Properties of wholegrain bread baked with various methods: Assessment to phytonutrient, antioxidant, shelf life and quality.

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Abstract

Wholegrain foods are sources of dietary fiber and antioxidants. Consumers today have become more aware of the health benefits associated with wholegrain bread consumption as opposed to white bread consumption. This review aimed to access different bread-making methods on quality Properties of wholegrain bread and subsequent shelf life 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 seven 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. Thus, 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. In addition, all wholegrain bread products had significant increases in antioxidant properties compared with the wholegrain flour. The shelf life of bread made from wholegrain or fiber-enriched flours could also be influenced. 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. Generally, this review access helps better understand the effect of different bread-making methods on quality, shelf life, phytonutrients and antioxidants properties in wholegrain bread products. So that, it would help identify preferred bread-making method(s) that would preserve nutritional and bio-functional properties of wholegrain bread; which is useful for the baking industry and wheat researchers to help make informed decisions with regard to the production of improved wholegrain bread products.

Keywords: Wholegrain, Antioxidant, Phytonutrient, shelf life.

Abbreviations

LAB: Lactic Acid Bacteria; AACCi: American Association of Cereal Chemists International; TPA: Texture Profile Analyses; LV: Loaf Volume; SLV: Specific Loaf Volume; ORAC: Oxygen Radical Absorbance Capacity; GPT: Gluten Peak Tester; MT: Maximum Torque; PMT: Peak Maximum Time; CBP: Chorleywood Bread Method.

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 [1-4]. 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. 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 [5]. Many studies have reported that sourdough improves loaf volume and crumb firmness [6,7], as well as shelf life [8,9]. 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 [5].

Baking also affects content and composition of phytonutrients in wholegrain products. Baking increased free ferulic acid of wholegrain bread [10]. Similarly, an increase in total phenol content of wholegrain bread regardless of baking time (10, 20 or 35 min) [11]. Sourdough fermentation during wheat and rye bread-making method showed an increase in phenolic acids [12-15]. Increasing free phenolic compounds would result in boosting their bioavailability since the bioavailability of bound 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. 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 288°C increased the antioxidant properties by as much as 82% [18]. Baking method decreased the DPPH scavenging capacity, but increased the ORAC values of wholegrain bread [19]. The authors found that wholegrain flour resulted in bread with higher antioxidant properties compared to the refined flour. This review helps for better understand the effect of different bread-making methods on quality, shelf life, phytonutrients and antioxidant capacity of wholegrain bread products.

Methods
This review report was formulated through literature searches using Available scientific information and pertinent literatures on the keywords: wholegrain; methods of bread-making (sourdough, straight dough, sponge dough); Antioxidant; Phytonutrient; bread quality, bread shelf life”. The logical term operant and was used in the search of items to match keywords.

Overview to bread
Bread is an essential part in human diets. 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. The health benefits associated with the consumption of wholegrain food have been well documented. Wholegrain foods have been linked with reduced risk of coronary heart disease, diabetes, and cancer. Nutritionally, wholegrain wheat flour contains higher concentrations of dietary fiber, vitamins, and minerals, physic acid and phenolic compounds than refined wheat flour [20,21]. 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.

Similarly, the incorporation of fiber into white flour negatively affects bread quality. 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 [22]. 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 [23,24]. During storage, there is an increase in crumb hardness and loss of bread freshness known as staling that reduces palatability [25]. 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 [26]. 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.

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 AACC approved method 10.10.03 [27]. 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 [28].

Quality of wholegrain flour
Flour quality affects bread quality when making bread. The amount of water required to fully dehydrate flour and form developed gluten is key in making quality bread. Since wholegrain flours contain soluble fiber, it is crucial to efficiently determine its water absorption. Similar observations when 10%–40% of wheat flour was replaced by wheat or oat bran [29,30]. Water absorption increased from 63% for hard wheat refined flour to 72% for wheat flour containing 40% wheat bran [30]. The increase in the water absorption of wholegrain or bran-rich flour was probably due to the high fiber 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. High
torque response and short PMT are indications of high quality gluten [31]. The addition of wheat bran resulted in higher MT and shorter PMT compared to the refined hard wheat flour [32].

**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 [23]. Studies have shown that high SLV (3.40 cm$^3$/g) was observed with a lower level (10%-15%) of wheat sourdough starter [33] and oat-wheat sourdough starter [28]. Moreover, increasing sourdough level to 40% resulted in lower loaf volume compared to straight dough breads [23]. Sponge dough and straight dough breads had LV about 15% and 10% greater than sourdough bread [34]. However, the negative effect of sourdough due to the high levels of sourdough starter (40%-80%) applied. Meanwhile, 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 [35]. The use of sourdough at high levels could affect bread quality due to acidity-induced activation of proteolytic enzymes in the flour [6].

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 [9,28,29,36-38] 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. Loaf volume was positively correlated with softness of bread crumb [39]. 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 [6,7] and oat-wheat sourdough bread [28]. The sourdough fermentation increased acidity in the dough, thus improving protein solubility and enhancing proteolysis. The proteolytic enzymes present in wheat flour [6] or originated by lactic acid bacteria [26] modify the gluten network, resulting in changes in the physical properties of gluten and a decrease in the firmness of the bread [40,26,6]. 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. Differences in moisture content between yeast-leavened, sourdough, and yeast/sourdough bread products were previously reported being 44%, 43%, and 42%, respectively [41]. On the contrary, no changes in the moisture content of all wheat bread studied using bakers’ yeast, chemical acidification, or various LAB strains [7]. 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 [42]. Similar observation; the study showed that crust lightness of wheat sourdough breads was lower than that of control breads [43]. During baking, crust coloring results from the Maillard reactions between reducing sugars and amino acids. In the absence of salt, yeast activity increases resulting in a reduction in the amount of free reducing sugars remaining for Maillard reaction [44].

**PH of dough and baked bread**

Dough acidity can affect the final bread product. In sourdough lactic acid bacteria in the starter produce more organic acids compared with yeast in straight or sponge dough. Increasing the level of sourdough (10%-40%) increased the acidity of the bread and similar pH values were reported [33,28]. The acidity may have positive or negative effects on the volume of sourdough bread depending on the gluten network and acidity profile.

**Adjusted  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. However, the method of Flander and others was used to bake refined wheat flour supplemented with oat flour. Thus, Optimizing proofing time and temperature play a significant role in final bread quality [45].

**Effect of bread-making method on bread shelf life**

The use of sourdough fermentation has been reported to increase shelf-life of wheat bread [7,33]. The crumb firmness changes during storage and found that sourdough bread crumb were softer than wheat straight dough bread [33]. 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 [33]. Sourdough breads were softer than the chemically acidified gluten-free breads after 5 days of storage [46]. 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 [47]. The reduction of moisture content occurred in bread crust during storage could be due to the redistribution of moisture from crust to crumb. Thus it is critical to minimize these changes during storage of breads.

**Results**

**Effect of bread-making methods on bread quality**

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Moisture content and water activity are important factors that affect bread quality, consumer acceptance and shelf life. Differences in moisture content between yeast-leavened, sourdough, and yeast/sourdough bread products were previously reported being 44%, 43%, and 42%, respectively [48]. On the contrary, Corsetti et al., reported no changes in the moisture content of all wheat bread studied using bakers’ yeast, chemical acidification, or various LAB strains [7]. 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 Determined overall bread quality. Color of bread products depends on baking conditions and dough characteristics [42]. Coda et al., reported similar observation; the study showed that crust lightness of wheat sourdough breads was lower than that of control breads [43]. During baking, crust coloring results from the Maillard reactions between reducing sugars and amino acids. In the In the absence of salt, yeast activity increases resulting in a reduction in the amount of free reducing sugars remaining for Maillard reaction [44].

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**Discussion**

**Wholegrain and wholegrain products**

**Wholegrain:** The American Association of Cereal Chemists International (AACCI) defines wholegrain as “whole grains 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” [49]. The grain or kernel contains three main parts: endosperm (80%-85% of the grain), germ (2%-3%) and outer layers or...
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 semi-celluloses. In addition, the outer layers are rich in bioactive components such as phenol compounds, anthocyanins, β-glucan and dietary fiber [50,51].

The bran (13%-17%). American Association of Cereal Chemists International defined wholegrain food as “A wholegrain food must contain 8 grams or more of wholegrain per 30 grams of product” [52]. 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 [53]. According to Canada’s Food Guide, the daily intake of wholegrain 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 [3] and bioactive components such as phenolic acids [41].

Wholegrain products: Wholegrain products are considered a good source of phytonutrients such as tocopherols, tocotrienols, carotenoids, plant sterols, and lignans [2,54-56]. These compounds play significant roles in human health and disease prevention such as cardiovascular disease, cancer and chronic diseases [57]. As consumer became more health alert, baking industry is facing new challenges [58] to obtain an acceptable wholegrain bread quality with improved nutritional properties [23].

Bread-Making methods

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 dough, and Chorleywood method [58,59] 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 [58].

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 [58].

In Chorleywood bread method (CBP) or “no-time dough” method all the ingredients are mixed in a high-speed mixer for few minutes [58]. 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 [60].

The sourdough method has been used for thousands of years, where fermentation occurs by yeast and lactic acid bacteria. Sourdough usually used for rye bread production [61]. 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 [58].

According to De Vuyst and Neyesens, there are three types of sourdough depending on the method and technology used by industry [62]. 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 3-48 hours and the pH is about 4.0. Traditional sourdough produced using traditional technique (back slopping) by continuing refreshments daily to keep the microorganism’s active [62]. For instance products produced by the three types of sourdough: type I sourdough are sourdough rye bread, San Francisco sourdough, French bread, Toscanon and Altamura bread, and Panettone [62].

Type II sourdough is a semi-fluid silo preparation that is fermented at >30°C to speed up the fermentation methods to 24 hour 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 [63].

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 [62].

Type III sourdough is a dried sourdough in a powder form that is initiated by “defined starter cultures” [62]. Stolz and Böcker, 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 [64]. 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 [65].

The ratio of lactic acid bacteria to yeast in sourdough fermentation is generally 100:1 [66]. However, yeast preparations often contain Lactic acid bacteria such as lactobacillus spp [61]. The microflora in sourdough, represented by yeast and lactic acid bacteria, is responsible for the dough characteristics [61].

Bread quality

Loaf Volume (LV), specific loaf volume (SLV) and crumb firmness are the main quality characteristic of bread [23]. The most common method of assessing whole product volume is by the rapeseed displacement method [27]. 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 [44].

Moreover, bread texture is a critical factor for consumer acceptance [44]. Crumb firmness is important in bread texture assessment because it is associated with the perception of bread freshness. 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” [27]. 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 [47]. 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 [47]. Since the addition of wheat bran decreases loaf volume [23].

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 [67]
3. The higher water binding ability of fiber (both soluble and insoluble) causes less water available for the development of gluten network and less steam production (Gill et al 2002).

Bread improvers [22] or commercial enzyme mixtures [23] can be used to improve the quality of wholegrain or high-fiber bread. However, Prefermentation of wheat bran with lactic acid bacteria and yeast improved loaf volume and crumb softness [23,68]. Clarke et al, reported that adding sourdough during wheat bread-making method increased the loaf volume more than chemical acidification alone [6].

Sourdough is a key element in wholegrain bread-making (especially, rye) to contribute flavor and texture to the bread product [68]. Sourdough can be used for improving quality of bread products without using other improvement additives [29]. Sourdough bread-making method produced improved bread with good quality such as improved texture, flavor, and shelf life [23], besides preserving some bioactive compounds such as phytate, folates, tocopherols, and phenolics [12-14,69,70]. 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 [71]. 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 [71].

**Phytonutrients in wholegrain**

Phytonutrients are natural compounds produced by plants as protective substances against external stress and/or pathogenic attack [72]. 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 whole grains are phenolics (e.g., phenolic acids, flavonoids, and alkylresorcinols), carotenoids, tocols (vitamin E) and lignans [20,55]. Phenolics are the most studied phytonutrient s in wholegrains [55]. They exist in soluble free compounds, soluble conjugated esterifies to sugars and other low molecular weight compounds, and insoluble bound compounds. The most common phenolics in wholegrains are phenolic acids and flavonoids [20]. The type and concentration ofphytonutrient s vary among grains and genotypes [73].

**Phenolic acids:** Phenolic acids are hydroxybenzoic acid or hydroxycinnamic acid derivatives. 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 hemicelullose through ester links in the cell wall [20]. Ferulic acid is the major phenolic acid found in grains [20]. In wheat, ferulic acid is the main phenolic acid, which as accounts for 90% of the total phenolic acids present in the wheat grain [41]. Other studies found that ferulic acid present in free and bound forms in cereal grains [74,20]. Ferulic acid is mostly concentrated in the aleurone and pericarp of the grains, while minor quantity is present in the starchy endosperm [20]. Manach et al., 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 [74].

**Carotenoids:** Carotenoids, in general, are a collection of tetramerpenoid 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. 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 [75]. Lutein is the main yellow pigment in wheat. 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 [75].
Flavonoids: Flavonoids are phenolic compounds containing two aromatic rings linked by a threecarbon structure and exist in an oxygenated heterocyclic ring [55]. Flavonoids include flavonols, flavanones, flavones, isoflavones, flavans, and anthocyanins [76]. Flavonols such as quercetin, kaempferol and myricetin are very common flavonoids in foods [77]. Tricin or 5,7,4’-tri hydroxyl 3’,5’-dimethoxyflavone is known as the dominant flavone pigment in wheat. In addition, two Cglycosylflavones, 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 ± 10 µmol/g (catechin equivalents) to 149 ± 17 µmol/g (catechin equivalents) [73]. 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 [55]. Anthocyanins, a group of water-soluble flavonoids impart red, purple and blue colors in plants [50,77,78].

Tocopherols and tocotrienols: Tocopherols and tocotrienolsor vitamin E precursors are fat-soluble compounds that include a 6-hydroxychromen group and a phytyl side chain made of isoprenoid units [20]. 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 [55]. Tocopherols and Tocotrienols can be found in different types of foods and wholegrains, especially in the germ fraction [20].

Alkyl resorcinol (s): 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. Alkylresorcinols are mostly found in the grain bran which explains why they are not found in refined wheat products. Rye contains the highest total alkylresorcinol content (734 ± 23 µg/g) followed by wheat (583 ± 82 µg/g) and barley (45 ± 5 µg/g) on a dry matter basis (Ross et al 2003). However, in corn, millet, oats, rice, and sorghum no alkylresorcinols were detected [55].

Ignans and lignin: Lignans are compounds that contain two coupled C6C3 units and they are considered as a kind of dietary phytostrogen. 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 [20]. Lignin is an aromatic polymer derived from hydrocinnamic acids in plants [79]. It is considered as insoluble dietary fiber in cereal bran [79].

Antioxidant properties of wholegrain

Research on dietary antioxidants has received much attention since the free radical theory of aging introduced in 1956 [80]. 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. Accordingly, free radicals are atoms, ions or molecules with unpaired electrons on an open shell configuration [81]. 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 [41].

Phenolic compounds especially phenolic acids are the main antioxidant contributors in whole-grain products [82,83]. 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 [20]. Beside ferulic acid, wheat grain contains other hydroxycinnamic acids with antioxidant activity such as coumaric, sinapic and caffeic acid. Additionally, vitamin E or tocols are strong antioxidants because of their ability to scavenge lipid peroxyl radicals and reactive nitrogen and oxygen species. The hard wheat flours had slightly higher radical scavenging capacity, as measured by DPPH and ABTS, than soft wheat flour [84]. Thus, the study found a correlation between the levels of free phenolic acids of wheat bran and antioxidant capacity [18]. The study showed that the greater antioxidant capacity, the greater the release of bound phenolic acids. Additionally, Adom et al., discovered the highest phenolic content and radical scavenging capacity occurred with the white spring durum cultivar [73]. IQbal et al., measured the radical scavenging capacity of wheat grown in Pakistan by ABTS, DPPH, and ORAC [85]. The authors indicted that the antioxidant capacity was related to total phenol and anthocyanin content.

Wholegrain phytonutrients and human health

Wholegrain contain unique phytonutrient s that is not found in fruits and vegetables such as fornic acid and diferulates [86]. Several studies reported that phytonutrient s in wholegrain could exhibit bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties [55,87,88]. The antioxidant properties of wholegrain phytonutrient s play a significant role in cancer prevention [89]. For example, wholegrain flavonoids have been demonstrated to act as antioxidant and anticancer compounds [86]. The antioxidant ability of flavonoids depends on the number and position of hydroxyl groups in the molecule. The eating foods rich in anthocyanin reduced the risk of colon cancer by inhibiting cancer cell production in the colon. 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 [86]. Ferulic and diferulates acids in cereal bran can be released by gastrointestinal esterase particularly microflora and intestinal mucosa [20]. This provides an explanation for...
the relationship between wholegrain consumption and the reduced risk of colon cancer [86].

There was an inverse association between serum concentrations of α-carotene, β-carotene, lutein, lycopene and β-cryptoxanthin and the risk of lung cancer [90]. A study proved that the intake of carotenoids, especially lutein and zeaxanthin, is correlated with reduced risk of premenopausal breast cancer [91]. 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 [56]. Moreover, tocotrienols and tocopherols inhibit the oxidative damage of cell membranes [56]. Naderi et al., found that increasing flavonoids in dietary intake can help to decrease coronary artery disease [71].

**Effect of bread-making on phytonutrients and antioxidants**

**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 phytonutrient s such as carotenoids, tocols, phenols [92,93]. The effect of bread-making method on flavonoid content (rutin and quercetin) using tartary buckwheat flour [94]. They found that rutin decreased during bread-making (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 µg/g) was observed after mixing [70]. 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%) [95]. In the same study, there was a strong correlation between lipoxygenase activity and carotenoid losses (r²=0.97).

**Fermentation:** Fermentation is the next step that follows the formation of dough, in which the dough is left to rise before baking. The effect of proofing on the antioxidant properties of pizza dough made with wholegrain wheat flour [18]. 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 [18]. The content of bioactive compounds including phenolic acids, alkylresorcinols, tocols, sterols, folate, lignans, and thiamin could be modified by sourdough fermentation [14]. There can be an increase or decrease of these compounds level depending on the sourdough method or on the compounds nature itself. Wheat and rye sourdough fermentation increases total content of phenolics [43], free ferulic acid content and antioxidant properties [13,15]. Katina et al., 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 [13]. Similarly, Liukkonen et al., examined the effect of sourdough fermentation on several bioactive compounds such as phenolic acids, sterols, folates, tocopherols and tocotrienols, alkylresorcinols, and lignans [12]. 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 [12].

**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 [96,94]. The changes in free and bound phenolic acids that occurred during baking in wholegrain breads, cookies, and muffins [83]. 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. Baking increased the concentration of phenolic compounds in bread when compared with the flour, regardless of baking time [11]. 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 [11]. 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 [97]. The effect of baking on phenolic acids in whole wheat pizza dough studied [18]. 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 [18]. 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 [18].

Another study found that polyphenol content decreased during baking for breads containing tartary buckwheat [94]. The authors reported that rutin was degraded during mixing and about 85% was transformed to quercetin, while quercetin did not change during baking. The stability of phenolic acids and flavonoid compounds in amaranth, quinoa, and buckwheat...
during the bread-making process [98]. 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. 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 [99]. 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. Losses ranging from 35% to 45%, depending on the wheat species used [95].

The 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 [100]. Thus, vitamin E losses of between 24% and 47% in white breads and between 10% and 15% in wheat and rye breads because of baking [101]. 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.

Future Consideration

Research to gain a thorough understanding of the relationships between baking methods 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.

Conclusion

Whole grain products are considered a good source of phytonutrients such as phenolic compounds, tocopherols, tocotrienols, carotenoids, plant sterols, and lignans. 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. Wholegrain breads are good sources of dietary fiber and antioxidants. Thus, Wholegrain foods have been linked with reduced risk of chronic disease such as cardiovascular disease, cancer, and diabetes.

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 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. 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. 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. Generally, sourdough fermentation can effectively modify the quality and nutritional properties of wholegrain bread products.

Conflicts of Interest

The authors declare no conflict of interest.

References

3. Liu RH. Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. Am J Clin Nutri. 2003;78:517S520S.
41. Abdel-Aal ESM, Abou-Arab A, Gamel TH, et al. Fractionation of blue wheat anthocyanin compounds and
52. AACC International. 2013.
60. Cauvain SP, Young LS. Technology of bread making. 2007.
78. Cauvain SP, Young LS. Technology of bread making. 2007;52:105-111.


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