson 1988). As expected, the predominant phenolic acid in wholegrain flour was ferulic acid in both the free and bound extracts. Other phenolic acids found in wholegrain flour in quantifiable amounts were vanillic, protocatechuic, caffeic, and *p*-coumaric acids in the free extracts (Table 4.1) and *p*-coumaric, vanillic, syringic, sinapinic, and caffeic acids in the bound extracts (Table 4.2). Earlier study by Abdel-Aal and Rabalski (2013) reported that ferulic and *p*-coumaric acids are the primary two phenolic acids found in wholegrain flour.

It has been reported that the processing of cereals may positively or negatively affect the

content of phenolic compounds, which possibly affects their bioactive properties and health benefits (Duodu 2011; Ragaee et al 2014). Additionally, processing could affect bioavailability of ferulic acid in wheat products (Anson et al 2009; Hamill et al 2009). However, only a few studies have investigated the impact of bread-making processes on free and bound phenolic compounds in bakery products. In the current study, changes in free and bound phenolic acids were investigated to study the effect of three bread-making methods, straight dough, sponge dough, and sourdough.

The bread-making methods affected the amount of free and bound phenolic acids in bread products. All bread products contained significantly higher amounts of free phenolic acids than wholegrain flour (Table 4.1) but similar amounts of bound phenolic acids to wholegrain flour were observed (Table 4.2). Comparisons of baked breads showed that the highest content of free ferulic acid occurred in straight dough and sponge dough, whereas the highest content of bound ferulic acid occurred in sourdough breads fermented with 25 or 35% starter. When the content of phenolic acids of bread products was adjusted based on 100% flour to determine the effect of the bread-making methods, free ferulic acid (the dominant phenolic compound) showed an increase of about 397 to 424% in straight dough and sponge dough bread, respectively (Table 4.3). Sourdough breads also showed an increase between 215% and 244% depending on the amount of starter used. Bound ferulic acid slightly increased by 17% in straight dough and sponge dough bread. Sourdough breads also showed an increase between 16% and 27% (Table 4.3). The bread-making method increased total free phenol content regardless of the fermentation type (yeast or lactic acid bacteria) (Figure 4.2A). However, straight dough and sponge dough bread-making methods resulted in a decrease in the content of total bound phenols (Figure 4.2B). Sourdough breads made with 15–25% starter showed significant increases of total bound phenol

content.

Abdelaal and Rablaski (2013) reported an increase in free ferulic acid but decreases in bound ferulic acid of wholegrain unleavened nonfermented one-layer flat bread. Another study indicated that fermentation either by yeast or sourdough and baking resulted in the release of bound ferulic acid of wholegrain wheat and rye breads (Konopka et al 2014). Gelinas and McKinnon (2006) reported an increase in total phenol content of wholegrain bread after baking regardless of baking time (10, 20, or 35 min). Additionally, Banu et al (2010) found that sourdough fermentation increased total phenol content compared to the control rye bread. The authors found that increasing the level of sourdough starter from 20–40% resulted in improving the total phenol content. By contrast, a reduction in free phenolic acids after pan and hearth bread baking has been reported (Holtekjølen et al 2008; Menga et al 2010). In these studies, however, bread products were fermented and supplemented with either wheat bran (Menga et al 2010) or barley flour (Holtekjølen et al 2008). Ragaee et al (2011) found that straight dough bread-making methods resulted in an increase in the content of free and bound phenolics of fiber-rich bread. It appears that the effect of baking on free or bound phenolic acids could be due to the nature and source of phenolic compounds as well as the baking method (Abdel-Aal and Rabalski 2013). Cheng et al (2006) found that heat stress could cause degradation of conjugated polyphenolic compounds, resulting in an increase in free phenolic acids in wheat. It has been suggested that sourdough fermentation increases the level of easily extractable phenolic compounds, especially ferulic acid monomers (Banu et al 2010; Konopka et al 2014). This would improve the bioavailability of phenolic compounds because it is believed that free phenolic acids are more readily available than bound phenolic compounds (Anson et al 2009).

4.4.2. Effect of Bread-Making Methods on Carotenoid and Flavonoid content of wholegrain bread

The total carotenoid content of the breads made with yeast (straight dough and sponge dough) was significantly lower compared with the breads fermented with sourdough (Figure 4.3). For sourdough breads, however, no significant differences were observed among the three levels used, 15%, 25%, and 35%. These results indicate that type of fermentation (e.g. yeast versus bacteria) has a major effect on carotenoids in wholegrain bread. Bread leavening has shown only a small effect on carotenoids (Hidalgo et al 2010). However, Abdel-Aal et al (2010) reported significant carotenoid losses occur during baking. Leenhardt et al (2006) found that the carotenoid content decreases after mixing during the process of making wholegrain French bread, and the greatest loss (66%) of carotenoids was observed in the dough. The authors highlighted a strong correlation between carotenoid losses and lipoxygenase activity ($R^2 = 0.97$). Lipoxygenase and peroxidase could lead to carotenoid oxidation in the presence of oxygen and water in the dough. During fermentation, consumption of oxygen by yeast prevents further oxidation by oxidative enzymes, with a minimal reduction in phytonutrient levels (Leenhardt et al 2006; Hidalgo et al 2010). The increase in total carotenoids content could be due to the fermentation process. Several bacteria, yeasts and fungi are good producers of carotenoid (Johnson and Schroeder, 1996). A study on improving nutritive properties of vegetable juice using beetroot and carrot juices fermented with brewer's yeast and lactic acid bacteria (*Lactobacillus acidophilus* strain) showed that fermented carrot juice had higher contents of β -carotene and some minerals (Ca, P, Fe) than beetroot juice (Rakin et al 2007). Other study conducted on cheese whey, the byproduct of cheese making, showed that total carotenoids (mainly β -carotene) increased during fermentation with Lactoso-Negative Yeasts Co-Cultivated

with Lactic Acid Bacteria (Frengova et al 2003). Eighteen strains of *Lactobacillus plantarum* from different origins were screened for carotenoid production (Garrido-Fernández et al 2010). The study showed most of the *L. plantarum* strains studied produced C30 carotenoid regardless their origin. The current study suggests that sourdough fermentation with baker's yeast and lactic acid bacteria play a significant role in improving total carotenoids content.

In addition to total carotenoids, flavonoids content was also affected by the different bread-making methods. In this study, rutin and catechin were used as standards for the quantification of flavonoids. Higher values were obtained with the rutin than with catechin. The results showed that wholegrain flour contains approximately 331 ug/g rutin equivalents and 122 ug/g catechin equivalents. In general, there was a significant ($P < 0.05$) reduction in total flavonoids content (rutin or catechin equivalent) among all bread products (Figure 4.4). However, when the products were adjusted to 100% flour, the straight-dough bread process showed 5% increase of flavonoids, whereas the sourdough bread-making method resulted in the greatest loss (9%–13%) (Table 4.3). Vogrincic et al (2010) monitored the polyphenol content, particularly the flavonoids rutin and quercetin, during the bread-making process of tartary buckwheat bread. The study showed that rutin decreased during the wholegrain bread-making process (mixing, proofing, and baking). Moreover, flavonoids in breads made with 70%–100% wheat flour were not detectable after baking (Vogrincic et al 2010). The authors suggested that buckwheat contains rutin-degrading enzymes. The addition of water and yeast led to the degradation of rutin, and approximately 85% was transformed to quercetin (Vogrincic et al 2010).

4.4.3. Effect of Bread-Making Methods on Antioxidant Properties of Wholegrain Bread

Dietary antioxidants have the ability to scavenge reactive oxygen species and free

radicals that could damage cellular components. Wholegrain bread could play a significant role in human diet as a good source of antioxidant components such as phenolic acids. The current study investigated the effect of different bread-making methods on the antioxidant properties of wholegrain flour. Currently, there is no single test that characterizes the antioxidant properties of foods due to the presence of multiple free radicals and oxidants in foods or biological systems. Such complex systems require more than one test for antioxidant measurement and characterization (Abdel-Aal and Rabalski 2013). In this study, three different antioxidant capacity assays, DPPH, ABTS, and ORAC, were used to measure the antioxidant properties of wholegrain bread products.

4.4.3.1. Antioxidant Properties Measured by DPPH Assay

Scavenging of DPPH radicals allows evaluation of the electron-donating potency of food components (Brand-Williams et al 1995). The ability of food or food extract to scavenge the DPPH free radical is measured through the reduction of the color intensity of the radical reduced in the presence of an antioxidant. The color intensity of DPPH radicals with no antioxidants or grain extracts should remain stable over the examination time (Ragae et al 2006). The DPPH-food reaction was not linear and rapidly increased during the first 10 minutes, after which the reaction rate steadily increased to 60 minutes. More than one inflection point was determined which indicates various reaction rates over the test period subject to the release of antioxidant components from food incubated with the radical. The bread products showed higher scavenging capacity for DPPH radicals than wholegrain flour. In general, the straight dough bread had lower DPPH scavenging capacity than did other bread products (Figure 4.3 A). The sourdough bread-making method had the greatest effect on scavenging DPPH radicals. The results are in agreement with Liukkonen et al (2003) study on methanol extract, which showed that sourdough

fermentation increased the DPPH radical scavenging capacity of rye sourdough. However, Yu et al (2013) reported that the average scavenging ability decreased by 32% for wholewheat bread during the bread-making process. This loss might be because of the loss of phytonutrients due to the high temperature during baking. The extraction of antioxidants from various food matrices (e.g., wheat flour versus bread) may also affect the level of antioxidant capacity (Yu et al 2013).

4.4.3.2. Antioxidant Properties Measured by ABTS Assay

The ABTS assay measures the ability of antioxidant compounds to scavenge ABTS free radical cation based on electron transfer donated by antioxidant compounds (Brand-Williams et al 1995). The ABTS radical cation has a relatively stable blue-green color which is in the presence of an antioxidant such as 6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid (trolox) or potential antioxidants in material extracts, the color production will be suppressed to a certain extent in proportional to the concentration of antioxidants (Ragae et al 2006). Similar to DPPH assay, the ABTS-food reaction reaction was not linear and rapidly increased during the first 15 minutes, after which the reaction rate steadily increased to 60 minutes. More than one inflection point was determined which indicates various reaction rates over the test period. There were no significant differences observed in straight dough (16.4 ug trolox equivalents /g sample) and sourdough breads (14.5-16.8 trolox equivalents ug/g sample) compared to whole-grain flour (14.7 trolox equivalents ug/g sample) (Figure 4.3 B). However, the sponge dough bread-making method slightly by significantly ($P \leq 0.05$) increased the ABTS scavenging capacity to 18.5 trolox equivalents ug/g sample. Abdel-Aal and Rabalski (2008) reported that scavenging capacity against ABST radical cation by wheat antioxidants would vary depending on the concentration of individual bioactive compounds in wheat extracts and their synergic effects.

4.4.3.3. Antioxidant Properties Measured by ORAC Assay

The ORAC assay measures the ability of compounds to react with physiologically relevant peroxy radicals (Moore et al 2009). The ORAC assay measures the scavenging capacity of peroxy radicals (ROO[•]) by calculating the net protection area under the time recorded for the fluorescein decay curve in the presence of antioxidants (e.g., pure chemical or plant extract). The ORAC values of breads made by sponge dough and sourdough bread-making methods were significantly higher than those produced by the straight dough method (Figure 4.3 C). Long fermentation time and presence of lactic acid bacteria resulted in higher ORAC scavenging capacity. According to Yu et al (2013), phytonutrients are more likely to be detected by the ORAC method after the bread-making process, indicating that bread-making process is associated with an increase in antioxidant capacity. Additionally, the increase in antioxidant capacity after baking, as measured by ORAC assay, could be the result of different factors such as Millard reactions, pH, water activity, and temperature (Yilmaz and Toledo 2005). On the other hand, the relative ORAC value results showed no change in straight dough bread compared to the wholegrain flour, whereas there was a reduction in sponge dough and sourdough breads. Similarly, Abdel-Aal and Rabalski (2013) found that relative ORAC values of the free phenol extracts were not significantly different between lutein-fortified and control wholegrain straight dough bread products. The authors suggested that insignificant differences between lutein-fortified and unfortified products could be due to the low extractability of lutein in aqueous methanol and the low solubility of lutein in the buffer solution used in the ORAC assay (Abdel-Aal and Rabalski 2013).

4.4.4. Effects of Storage on Phytonutrient and Antioxidant Properties of Wholegrain bread

There are limited studies available on the stability of phytonutrients and antioxidants during bread storage. The current study investigated the stability of phytonutrients and

antioxidants in wholegrain breads stored at room temperature for up to 7 days. However, sponge dough bread lasted for only 5 storage days due to the spoilage growth noticed after day 5. Storage had no effect on free (Table 4.3) and bound (Table 4.4) ferulic acid content in wholegrain breads. Significant reduction was observed free vanillic acid in straight dough and sourdough (15% and 25% starter), sinapinic acid in sourdough bread with 25% starter, and caffeic acid in sourdough with 15% starter. Storage had no effect on any bound phenolic acids except *p*-coumaric acid in sponge dough bread. Regarding total phenol content, no changes were observed in the total free phenol content (Figure 4.6A) of bread products during storage. Nevertheless, there was a significant reduction in total bound phenol content (Figure 4.6B) in straight dough only, whereas no changes were noticed among other bread products during storage.

The total carotenoid content of straight dough bread was significantly reduced during the 7-day storage. This finding is similar to that of Abdel-Aal et al (2010), who reported that storage for 5 days at room temperature resulted in a reduction in lutein, the main carotenoid in wheat, in bread made by straight dough method. As shown in Figure 4.7, storage had no effect on total carotenoid content in sponge dough and sourdough breads products. The total flavonoid content of sponge dough bread significantly decreased after storage (day 1), with no change between day 1 and day 5 (Figure 4.8). Storage gradually decreased the total flavonoid content of sourdough bread products but had no effect on straight dough bread stored for 7 days. The storage results showed different effects on phytonutrient content depending on the nature of the phytonutrient and the bread-making method applied.

Like phytonutrients, the antioxidant properties of wholegrain bread were affected by storage. The effect of storage on antioxidant properties is presented in Figure 4.9. The storage of

sponge dough bread for 5 days significantly reduced antioxidant capacity, measured by DPPH and ABTS assays, whereas no effect was reported in bread made by the straight dough and sourdough methods. On the other hand, the storage of sponge dough bread had no significant effect on the ORAC value, whereas straight dough and sourdough bread products showed significantly decreased in ORAC values. The results are in agreement with Jensen et al (2011), who studied the effect of storage on wholegrain bread and antioxidant capacity. The breads were packed in vacuum-grade plastic bags in a modified atmosphere (nitrogen) and stored for 5 weeks. Jensen et al (2011) showed that the storage (for up to 5 weeks) of wholegrain bread made with the sponge dough method had no effect on bread crumb antioxidant capacity, as measured by Trolox equivalent antioxidant capacity (TEAC) and ORAC assays; however, a significant decrease in antioxidant capacity was reported in bread crust.

4.5. CONCLUSION

The current study showed that bread-making methods might positively or negatively affect phytonutrient levels and antioxidant properties. The fermentation type (yeast versus sourdough) could liberate phenolic acids and their derivatives, resulting in an increase in free phenolic acids. Generally, the sourdough bread-making method was effective in releasing bound phenolic acids in wholegrain flour, resulting in a significant increase in free phenolic acids and a slight improvement in the extractability of the bound form of phenolic acids. Moreover, wholegrain breads made by the sponge dough and sourdough methods also exhibited the highest DPPH scavenging capacity and ORAC values, possibly as a result of the increase in easily extractable phenolic compounds. The ABTS scavenging capacity, however, did not significantly influence the bread-making method, whereas all the wholegrain breads (straight, sponge, and sourdough) had similar scavenging capacities. In general, sourdough fermentation showed

significant improvements in liberating phytonutrients during baking, which could result in improved bioavailability and an increase in the beneficial health effects of wholegrain bread.

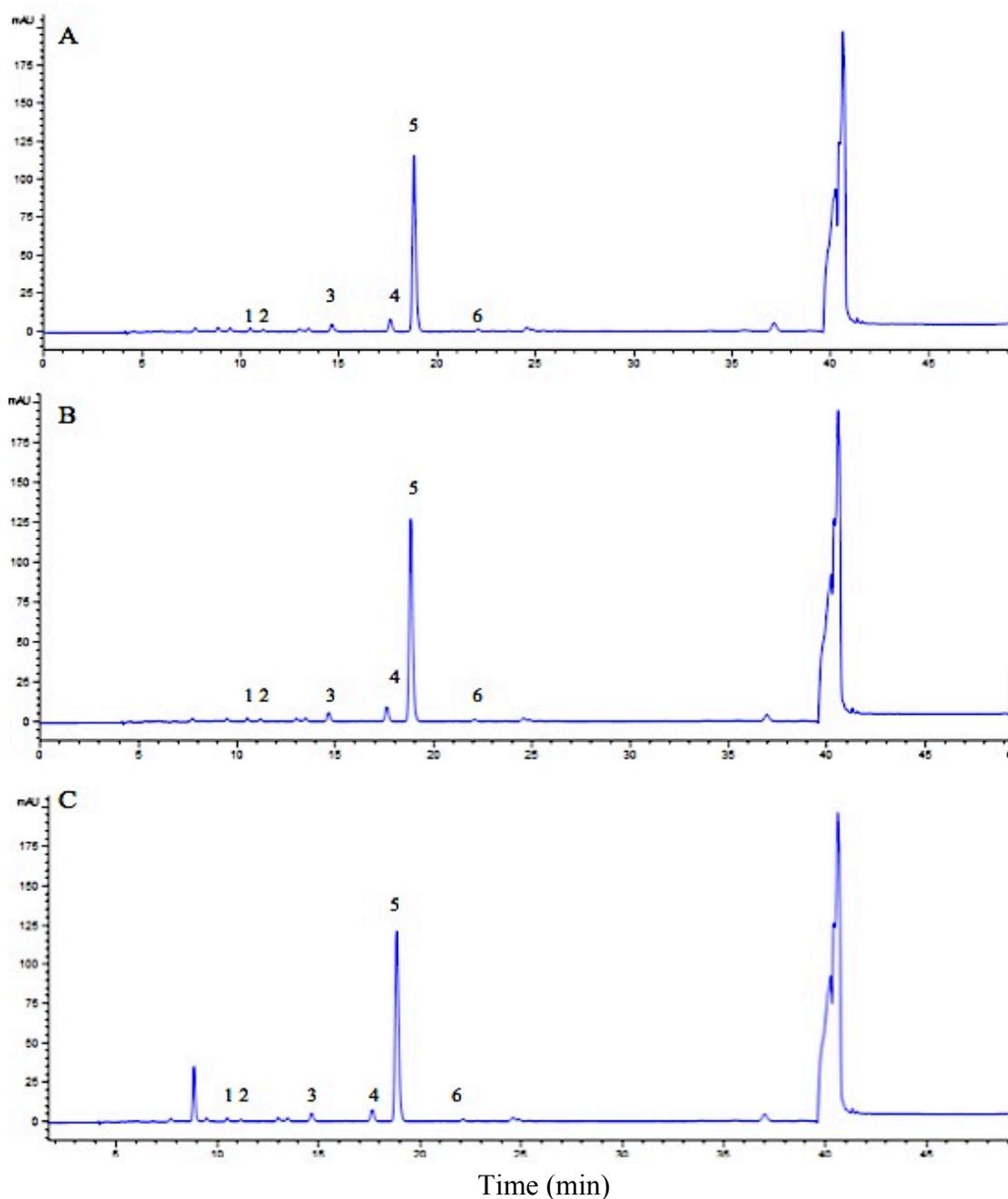


Figure 4.1 Typical HPLC chromatograms showing the separation of bound phenolic acids from different bread products, (A) straight dough (B) sponge dough (C) sourdough (25%). 1 = Vanillic 10.42 min, 2 = Caffeic 11.4 min, 3 = Syringic 14.6 min, 4 = *p*-coumaric 17.5 min, 5 = Ferulic 18.7 min, 6 = Sinapinic 22 min.

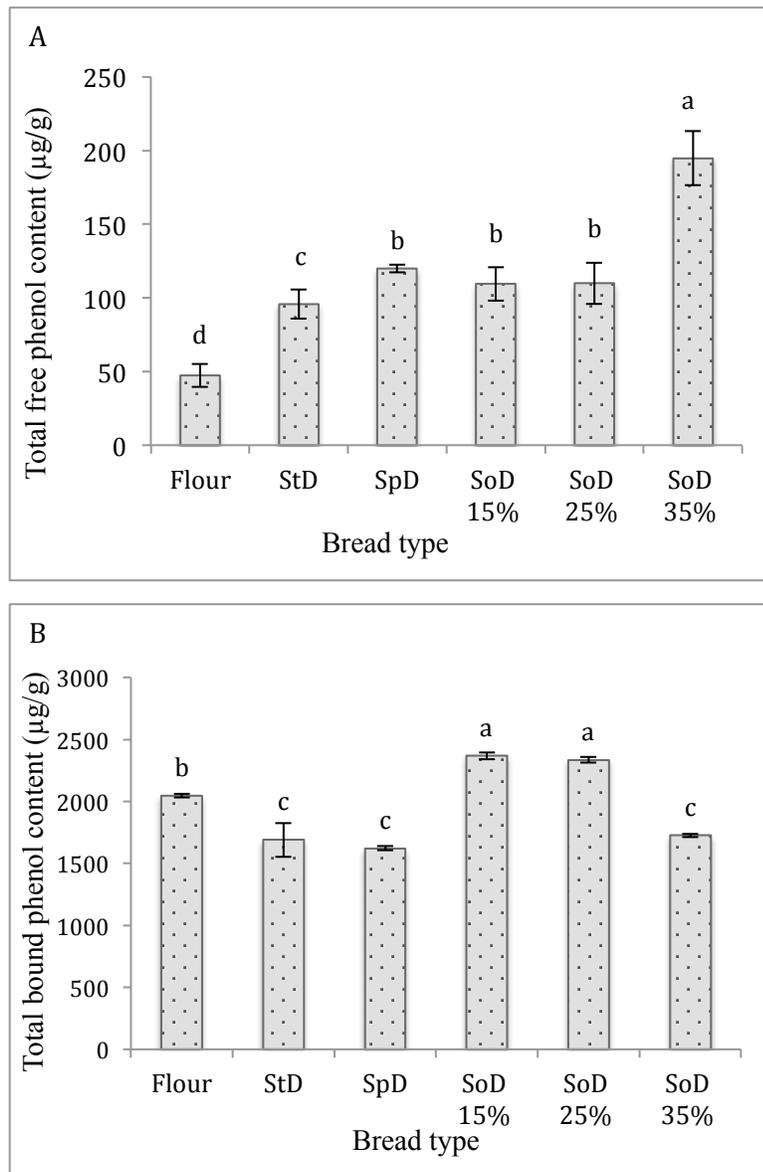


Figure 4.2 Effects of Bread-Making Methods on Total Free (A) and Bound (B) Phenol Content of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

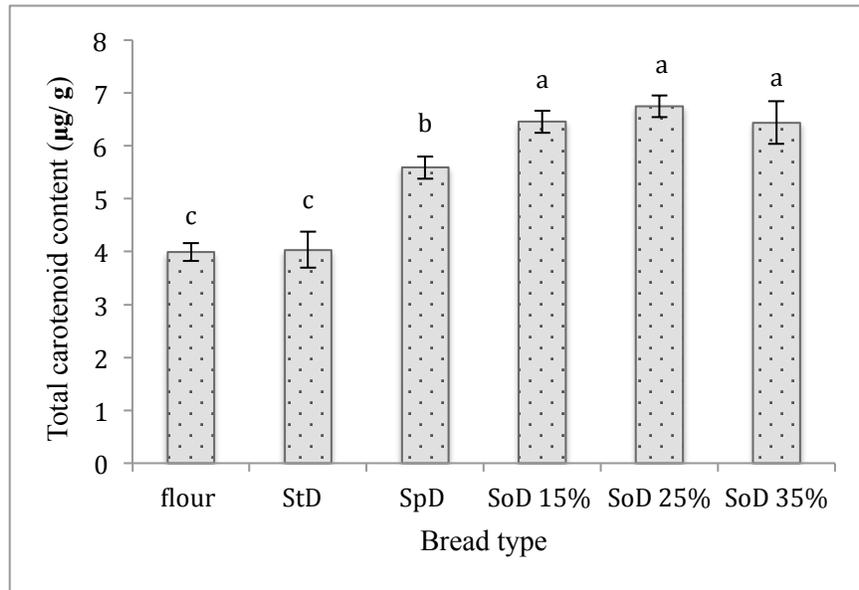


Figure 4.3 Effects of Bread-Making Methods on Total Carotenoid Content of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

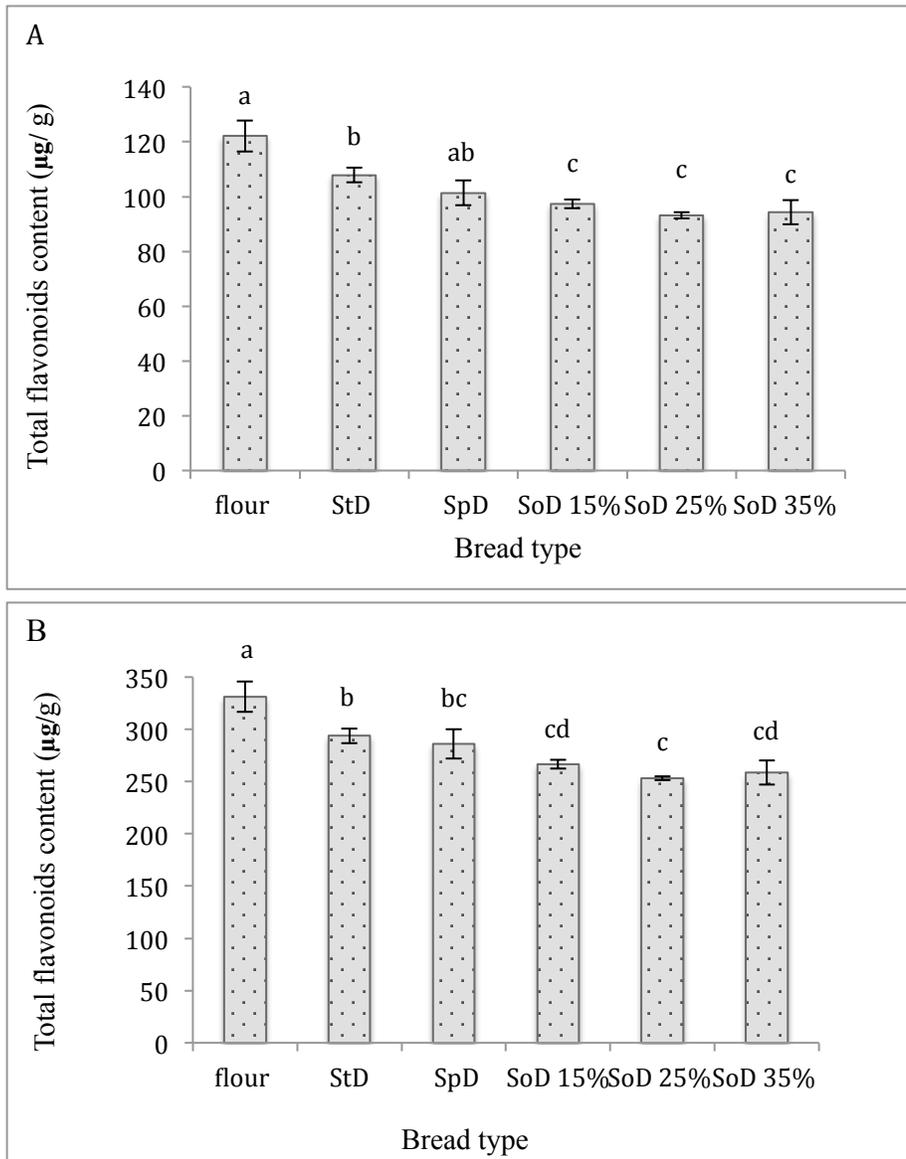


Figure 4.4 Effects of Bread-Making Methods on Total Flavonoids Content as Catechin equivalent (A) and Rutin equivalent (B) of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

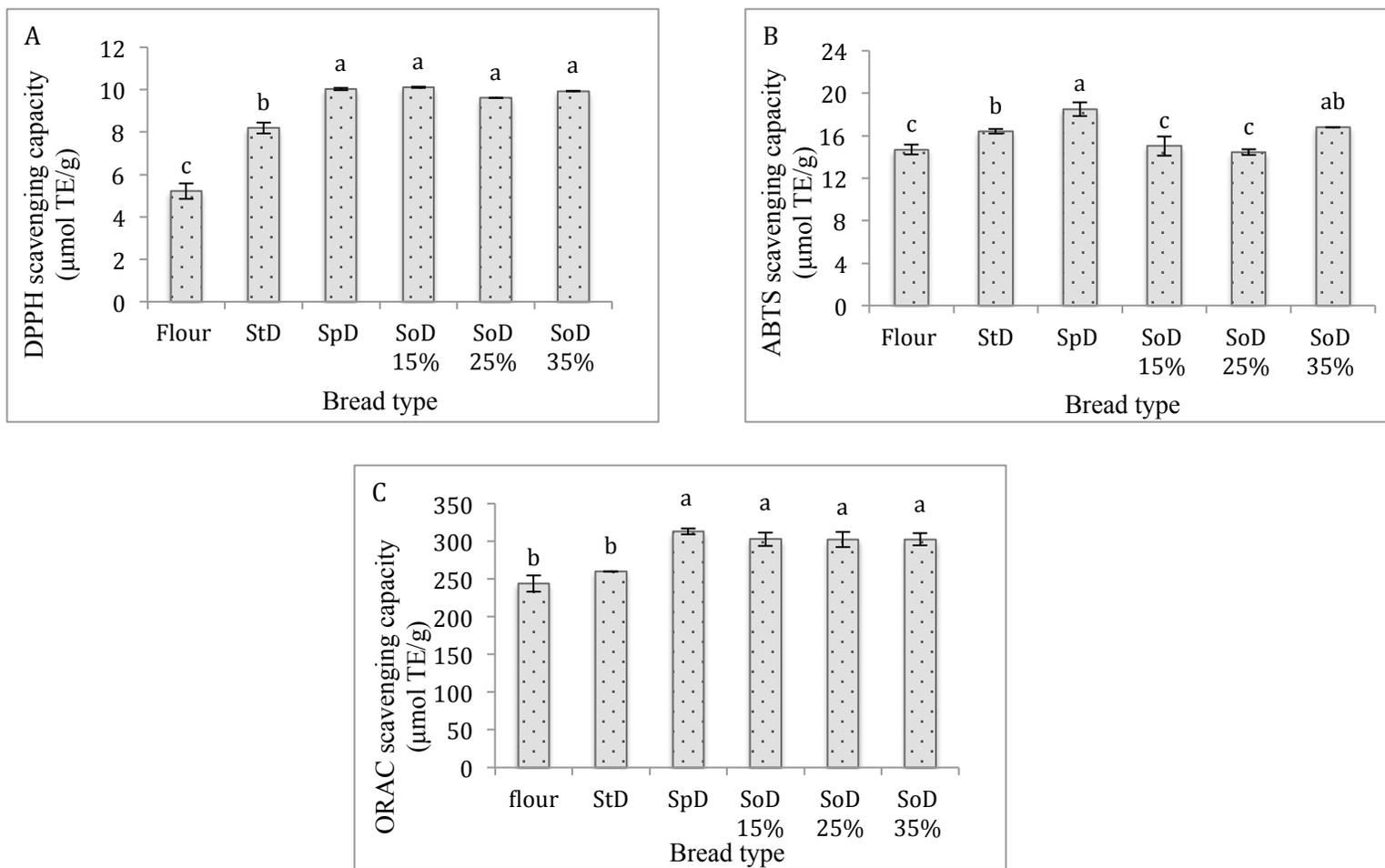


Figure 4.5 Effects of Baking-Making Methods on DPPH (A), ABTS (B), and ORAC (C) scavenging capacity of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

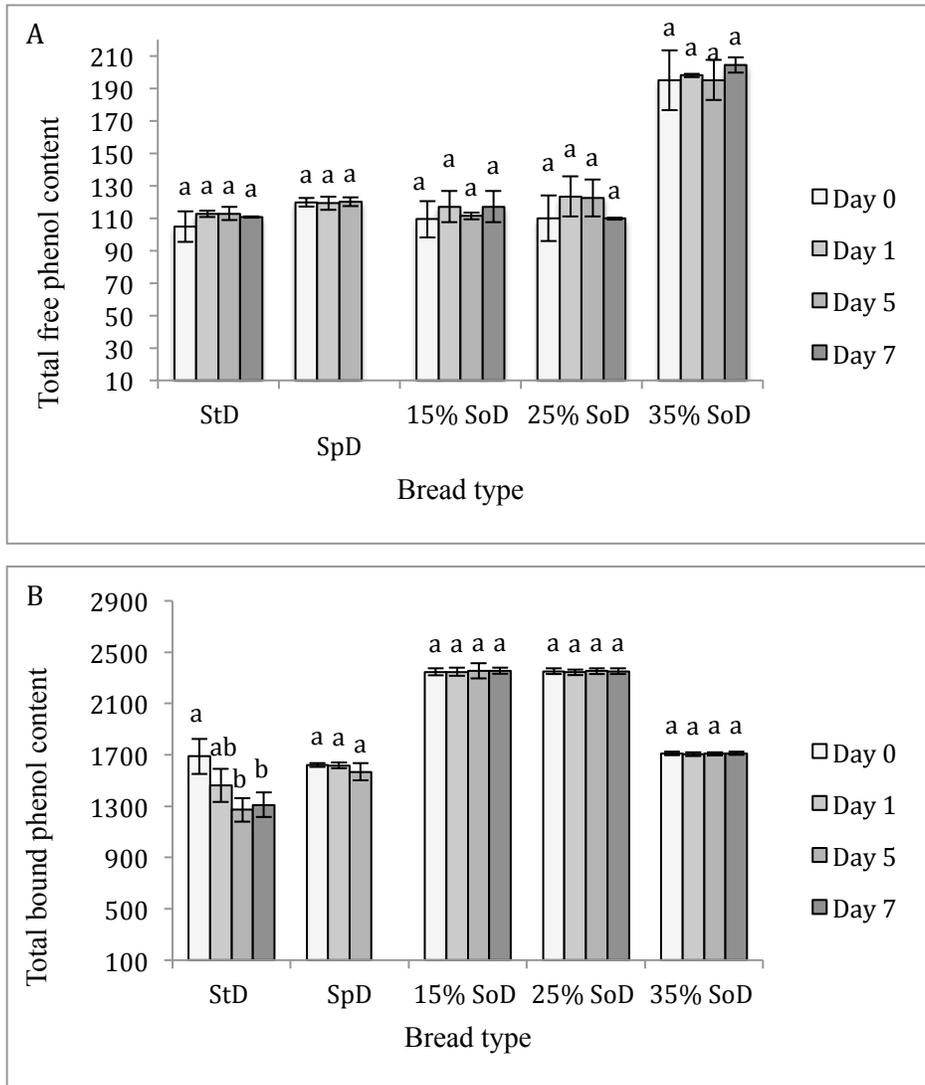


Figure 4.6 Effects of storage at room temperature on Total Free (A) and Bound (B) Phenol Content of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

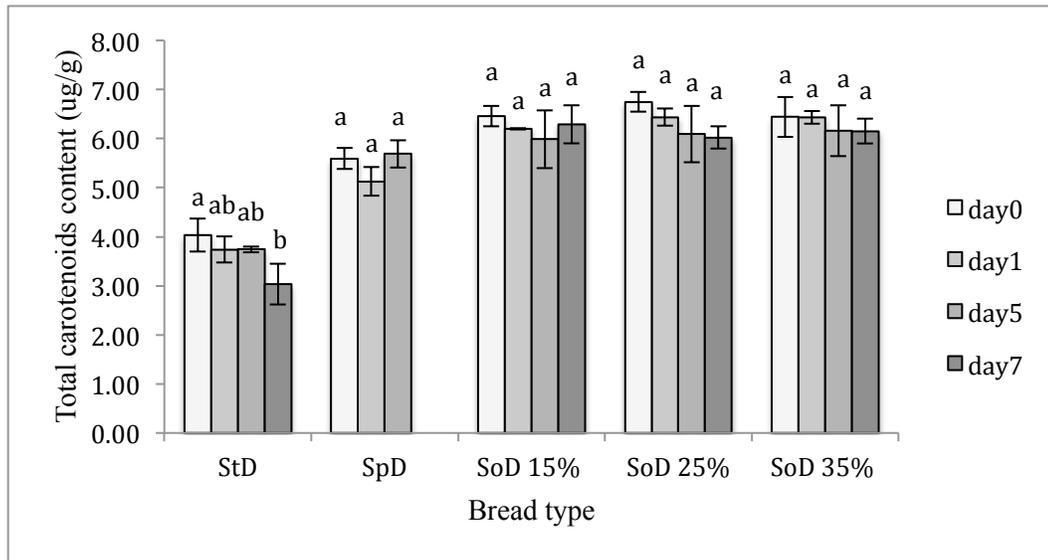


Figure 4.7 Effects of storage at room temperature on Total Carotenoid Content of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

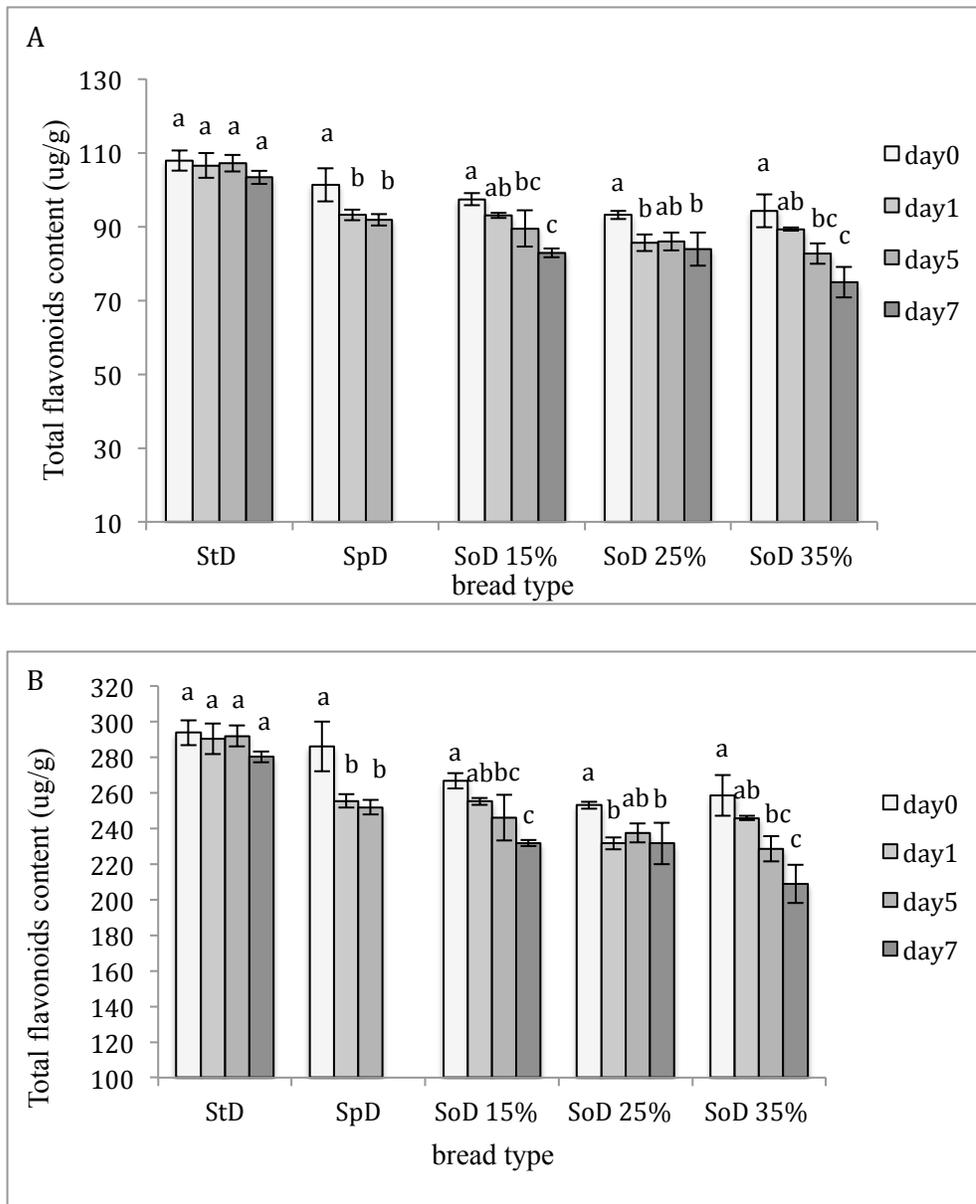


Figure 4.8 Effects of storage at room temperature on Total Flavonoid Content as Catechin Equivalent (A) and Rutin Equivalent (B) of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

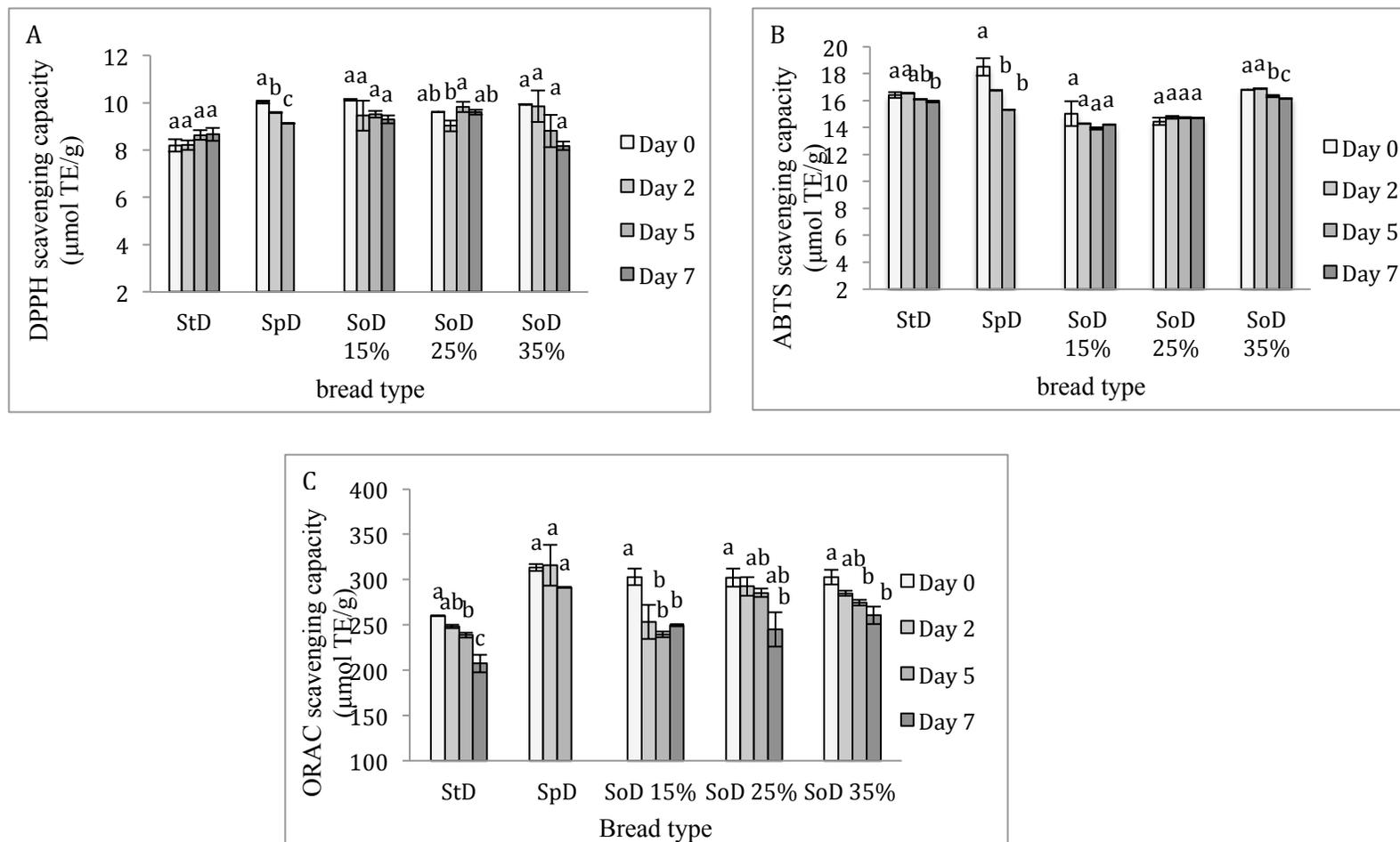


Figure 4.9 Effects of storage at room temperature on DPPH (A), ABTS (B), and ORAC (C) scavenging capacity of Wholegrain Bread Products. (Data are reported as means and standard deviation values presented by error bars). Different letters indicate significant differences between products at $p \leq 0.05$. StD= Straight dough; SpD= Sponge dough; and SoD= Sourdough.

CHAPTER 5

CONCLUSIONS AND FUTURE WORK

Consumers have become more aware of the health benefits associated with wholegrain bread consumption as opposed to white bread consumption. Wholegrain foods have been linked with reduced risk of chronic disease such as cardiovascular disease, cancer, and diabetes (Borneo and León 2012; Gani et al 2012). Thus, the development of improved wholegrain bread with superior quality and enhanced nutritional properties is needed to increase consumer appeal and to boost the daily consumption of wholegrain foods. Bread is commercially produced using different baking formulas and methods to produce numerous flavors, tastes, and textural properties. Duodu (2011) reported that different methods of cereal processing, including bread making, may positively or negatively affect the content of phytonutrients, which in turn affect their bioactive properties and health benefits. In the current study, research looking into bread-making techniques has been carried out to improve the quality and the nutritional and antioxidant properties of wholegrain bread.

Several methods are used in the production of bread including straight dough, sponge dough, Chorleywood process, and sourdough. Bread made from wholegrain wheat flour often has a lower loaf volume, firmer dense crumb, and darker crumb and crust compared to bread made from refined wheat flour (Heinio 2006; Cai et al 2014). As a result, research has been carried out to improve the quality characteristics of wholegrain bread products using various baking methods. Sourdough bread-making methods were more effective in improving wholegrain bread quality compared to straight or sponge dough (yeast-leavened) methods if the appropriate amount of starter and conditions were utilized. The optimum volume of all

sourdough breads was obtained when moderate acidity was achieved in sourdough bread containing 15% starter. The bread's improved volume and softness was probably due to the appropriate acidity, which modifies dough gluten through the enzymatic activity of flour. Furthermore, improved bread softness during storage was obtained with the sourdough bread-making method.

The phytonutrient and antioxidant properties of wholegrain bread could be altered during the baking process. Different baking processes would produce various reactions among ingredients during fermentation and oven baking, which causes changes in phytonutrients level and antioxidant capacity. In the current study, all bread-making methods displayed a positive effect on free phenolic acids. Sponge and sourdough breads increased total free phenol content, total carotenoids, and antioxidant properties as determined by the DPPH and ORAC assays whereas different bread-making methods did not affect ABTS scavenging capacity. In addition, all the bread-making methods applied in this study negatively affected total flavonoid content, with the most loss reported when sourdough fermentation was applied. However, the straight dough method had no effect on the total carotenoid content of wholegrain flour. In general, the study showed that phytonutrients in wholegrain bread could be manipulated via the chosen bread-making method. This was particularly true with ferulic acid, the predominant antioxidant in wheat, whereby the level of its free form was increased, which could improve its bioavailability.

This result has implications for future applications of sourdough in terms of the improved nutritional properties of bread products made from either refined or wholegrain flour.

In this study, it was hypothesized that the different fermentation types (e.g., yeast versus sourdough) and different bread-making methods (e.g., straight dough, sponge dough, and

sourdough) might affect bread quality, phytonutrient content, and antioxidant properties. The outcome findings confirmed the previous proposed hypothesis and proved that sourdough fermentation can effectively modify the quality and nutritional properties of wholegrain bread products. Likewise, using different sourdough levels (e.g., 15%, 25%, and 35%) was hypothesized to affect wholegrain bread's quality and nutritional value. The results suggest that level of sourdough starter used in the bread making is key to improving quality and phytonutrient content. Sourdough level has to be adjusted to obtain improved wholegrain bread quality while maintaining a minimal effect on phytonutrient levels. In this way, the health benefits of wholegrain products can be optimized. Future research to gain a thorough understanding of the relationships between sourdough fermentation and bread dough rheology and how they relate to improved product and nutritional quality would be significantly useful for the baking industry. Another future prospect could be to investigate the potential of sourdough to improve the stability of incorporated functional ingredients into bread formulas.

REFERENCES CITED

- AACCI International. 2013, <http://www.aaccnet.org/definitions/wholegrain.asp>.
- AACCI International. 2011. Approved methods of analysis. Available online; <http://methods.aaccnet.org/toc.aspx>.
- Abdel-Aal, E. S. M., and Gamel, T. H. 2008. Effects of Selected Barley Cultivars and Their Pearling Fractions on Inhibition of Human LDL Oxidation in Vitro Using a Modified Conjugated Dienes Method. *Cereal Chemistry*, 85(6), 730–737.
- Abdel-Aal, E. S. M., and Rabalski, I. 2013. Effect of Baking on Free and Bound Phenolic Acids in Wholegrain Bakery Products. *Journal of Cereal science*. 57(3), 312–318.
- Abdel-Aal, E. S. M., Young, JOURNAL C., Rabalski, I. 2006. Anthocyanin Composition in Black, Blue, Pink, Purple, and Red Cereal Grains. *Journal of Agricultural and Food Chemistry*. 54(13), 4696–4704.
- Abdel-Aal, E. S. M., Young, JOURNAL C., Rabalski, I., Hucl, P., Fregeau-Reid, JOURNAL 2007. Identification and Quantification of Seed Carotenoids in Selected Wheat Species. *Journal of Agricultural and Food Chemistry*. 55(3), 787–794.
- Abdel-Aal, E. S. M., Abou-Arab, A. a., Gamel, T. H., Hucl, P., Young, JOURNAL C., Rabalski, I. 2008. Fractionation of Blue Wheat Anthocyanin Compounds and Their Contribution to Antioxidant Properties. *Journal of Agricultural and Food Chemistry*. 56(23), 11171–11177.

Abdel-Aal, E. S. M., Young, JOURNAL C., Akhtar, H., Rabalski, I .2010. Stability of Lutein in Wholegrain Bakery Products Naturally High in Lutein or Fortified with Free Lutein. *Journal of Agricultural and Food Chemistry*. 58(18), 10109–10117.

Abdel-Aal, E. S. M., Choo, T. M., Dhillon, S., Rabalski, I. 2012. Free and Bound Phenolic Acids and Total Phenolics in Black, Blue, and Yellow Barley and Their Contribution to Free Radical Scavenging Capacity. *Cereal Chemistry*. 89(4., 198–204.

Abdel-Aal, E. S. M., Akhtar, H., Zaheer, K., Ali, R. 2013. Dietary Sources of Lutein and Zeaxanthin Carotenoids and Their Role in Eye Health. *Nutrients*. 5(4), 1169–1185.

Abdel-Aal, E. S. M., Rabalski, I. 2008. Bioactive Compounds and Their Antioxidant Capacity in Selected Primitive and Modern Wheat Species. *Open Agriculture Journal*. 2(1), 7–14.

Abdel-Aal, E. S. M., Rabalski, I. 2013. Antioxidant Properties of High-Lutein Grain-Based Functional Foods in Comparison with Ferulic Acid and Lutein. *American Journal of Biomedical Science*. 5(2), 109–125.

Abdel-Aal, E. S. M., Rabalski, I. 2013. Effect of Baking on Free and Bound Phenolic Acids in Wholegrain Bakery Products. *Journal of Cereal Science*. 57 (3), 312–318.

Adams, V., Ragaee, S., & Abdelaal, E. M. 2015. Impact of Wheat Fiber on Frozen Dough Shelf Life and Bread Quality. *Cereal Chemistry*, (ja).

Adom, K. K., Sorrells, M. E., & Liu, R. H. 2003. Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry*, 51(26), 7825-7834.

Adom, K. K., & Liu, R. H. 2002. Antioxidant activity of grains. *Journal of agricultural and food chemistry*, 50(21), 6182-6187.

Alvarez-Jubete, L., Wijngaard, H., Arendt, E. K., Gallagher, E. 2010. Polyphenol Composition and in Vitro Antioxidant Activity of Amaranth, Quinoa Buckwheat and Wheat as Affected by Sprouting and Baking. *Food Chemistry*. 119(2), 770–778.

Andersson, A. a M., Dimberg, L., Åman, P., Landberg, R. 2014. Recent Findings on Certain Bioactive Components in Whole Grain Wheat and Rye. *Journal of Cereal science*. 59(3), 294–311.

Anson, N.M., van den Berg, R., Havenaar, R., Bast, A., Haenen, G.R.M.M., 2009. Bioavailability of ferulic acid is determined by its bioaccessibility. *Journal of Cereal Science*. 49, 296-300.

Arendt, E. K., Ryan, L. a M., Dal Bello, F. 2007. Impact of Sourdough on the Texture of Bread. *Food Microbiology*. 24(2), 165–174.

Armero, E., Collar, C. Texture Properties of Formulated Wheat Doughs - Relationships with Dough and Bread Technological Quality. 1997. *Zeitschrift Fur Leb. Und-forsch. a-Food Res. Technol.*, 204(2), 136–145.

Banu, I., Vasilean, I., Aprodu, I. 2010. Effect of Lactic Fermentation on Antioxidant Capacity of Rye Sourdough and Bread. *Food Science and Technology*. 16(6), 571–576.

Belz, M. C. E., Ryan, L. a M., Arendt, E. K. 2012. The Impact of Salt Reduction in Bread: A Review. *Crit. Rev. Food Science and Nutrition*. 52(6), 514–524.

Borneo, R., León, A. E. 2012. Whole Grain Cereals: Functional Components and Health Benefits. *Food Functional*. 3(2), 110.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. L. W. T. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*. 28(1), 25-30.

Cai, L., Choi, I., Lee, C., Park, K., Baik, B. 2014. Bran Characteristics and Bread-Baking Quality of Whole Grain Wheat Flour. *Cereal Chemistry*. 91(4), 398–405.

Cauvain, S. P., & Young, L. S. 2007 . *Technology of breadmaking*.

Chandi, G. K., & Seetharaman, K. 2012. Optimization of gluten peak tester: a statistical approach. *Journal of Food Quality*, 35(1), 69-75.

Cheng, Z., Su, L., Moore, JOURNAL, Zhou, K., Luther, M., Yin, JOURNAL JOURNAL, & Yu, L. 2006. Effects of postharvest treatment and heat stress on availability of wheat antioxidants. *Journal of agricultural and food chemistry*. 54(15), 5623-5629.

Chew, Y. L., Goh, JOURNAL K., & Lim, Y. Y. 2009. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. *Food Chemistry*, 116(1), 13-18.

Clarke, C.I., Arendt, E., 2005. A review of the application of sourdough technology to wheat breads. *Advanced Food And Nutrition. Res.* 49, 137–161

Clarke, C. I., Schober, T. JOURNAL, & Arendt, E. K. 2002. Effect of single strain and traditional mixed strain starter cultures on rheological properties of wheat dough and on bread

quality. *Cereal Chemistry*. 79(5), 640-647.

Coda, R., Rizzello, C. G., Gobbetti, M. 2010. Use of Sourdough Fermentation and Pseudo-Cereals and Leguminous Flours for the Making of a Functional Bread Enriched of Gamma-Aminobutyric Acid (GABA). *International Journal of Food Microbiology*. 137(2-3), 236–245.

Corsetti, a., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J. 2000. Combined Effect of Sourdough Lactic Acid Bacteria and Additives Bread Firmness and Staling. *Journal of Agricultural and Food Chemistry*. 48(7), 3044–3051.

Corsetti, A., Settanni, L. 2007. Lactobacilli in Sourdough Fermentation. *Food Res. Int.* 40(5), 539–558.

Corsetti, A., Gobbetti, M., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J. C.b, L. 1998. Sourdough Lactic Acid Bacteria Effects on Bread Firmness and Staling. 63(2), 347–351.

Courtin, C. M., & Delcour, J. A. 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science*. 35(3), 225-243.

Crowley, P., Schober, T. J, Clarke, C. I., Arendt, E. K. 2002. The Effect of Storage Time on Textural and Crumb Grain Characteristics of Sourdough Wheat Bread. *Eur. Food Res. Technology*. 214(6), 489–496.

Dal Bello, F., Clarke, C. I., Ryan, L. A. M., Ulmer, H., Schober, T. J., Ström, K., & Arendt, E. K. 2007. Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. *Journal of Cereal Science*. 45(3),

309-318.

De Vuyst, L., Neysens, P. 2005. The Sourdough Microflora: Biodiversity and Metabolic Interactions. *Trends in Food Science and Technology*. 16(1-3), 43–56.

Gellynck, X. 2008. Nutritional Value of Bread: Influence of Processing, Food Interaction and Consumer Perception. *Journal of Cereal science*. 48(2), 243–257.

Duodu, K. G. 2011. Effects of processing on antioxidant phenolics of cereals and legume grains. *Advances in cereal science: Implications to food processing and health promotion*. American Chemistry Society. 31-54.

Dykes, L., & Rooney, L. W. 2007. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World*. 52(3), 105-111.

Feng, Y., & McDonald, C. E. 1989. Comparison of flavonoids in bran of four classes of wheat. *Cereal chemistry*, 66(6), 516-518.

Fardet, A. 2010. New Hypotheses for the Health-Protective Mechanisms of Whole-Grain Cereals: What Is beyond Fibre? *Nutrition.Res. Rev.* 23(1), 65–134.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., & Autio, K. 2007. Optimization of ingredients and baking process for improved wholemeal oat bread quality. *LWT-Food Science and Technology*, 40(5), 860-870.

Flander, L., Suortti, T., Katina, K., Poutanen, K. Effects of Wheat Sourdough Process on the Quality of Mixed Oat-Wheat Bread. *LWT - Food Science Technol.* 2011, 44(3), 656–664.

Frengova, G. I., Emilina, S. D., & Beshkova, D. M. 2003. Carotenoid production by lactoso-negative yeasts co-cultivated with lactic acid bacteria in whey ultrafiltrate. *Zeitschrift für Naturforschung C*, 58(7-8), 562-567.

Freudenheim, J. L., Marshall, J. R., Vena, J. E., Laughlin, R., Brasure, J. R., Swanson, M. K., Nemoto, T., Graham, S. 1996. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. *Journal of the National Cancer Institute*. 88(6), 340-348.

Galle, S., Schwab, C., Arendt, E. K., Gänzle, M. G. 2011. Structural and Rheological Characterisation of Heteropolysaccharides Produced by Lactic Acid Bacteria in Wheat and Sorghum Sourdough. *Food Microbiology*. 28(3), 547–553.

Gamel, T. H., Abdel-Aal, E. S. M., Tosh, S. M. 2015. Effect of Yeast-Fermented and Sour-Dough Making Processes on Physicochemical Characteristics of B-Glucan in Whole Wheat/oat Bread. *LWT - Food Science Technology*. 60(1), 78–85.

Gani, A., Sm, W., Fa, M. 2012. Whole-Grain Cereal Bioactive Compounds and Their Health Benefits: A Review. *Journal of Food Processing and Technology*. 03(03).

Garrido-Fernández, J., Maldonado-Barragán, A., Caballero-Guerrero, B., Hornero-Méndez, D., & Ruiz-Barba, J. L. 2010.. Carotenoid production in *Lactobacillus plantarum*. *International journal of food microbiology*, 140(1), 34-39.

Gelinas, P., McKinnon, C. M. 2006. Effect of Wheat Variety, Farming Site, and Bread-Baking on Total Phenolics. *Int. Journal of Food Science and Technology*. 41(3), 329–332.

Giannou, V., Kessoglou, V., Tzia, C., 2003. Quality and safety characteristics of bread

made from frozen dough. *Trends in Food Science and Technology*. 14(3), 99–108

Gobbetti, M., Rizzello, C. G., Di Cagno, R., De Angelis, M. 2014. How the Sourdough May Affect the Functional Features of Leavened Baked Goods. *Food Microbiol.* 37, 30–40.

Gómez, M., Ronda, F., Blanco, C. A., Caballero, P. A., & Apesteguía, A. 2003. Effect of dietary fibre on dough rheology and bread quality. *European Food Research and Technology*, 216(1), 51-56.

Halliwell, B. 1994. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?. *The lancet*, 344(8924), 721-724.

Hammes, W. P., & Gänzle, M. G. 1997. Sourdough breads and related products. In *Microbiology of fermented foods* (pp. 199-216). Springer US.

Hansen, H. B., Andreasen, M. F., Nielsen, M. M., Larsen, L. M., Knudsen, K. E. B., Meyer, A. S., Christensen, L. P. and Hansen, A 2002. Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. *European Food Research and Technology*, 214(1), 33-42.

Harman, D. 2009. Origin and evolution of the free radical theory of aging: a brief personal history, 1954–2009. *Biogerontology*, 10(6), 773-781.

Hebeda, R. E., Bowles, L. K., & Teague, W. M. 1990. Developments in enzymes for retarding staling of baked goods. *Cereal Foods World*. 35(5), 453-457

Health Canada, 2012 <http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/basics-base/quantit->

[eng.php](#)

Hidalgo, A., Brandolini, A., and Pompei, C. 2010. Carotenoids evolution during pasta, bread and water biscuit preparation from wheat flours. *Food Chemistry*. 121(3), 746-751.

Holtekjølen, A. K., Bævre, A. B., Rødbotten, M., Berg, H., & Knutsen, S. H. 2008. Antioxidant properties and sensory profiles of breads containing barley flour. *Food Chemistry*, 110(2), 414-421.

Hüttner, E. K., Bello, F., Arendt, E. K. 2010. Identification of Lactic Acid Bacteria Isolated from Oat Sourdoughs and Investigation into Their Potential for the Improvement of Oat Bread Quality. *Eur. Food Res. Technol.* 230(6), 849–857.

Iqbal, S., Bhangar, M. I., & Anwar, F. 2007. Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. *LWT-Food Science and technology*, 40(2), 361-367.

Jaekel, L. Z., Batista, C., Steel, C. J., Chang, Y. K. 2012. Influence of Xylanase Addition on the Characteristics of Loaf Bread Prepared with White Flour or Whole Grain Wheat Flour. 32(4), 844–849.

Jensen, S., Oestdal, H., Clausen, M. R., Andersen, M. L., Skibsted, L. H. 2011. Oxidative Stability of Whole Wheat Bread during Storage. *LWT - Food Science Technol.* 44 (3), 637–642.

Jideani, A. I., Silungwe, H., Takalani, T., Anyasi, T. A., Udeh, H., & Omolola, A. 2014. Antioxidant nich natural grain products and human health. *Antioxidant-Antidiabetic Agents and Human Health*. InTech Croatia, 167-186.

Johnson, E. A., & Schroeder, W. A. 1996. Microbial carotenoids. In Downstream processing biosurfactants carotenoids (pp. 119-178). Springer Berlin Heidelberg.

Jones, J. M., Reicks, M., Adams, J., Fulcher, G., Marquart, L. 2004. Becoming Proactive With the Whole-Grains Message. *And Nutrition.Today.* 39 (1), 10–17.

Katina, K., Salmenkallio-Marttila, M., Partanen, R., Forssell, P., Autio, K. 2006. Effects of Sourdough and Enzymes on Staling of High-Fibre Wheat Bread. *LWT - Food Science Technol.*, 39(5), 479–491.

Katina, K., Liukkonen, K. H., Kaukovirta-Norja, a., Adlercreutz, H., Heinonen, S. M., Lampi, a.-M., Pihlava, J. M., Poutanen, K. 2007. Fermentation-Induced Changes in the Nutritional Value of Native or Germinated Rye. *Journal of Cereal science.* 46 (3), 348–355.

Koletta, P., Irakli, M., Papageorgiou, M., Skendi, A. 2014. Physicochemical and Technological Properties of Highly Enriched Wheat Breads with Wholegrain Non Wheat Flours. *Journal of Cereal science.* 60(3), 561–568.

Konopka, I., Tańska, M., Faron, A., Czaplicki, S. 2014. Release of Free Ferulic Acid and Changes in Antioxidant Properties during the Wheat and Rye Bread Making Process. *Food Science Biotechnol.* 23(3), 831–840.

Ktenioudaki, A., Alvarez-Jubete, L., Gallagher, E. 2014. A Review of the Process-Induced Changes in the Phytochemical Content of Cereal Grains: The Breadmaking Process. *Crit. Rev. Food Science And Nutrition.* 55(5), 611–619.

Leenhardt, F., Lyan, B., Rock, E., Boussard, A., Potus, J., Chanliaud, E., Remesy, C.

Wheat Lipoxygenase Activity Induces Greater Loss of Carotenoids than Vitamin E during Breadmaking. *J. Agric. Food Chemistry*. 2006,54 (5), 1710–1715.

Lillioja, S., Neal, A. L., Tapsell, L., & Jacobs, D. R. 2013. Whole grains, type 2 diabetes, coronary heart disease, and hypertension: links to the aleurone preferred over indigestible fiber. *Biofactors*, 39(3), 242-258.

Lindenmeier, M., & Hofmann, T. 2004. Influence of baking conditions and precursor supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. *Journal of Agricultural and Food Chemistry*, 52(2), 350-354.

Liu, R. H., 2003.. Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *American Journal of Clinical Nutrition*. 78:517S-520S.

Liu, R. H. 2004. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *Journal And Nutrition*.134:3479S-3485S.

Liu, R. H., 2007. Whole Grain Phytochemicals and Health. *Journal of Cereal Science*.46(3), 207–219.

Liukkonen, K.H., Katina, K., Wilhelmsson, A., Myllymaki, O., Lampi, A.M., Kariluoto, S., Piironen, V., Heinonen, S.M., Nurmi, T., Adlercreutz, H., Peltoketo, A., Pihlava, J. M., Heitaniemi, V. and Poutanen, K. 2003.. Process-induced changes on bioactive compounds in whole grain rye. *Processing And Nutrition Society*. 62, 117-122.

Maleki, M., Hosoney, R. C., & Mattern, P. J. 1980. Effects of loaf volume, moisture content, and protein quality on the softness and staling rate of bread. *Cereal Chemistry* 57(2),

138-140.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. 2004. Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*. 79(5), 727-747.

Maraschin, C., Robert, H., Boussard, A., Potus, J., Baret, J., & Nicolas, J. 2008. Effect of storage temperature and flour water content on lipids, lipoxygenase activity, and oxygen uptake during dough mixing. *Cereal chemistry*, 85(3), 372-378.

Martel, F., Monteiro, R., & Calhau, C. 2010. Effect of polyphenols on the intestinal and placental transport of some bioactive compounds. *Nutrition research reviews*, 23(01), 47-64.

Menga, V., Fares, C., Troccoli, A., Cattivelli, L., Baiano, A. 2009. Effects of Genotype, Location and Baking on the Phenolic Content and Some Antioxidant Properties of Cereal Species. *Int. J. Food Science Technol.*, 45(1), 7–16.

Michalska, A., Ceglinska, A., Amarowicz, R., Piskula, M. K., Szawara-Nowak, D., Zielinski, H. 2007. Antioxidant Contents and Antioxidative Properties of Traditional Rye Breads. *Journal of Agricultural and Food Chemistry*, 55(3), 734–740.

Mondal, A., Datta, a. K. 2008. Bread Baking - A Review. *Journal of Food Engineering*, 86(4), 465–474.

Moore, J, Luther, M., Cheng, Z., Yu, L. 2009. Effects of Baking Conditions, Dough Fermentation, and Bran Particle Size on Antioxidant Properties of Whole-Wheat Pizza Crusts. *Journal of Agricultural and Food Chemistry*. 57(3), 832–839.

Moore, M. M., Bello, F. D., Arendt, E. K. 2007. Sourdough Fermented by *Lactobacillus Plantarum* FST 1.7 Improves the Quality and Shelf Life of Gluten-Free Bread. *Eur. Food Res. Technology.* 226 (6), 1309–1316.

Naderi, G. A., Asgary, S., Sarraf-Zadegan, N., & Shirvany, H. 2003. Anti-oxidant effect of flavonoids on the susceptibility of LDL oxidation. *Molecular and cellular biochemistry.* 246(1-2), 193-196.

Najafi, M. A., Rezaei, K., Safari, M., Razavi, S. H. 2012. Effects of Several Starter Cultures on the Anti-Mold Activity and Sensory Attributes of a Traditional Flat Bread (Sangak) from Iran. *Food Science Biotechnol.* 21 (1), 113–121.

Okarter, N. and Liu, R. 2010. Health benefits of whole grain phytochemicals. *Crit. Rev. Food Science And Nutrition.* 50:193–208.

Ottogalli, G., Galli, A., & Foschino, R. 1996. Italian bakery products obtained with sour dough: characterization of the typical microflora. *Advances in food sciences,* 18 (5-6), 131-144.

Ou, B., Hampsch-Woodill, M., and Prior, R. L. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of agricultural and food chemistry.* 49(10), 4619-4626.

Peterson, J., Dwyer, J., Adlercreutz, H., Scalbert, A., Jacques, P., & McCullough, M. L. 2010. Dietary lignans: physiology and potential for cardiovascular disease risk reduction. *Nutrition reviews,* 68(10)., 571-603.

Poutanen, K., Flander, L., Katina, K. 2009. Sourdough and Cereal Fermentation in a Nutritional Perspective. *Food Microbiol.* 26(7), 693–699.

Ragae, S., Abdel-Aal, E. S. M., Noaman, M. 2006, Antioxidant Activity and Nutrient Composition of Selected Cereals for Food Use. *Food Chemistry* 98 (1), 32–38.

Ragae, S., Guzar, I., Dhull, N., Seetharaman, K. Effects of Fiber Addition on Antioxidant Capacity and Nutritional Quality of Wheat Bread. *LWT - Food Science Technol.* 2011, 44 (10.), 2147–2153.

Ragae, S., Seetharaman, K., Abdel-Aal, E. S. M. 2014. The Impact of Milling and Thermal Processing on Phenolic Compounds in Cereal Grains. *Crit. Rev. Food Science And Nutrition.* 54 (7), 837–849.

Rakin, M., Vukasinovic, M., Siler-Marinkovic, S., & Maksimovic, M. 2007. Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate. *Food chemistry*, 100(2), 599-602.

Rastmanesh, R. 2011.. High polyphenol, low probiotic diet for weight loss because of intestinal microbiota interaction. *Chemico-biological interactions*, 189(1), 1-8.

Ravyts, F., De Vuyst, L. 2011. Prevalence and Impact of Single-Strain Starter Cultures of Lactic Acid Bacteria on Metabolite Formation in Sourdough. *Food Microbiol.* 2011, 28 (6), 1129–1139.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free*

radical biology and medicine. 26(9), 1231-1237.

Read, A., Wright, A., & Abdel-Aal, E. S. M. 2015.. In vitro bioaccessibility and monolayer uptake of lutein from wholegrain baked foods. Food chemistry, 174, 263-269.

Rieder, A., Holtekjølen, A. K., Sahlstrøm, S., Moldestad, A. 2012. Effect of Barley and Oat Flour Types and Sourdoughs on Dough Rheology and Bread Quality of Composite Wheat Bread. Journal of Cereal science. 55 (1), 44–52.

Rizzello, C. G., Calasso, M., Campanella, D., De Angelis, M., Gobbetti, M. 2014 Use of Sourdough Fermentation and Mixture of Wheat, Chickpea, Lentil and Bean Flours for Enhancing the Nutritional, Texture and Sensory Characteristics of White Bread. Int. Journal of Food Microbiology, 180, 78–87.

Ross, A. B., Kamal-Eldin, A., Aman, P. 2004 Dietary Alkylresorcinols: Absorption, Bioactivities, and Possible Use as Biomarkers of Whole-Grain Wheat- and Rye-Rich Foods. And Nutrition.Rev. 62(3), 81–95.

Schmiele, M., Jaekel, L. Z., Patricio, S. M. C., Steel, C. J., Chang, Y. K. 2012. Rheological Properties of Wheat Flour and Quality Characteristics of Pan Bread as Modified by Partial Additions of Wheat Bran or Whole Grain Wheat Flour. Int. Journal of Food Science Technol., 47(10.), 2141–2150.

Serpen, A., Gökmen, V., and Fogliano, V. 2012. Total antioxidant capacities of raw and cooked meats. Meat science. 90 (1), 60-65.

Serpen, A., Gökmen, V., Pellegrini, N., and Fogliano, V. 2008. Direct measurement of

the total antioxidant capacity of cereal products. *Journal of Cereal Science*. 48 (3), 816-820.

Shahidi, F., & Chandrasekara, A. 2010.. Hydroxycinnamates and their in vitro and in vivo antioxidant activities. *Phytochemistry Reviews*, 9(1), 147-170.

Singleton, V. L., & Rossi, J. A. 1965.. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158.

Slavin, J. Why Whole Grains Are Protective: Biological Mechanisms. *Processing And Nutrition Society*. 2003, 62(1), 129–134.

Slavin, J. L., Jacobs, D., & Marquart, L. 2000.. Grain processing and nutrition. *Critical Reviews in Food Science and Nutrition*, 40, 309–326

Stolz, P., & Böcker, G. 1996. Technology, properties and applications of sourdough products. *Advances in food sciences*, 18(5-6), 234-236.

Tronsmo, K. M., Færgestad, E. M., Schofield, J. D., Magnus, E. M. 2003. Wheat Protein Quality in Relation to Baking Performance Evaluated by the Chorleywood Bread Process and a Hearth Bread Baking Test. *Journal of Cereal science*.2003, 38 (2), 205–215.

USDA. Dietary Guidelines for Americans, 2010 <http://www.cnpp.usda.gov/DGAs2010-PolicyDocument.htm>.

Vitaglione, P., Napolitano, A., Fogliano, V. 2008, Cereal Dietary Fibre: A Natural Functional Ingredient to Deliver Phenolic Compounds into the Gut. *Trends Food Science*

Technol., 19(9), 451–463.

Vogrincic, M., Timoracka, M., Melichacova, S., Vollmannova, A., Kreft, I. 2010. Degradation of Rutin and Polyphenols during the Preparation of Tartary Buckwheat Bread. *Journal of Agricultural and Food Chemistry*. 58(8), 4883–4887.

Wennermark, B. H., & Jagerstad, M. (1992). Breadmaking and storage of various wheat fractions affect vitamin E. *Journal of food science*, 57(5), 1205-1209.

Woggon, W. D., & Kundu, M. K. 2004. Enzymatic Versus Chemical Cleavage of Carotenoids: Supramolecular Enzyme Mimics for b-Carotene 15, 150-Monooxygenase. *Carotenoids in health and disease*, 357.

Yazar, G., Tavman, Ş. 2012. Functional and Technological Aspects of Sourdough Fermentation with *Lactobacillus Sanfranciscensis*. *Food Eng. Rev.* 4(3), 171–190.

Yilmaz, Y., and Toledo, R. 2005. Antioxidant activity of water-soluble Maillard reaction products. *Food Chemistry*. 93(2), 273-278.

Yu, L., Nanguet, A.-L., Beta, T. 2013. Comparison of Antioxidant Properties of Refined and Whole Wheat Flour and Bread. *Antioxidants*. 2, 370–383.

Zhao, C. *JOURNAL*, Kinner, M., Wismer, W., & Gänzle, M. G. 2015. Effect of Glutamate Accumulation During Sourdough Fermentation with *Lactobacillus reuteri* on the Taste of Bread and Sodium-Reduced Bread. *Cereal Chemistry* 92(2), 224-230.

Zhou, K., Yin, J. J., Yu, L. 2005 Phenolic Acid, Tocopherol and Carotenoid Compositions,

and Antioxidant Functions of Hard Red Winter Wheat Bran. *Journal of Agricultural and Food Chemistry*, 53(10). 3916–3922.

APPENDIX

Appendix A: Supplementary to Chapter4

Appendix A-1: Inflection points of DPPH and ABTS reactions with wholegrain bread samples

Table A-1.1. Regression equations and determination coefficients (R^2) of DPPH reactions with wholegrain bread samples

	3rd order regression equation	R²	4th order regression equation	R²
Flour	$y = -4E-06x^3 + 0.0005x^2 - 0.0167x + 0.7676$	0.93063	$y = 2E-07x^4 - 3E-05x^3 + 0.0013x^2 - 0.0264x + 0.7863$	0.98069
Straight dough	$y = -4E-06x^3 + 0.0004x^2 - 0.0148x + 0.7672$	0.94283	$y = 2E-07x^4 - 3E-05x^3 + 0.0012x^2 - 0.0247x + 0.7862$	0.98141
Sponge dough	$y = -3E-06x^3 + 0.0004x^2 - 0.0166x + 0.7663$	0.9478	$y = 2E-07x^4 - 2E-05x^3 + 0.0012x^2 - 0.0263x + 0.7851$	0.98318
<i>Sourdough</i>				
15% starter	$y = -4E-06x^3 + 0.0005x^2 - 0.0174x + 0.7708$	0.96272	$y = 1E-07x^4 - 2E-05x^3 + 0.0011x^2 - 0.0254x + 0.7861$	0.9853
25% starter	$y = -5E-06x^3 + 0.0005x^2 - 0.0165x + 0.7675$	0.95796	$y = 1E-07x^4 - 2E-05x^3 + 0.0011x^2 - 0.024x + 0.7819$	0.97558
35% starter	$y = -4E-06x^3 + 0.0004x^2 - 0.0159x + 0.7736$	0.96425	$y = 1E-07x^4 - 2E-05x^3 + 0.001x^2 - 0.0225x + 0.7864$	0.98304

Table A-1.1. Inflection points of DPPH reaction determined by polynomial regression analysis

Sample	Inflection point (min) from 3rd order equation	Inflection points (min) from 4th order equation	
Flour	41	19	55
Straight dough	33	17	57
Sponge dough	44	-	-
15% sourdough	42	24	75
25% sourdough	33	24	75
35% sourdough	33	21	78

Table A-1.2. Inflection points of DPPH reaction determined by Hokey Stick Regression analysis (SAS software)

Sample	Inflection point (min) from 3rd order equation	Inflection points (min) from 4th order equation	
Flour	40	11	60
Straight dough	41	15	55
Sponge dough	38	7	45
15% sourdough	36	11	57
25% sourdough	40	6	60
35% sourdough	38	7	57

Table A-1.1. Regression equations and determination coefficients (R²) of ABTS reactions with wholegrain bread sample

	3rd order regression equation	R²	4th order regression equation	R²
Flour	$y = -6E-06x^3 + 0.0007x^2 - 0.0316x + 0.77$	0.98159	$y = 2E-07x^4 - 3E-05x^3 + 0.0017x^2 - 0.043x + 0.792$	0.99197
Straight dough	$y = -7E-06x^3 + 0.0009x^2 - 0.0353x + 0.7264$	0.96714	$y = 3E-07x^4 - 4E-05x^3 + 0.0021x^2 - 0.0507x + 0.7561$	0.98645
Sponge dough	$y = -7E-06x^3 + 0.0009x^2 - 0.0354x + 0.7166$	0.95292	$y = 4E-07x^4 - 5E-05x^3 + 0.0025x^2 - 0.056x + 0.7563$	0.98686
<i>Sourdough</i>				
15% starter	$y = -6E-06x^3 + 0.0007x^2 - 0.0284x + 0.7704$	0.97737	$y = 2E-07x^4 - 3E-05x^3 + 0.0015x^2 - 0.0378x + 0.7885$	0.98588
25% starter	$y = -3E-06x^3 + 0.0005x^2 - 0.0254x + 0.7371$	0.96813	$y = 2E-07x^4 - 3E-05x^3 + 0.0015x^2 - 0.0383x + 0.7619$	0.98351
35% starter	$y = -5E-06x^3 + 0.0006x^2 - 0.029x + 0.7625$	0.9933	$y = 1E-07x^4 - 2E-05x^3 + 0.0012x^2 - 0.0362x + 0.7764$	0.99814

Table A-1.3. Inflection points of ABTS Reaction determined by polynomial regression analysis

Sample	Inflection point (min) from 3rd order equation	Inflection points (min) from 4th order equation	
Flour	39	-	-
Straight dough	42	-	-
Sponge dough	42	-	-
15% sourdough	39	25	50
25% sourdough	55	25	50
35% sourdough	40	27	72

Table A-1.4. Inflection points of ABTS reaction determined by Hokey Stick Regression analysis (SAS software)

Sample	Inflection point (min) from 3rd order equation	Inflection points (min) from 4th order equation	
Flour	35	15	50
Straight dough	33	11	47
Sponge dough	39	7	47
15% sourdough	33	12	61
25% sourdough	40	8	40
35% sourdough	40	16	50