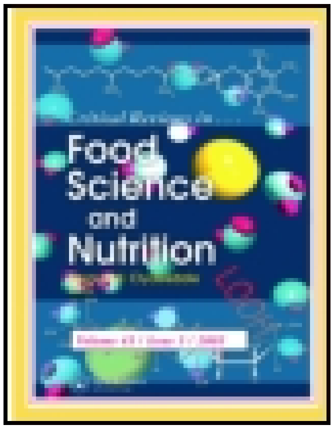

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

A review of the process-induced changes in the phytochemical content of cereal grains: The breadmaking process

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Accepted author version posted online: 02 Sep 2013.

A review of the process-induced changes in the phytochemical content of cereal grains: The breadmaking process

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Abstract

The importance of cereal grains in human nutrition is well documented. Especially the impact of whole grains consumption on human health has attracted much attention over the last decades. The health benefits are associated with the high content of cereal grains in dietary fibre but also recent research has shown that cereal grains are rich in bioactive compounds, which are unique in composition and different to phytochemicals from other sources such as fruit and vegetables. Cereal grains however are processed before human consumption. Depending on the process, the levels and the composition of the available bioactive compounds can be affected. Knowledge of the effect of these processes on the phytochemical compounds is essential to ensure the products generated are nutritious and can convey health benefits to the consumers. This article reviews the literature on the effect of milling and breadmaking processes on the levels and profile of bioactive compounds. Milling and breadmaking are two of the most common processes used universally to prepare the grains for consumption. These processes involve several stages and many variable factors, which can alter the levels and profile of the available phytochemicals in the end-product.

Introduction

The health benefits of consuming whole grains have been well documented, and are often associated with those health benefits conveyed by their dietary fibre content. However, in addition to fibre, whole grains are also rich in bioactive phytochemicals such as phenolic compounds, tocopherols, tocotrienols, carotenoids, plant sterols and lignans (Slavin et al., 2000). The benefits of phytochemicals from food sources in human health have been of major interest over the last years. Supporting evidence suggests that the consumption of food rich in phytochemical compounds plays a major role in the prevention of chronic diseases. The phytochemicals present in cereal grains differ in composition from the compounds found in fruit and vegetables and therefore whole grains are believed to contain unique phytochemical compounds (Liu, 2007).

Cereal grains are traditionally processed before human consumption. Most commonly, cereal grains are used for the production of flour, malt, beer, and a range of food products such as baked goods, breakfast cereals, extruded snacks, and many more. Grains undergo a range of processes that can alter their nutritional value. It is therefore important to assess the effect of these processes on the profile and levels of nutrients and phytochemicals. Breadmaking is the most common process during which cereal grains are used for the preparation of baked foodstuffs. Bread has always been a widely consumed staple food and recently there has been much focus on maximising its nutritional value. According to Lopez et al. (2001), ideal bread should have a low Glycaemic Index (GI), and be an essential source of proteins, dietary fibre, vitamins, trace elements and antioxidants. Breadmaking is a complex process that involves many physicochemical and structural changes which lead to the production of an aerated baked product from basic ingredients such as flour, water, yeast and salt. As cereal grains are known to be a rich source of phytochemicals, many researchers have explored the stability of these compounds during the baking process, as well as their bioavailability in the end-product.

In recent years, the cereal industry has focused on enhancing the nutritional value of bread and baked products. Functional ingredients, dietary fibre, antioxidants and bioactive compounds have been explored for their health promoting properties and for their effect on the quality of the end product. This article reviews the literature and evaluates the processes involved in breadmaking, and how each process affects the profile and levels of nutrients and phytochemicals present.

Phytochemical compounds in cereal milling fractions

Milling is the most common process to prepare the grains for human consumption. It is an essential step before the breadmaking process begins. The purpose of milling is to separate the starchy endosperm from the bran and the germ and reduce it to a desired granulation. High extraction rates in milling allow for higher percentage of the outer layers of the grain to be retained i.e 100 % extraction rate will result in whole grain flour. Studies have shown that decreasing the extraction rates leads to losses of dietary fibre and bioactive compounds.

Liukkonen et al. (2003) studied the effect of milling rye on the levels of bioactive compounds. They found that the bran fraction contained the highest level of bioactive compounds. The levels in the germ fraction were similar to those of the wholemeal flour, whereas the flour only contained traces of the bioactives. In particular, the outer bran fraction contained 3.3 to 4 times more alk(en)ylresorcinols and alkaline extractable total phenolics, and 1.6 to 2.1 times more sterols, folates, tocopherols and tocotrienols, lignans, phenolic acids, and easily-extractable total phenolics than the wholemeal rye flour. Similarly, Andreasen et al. (2000), showed that rye bran has a high content of phenolic acids and in particular, the levels of ferulic acid were 10 to 20 times higher in rye bran than in the endosperm.

Liyana-Pathirana et al. (2007) found the content of phenolics to be higher in the bran fraction for the two species of wheat and durum wheat examined. The predominant phenolic acids in all fractions were vanilic acid, p-coumaric, ferulic and sinapic acid. The bran fraction also exhibited the highest antioxidant activity as measured by ORAC method, and it was found to be 6.8 times higher than that of the flour fractions. Adom et al. (2005) examined the phytochemicals and antioxidant activity of milled fractions of different wheat varieties, and found that the total phenolic content, the flavonoid content and the content of ferulic acid were higher in the bran/germ fraction of all wheat varieties than in the endosperm. Previous work from Adom et al. (2003) has shown that bound phenolics contribute the most to the total phenolic content as well as to the antioxidant capacity of wheat varieties. Most bound phenolics are attached to the cell wall materials of the outer parts of the grain and therefore the bran/germ fraction exhibits higher total phenolic content. Van Hung et al. (2008) showed how the phenolic compounds in buckwheat exist mainly in free form, and are present in higher concentrations in the outer part of the grain. The antioxidant activity increased from the inner to the outer parts of the grain and it was higher for free phenolics than bound phenolics. Flavonoid compounds were also found in higher concentration in the outer parts of the grain, which are most likely removed during milling. Rutin was the most abundant flavonoid and its concentration was the highest in the outer part of the grain (2.5 μg/g of flour in the innermost fraction compared to 389.9 μg/g of flour in the outermost layer of the grain).

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Hidalgo and Brandolini (2008), studied the tocol distribution in two varieties of Einkorn seeds and a wheat variety. The authors found that the germ fraction contains the highest concentration of total tocols compared to the bran and the flour fractions. In particular, they showed that α- and β- tocopherols are found in high concentrations in the germ fraction of all the samples tested. The highest concentration of α-tocotrienol was found in the bran fraction (54.4 μg/g DM in Einkorn variety Monlis) but considerable amounts were also found in the germ fraction (25.1 μg/g DM in Einkorn variety Monlis). The bran fraction contained the highest concentration of β-tocotrienols (130 μg/g DM in Einkorn variety Monlis) but significant amounts were also found in the flour fraction (21 μg/g DM in Einkorn variety Monlis) and the germ fraction (31.7 μg/g DM in Einkorn variety Monlis). The tocol content of various cereals and the effect of processing were recently reviewed by Tiwari and Cummins (2009). They demonstrated that together with environmental and agronomic factors, processing will also affect the level of tocols in cereals. The authors also concluded that wholegrain flours contain higher quantities of tocols than refined flours due to the contribution of the bran and germ fractions.

Milling also affects the antioxidant capacity of the bioactive compounds in rice. Finocchiaro et al. (2007) found that milling reduces the antioxidant compounds and therefore the total antioxidant capacity for both red and white rice. Depending on the degree of milling (low to high), the reduction observed was 70% and 87% for the red rice and 52% and 51% for the white rice. Milling reduced the level of tocols, as they are concentrated in outer parts of the grain, and also resulted in a decrease in γ-oryzanols, and ferulic and sinapic acids.

The carotenoid content of cereals has gained much research interest lately, due to their proposed health benefits. Carotenoids serve as antioxidants, and are believed to protect against age-related macular degeneration (Siebenhandl et al., 2007; Leenhardt et al., 2006). Conflicting results have been reported in the literature regarding the distribution of carotenoids in the grain. Adom et al. (2005) and Abdel-Aal et al. (2002) found that the carotenoid content in the bran/germ of different wheat varieties was higher than that of the endosperm. In contrast to this, Leenhardt et al. (2006) and previously Pinzino et al. (1999) have shown that the carotenoid content was evenly distributed among the fractions of wheat. In addition, Leenhardt et al. (2006) found that carotenoid losses are linked to lipoxygenase activity and that the bran/germ fractions exhibit the highest lipoxygenase activity. Siebenhandl et al. (2007) examined the carotenoid content and their distribution in milled fractions of wheat and barley varieties. They described how carotenoids were evenly distributed among the fractions of wheat varieties, and although the carotenoid content of barley was higher in the shorts fraction, 51% was recovered in the flour. They also reported differences in the carotenoid distribution among varieties of both wheat and barley. Adom et al. (2005) found that when wheat wholemeal flour was assessed, the lutein concentration was the same as that present in the endosperm even though the lutein content was higher in the bran/germ fraction. Siebenhandl et al. (2007) suggested that these differences in the literature could arise due to the fact that many factors such as the genetic background, growing conditions, milling procedure, and the solvent used for extraction can affect the analysis of the carotenoid compounds.

Wholegrain cereals represent distinct nutritional and health-related advantages compared to refined flours. It has been widely accepted that the consumption of wholegrain foods increases fibre intake, which is considered to have several health benefits. In addition to the increased fibre intake, the health promoting effects of the bioactive compounds present in cereals, as well as their antioxidant activity, have attracted much interest recently from the food industry. It has been well documented in the literature that the outer part of the grain of cereals contains more bioactive compounds and contributes the most to the total antioxidant capacity of the grain. Nevertheless, bran and germ are still considered by-products of milling, and despite their high content of health promoting phytochemicals, the incorporation of bran/germ and generally the outer part of the grains in the formulation of cereal foods, presents a challenge for the food industry and the research community, due to the adverse properties which may be present in the end products.

Breadmaking

Generally, the breadmaking process is divided into three main steps: mixing, fermentation (proofing) and baking. Mixing is responsible for blending the ingredients together, hydrating the flour components and developing the matrix that leads to the formation of a smooth and elastic dough, which is able to expand and retain the gas produced by the yeast during the subsequent processing. Air is also incorporated into the dough during mixing, in the form of air bubbles that serve as nuclei for gas cells that grow during fermentation and the early stages of baking (Bloksma, 1990). During proofing, gas produced by the yeast activity diffuses into the gas bubbles, and causes them to expand. Finally, during baking, the dough continues to expand, formation of crust and crumb takes place and the final change to a sponge structure, characteristic of bread, occurs. The Maillard reaction occurs soon after the setting of the crust begins. This results in the browning of the crust and the formation of various compounds that are known to contribute to the flavour of the baked products (Sluimer, 2005). These basic steps of the baking process can affect the stability of many bioactive compounds and their presence in the end-product.

Mixing

Mixing involves the incorporation of water and oxygen in the dough, a practice which can affect the phytochemicals present in the cereal flours. The incorporation of water activates oxidative enzymes present in flours which may in turn affect phytochemical compounds such as phenols, carotenoids, tocols etc. Much attention has been drawn to lipoxygenase enzymes present in wheat flour. As previously mentioned by Maraschin et al. (2008), wheat lipoxygenase (LOX) uses the oxygen provided during mixing for the oxidation of linoleic and linolenic acids, either in the free or monoglyceride forms (Ameille et al., 2000; Graveland, 1970). Lipoxygenase enzymes become active with the incorporation of water and oxygen, and facilitate the lipoxygenase-catalyzed oxidation of fatty acids which can lead to oxidation of carotenoid pigments (Hidalgo and Brandolini, 2010; Leenhardt et al., 2006). Nicolas (1978) has shown that adding lipoxygenase to flour increased the rate of carotenoid bleaching during mixing, whereas Drapron et al. (1971) showed that α-tocopherol was destroyed in the presence of lipoxygenase after mixing.

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More recently Leenhardt et al. (2006) and Hidalgo et al. (2010) examined the effect of breadmaking on carotenoids. Leenhardt et al. (2006) found that the carotenoid content decreased after mixing during the breadmaking process of French bread using whole grain durum, einkorn, wholegrain wheat and wheat flour (white flour). The greatest loss (66%) of carotenoids was observed in the whole grain wheat dough; in the whole grain einkorn losses of approximately 7% occurred. The authors highlighted a strong correlation between carotenoid losses and lipoxygenase activity ($r^2 = 0.97$). Similarly Hidalgo et al. (2010) observed carotenoid losses after kneading, during bread, biscuit and pasta preparation. They used einkorn, wheat, and durum wheat (for pasta preparation only). The carotenoid losses were higher for wheat and durum wheat than the losses observed for einkorn. These species have higher content of linoleic acid (Hidalgo et al., 2009) which is a preferential lipoxygenase substrate and could be an additional reason for the higher carotenoid losses observed.

In addition to carotenoids, changes in tocopherol and tocotrienol levels have also been examined during the same processes as above for bread, biscuit and pasta preparation (Hidalgo and Brandolini, 2010). It was found that tocol content decreased during mixing for all products (21.4%, 28.2%, and 44.2% for bread, biscuit and pasta respectively). Smaller losses in vitamin E activity were observed after mixing in a study contacted by Leenhardt et al. (2006). The authors suggested that vitamin E is protected from lipoxygenase activity by carotenoid oxidation as carotenoids are better electron donors.

Angioloni and Collar (2010) and Hansen et al. (2002) both observed a reduction in phenol content due to oxidation in cereals and pseudocereals after mixing. Angioloni et al. (2010) assessed the polyphenol composition of multigrain breads which were prepared by replacing wheat flour with minor cereal and pseudocereals. They found that the phenol content was higher in the flour than the respective breads and pointed out how enzymes such oxygenase and peroxidase (which are present in flour) are activated by the addition of water during mixing, and possibly promote the decrease of phenol content. However Hansen et al. (2002) only observed a significant decrease in ferulic acid in rye wholemeal after mixing (from 1079 to 1022 µg/g); the rest of the phenolic acids remained unchanged.

Vogrincic et al. (2010) monitored the polyphenol content, and particularly the flavonoids rutin and quercetin through the process of baking breads containing tartary buckwheat. They found that rutin decreased through the whole breadmaking process (mixing and proofing) and in most samples, was not detectable after baking. Buckwheat contains rutin degrading enzymes and the authors discussed how rutin was degraded with the addition of water and yeast and approximately 85% was transformed to quercetin.

Fermentation

In bread production, mixing is usually followed by a period of rest during which the dough is left to rise and reach an optimum condition for baking. Bread dough fermentation is used to produce leavened dough through the action of a leavening agent (usually baker's yeast, also called *Saccharomyces cerevisiae*), which converts the fermentable sugars present in the dough into ethanol and CO_2. During this stage, the CO_2 produced by the yeast is diffused into the gas cells of the dough which were previously incorporated during the mixing stage. Yeast respiration consumes the oxygen present in the dough and within a few minutes after the end of mixing, all oxygen is consumed (Sluimer, 2005).

Due to the consumption of oxygen by yeast, further oxidation due to oxidative enzymes is prevented and therefore changes in the levels of phytochemical compounds are minimal during this stage (Leenhardt et al., 2006; Hidalgo et al., 2010; Hansen et al., 2002; Vogrincic et al., 2010; Hidalgo and Brandolini, 2010). Moore et al. (2009) have studied the effect of proofing and baking on the antioxidant properties of pizza dough made with whole wheat flour. They found that fermentation times of 18 and 48 hours at 4^{o}C significantly increased the soluble free ferulic acid content for the whole wheat varieties used in the trial (Trego and Lakin hard winter wheat varieties), whereas there were no significant changes in soluble conjugated ferulic acid. On the other hand, the insoluble bound ferulic acid decreased significantly (by 61%) after 48 hours of fermentation for the Lakin variety whereas there was no change for those baked with Trego. The authors attributed these differences to varietal variation in the enzymatic hydrolysis of the insoluble or the conjugated ferulic acid by enzymes produced from yeast or other micro-organisms and enzymes present in the doughs. The antioxidant capacity, however, remained unaffected for both varieties when measured with the ORAC assay, the $ABTS^{\bullet+}$ Scavenging Capacity and $DPPH^{\bullet}$ Scavenging Capacity.

Another practice traditionally used in breadmaking is sourdough fermentation. In a sourdough starter, a lactic acid bacteria exists in symbiotic combination with yeasts, resulting in the production of lactic and acetic acids during fermentation. This in turn results in the modification of many important quality and nutritional parameters.

Sourdough fermentation is a common practice especially in rye bread making (Poutanen et al., 2009). The use of a sourdough starter has been shown to improve the volume, texture and shelf-life of breads (Katina et al., 2006) and affect the levels of several bioactive compounds such as phytate, folates, and tocopherols (Liukkonen et al., 2003; Michalska et al., 2007; Hansen et al., 2002; Katina et al., 2007).

Kariluoto et al. (2004) found that folate content increased during sourdough fermentation of rye and wheat and that this was associated with the inclusion and growth of yeast. Yeast has a high content of folate, but also the ability to synthesize more during fermentation, and this compensated for folate losses during baking. Furthermore, Kariluoto et al. (2006) documented how the ability of yeast to synthesize folate can increase the content in rye sourdough by up to three-fold but the effect of sourdough bacteria was negligible.

Liukkonen et al. (2003) examined the effect of sourdough fermentation on several bioactive compounds such as sterols, folates, tocopherols and tocotrienols, alk(en)ylresorcinols, phenolic acids and lignans. The authors found that sourdough fermentation increased the content of folates and methanol extracted phenolic compounds, whereas the content of tocopherols and tocotrienols was reduced. There was no significant effect observed on the other bioactive compounds tested. An increase in the antioxidant activity was also observed during fermentation, most likely due to the increase of methanol extracted phenolic compounds. Similar results were obtained by Katina et al. (2007). They found that in general yeast fermentation or fermentation with yeast and lactic acid bacteria increased the levels of folates, sterols, lignans, free ferulic acids, and preserved the levels of alk(en)yl-resorcinols, of both native and germinated rye. However, the increase was more pronounced when fermentation and germination were combined. This phenomenon was

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attributed to the optimum conditions possibly created by germination. In particular, the high level of folates in germinated rye is possibly due to intensified yeast fermentation as a result of the higher content of nutrients (such as sugars and nitrogen sources) present in germinated rye kernels. In addition, the high levels of readily extractable phenolic compounds and free phenolic acids in germinated rye probably result from increased activity of cell wall degrading enzymes such as xylanases and cellulases.

Baking

Many authors have studied the effect of baking on bioactive compounds and antioxidant activity of breads from various cereals. Baking time and temperature varies for different bread making processes, and the major effect observed is the degradation of many of the heat and light sensitive bioactive compounds present in the system. This influences the levels of phytochemicals present in the final baked product. Another characteristic of baking is the production of Maillard reaction compounds which are shown to increase the antioxidant capacity of the finished baked product, in particular the antioxidant activity of compounds present in the crust as compared to bread crumb (Lindenmeier and Hofmann, 2004; Vogrincic et al., 2010; Vitali et al., 2009).

Moore et al. (2009) and Hansen et al. (2002) studied the effect of baking on phenolic acids in whole wheat pizza dough and wholemeal rye bread respectively. Both studies reported that the levels of total phenolic acids remained unaffected following the effect of baking. Moore et al. (2009) in particular reported an increase in the level of extractable free ferulic acid in one of the

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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 of baking has the potential to increase antioxidant activity of the final product. Another study by Vitali et al. (2009) investigated the effect of apple fibre, oat fibre, soya flour, amaranth, and carob on the nutritional characteristics of biscuits. The authors showed that the biscuit baking process did not affect total phenol content as calculated from the sum of soluble and insoluble phenols. However, a significant increase was found in the levels of the soluble phenols and a considerable decrease in insoluble phenols in all samples following baking. This observation was attributed to the high temperatures during baking which could destroy some of the complexes of insoluble phenols with other food components. The same authors also examined the bioavailability of the phenols and found that this increased significantly after baking for all samples, apart from the sample containing oat fibre. Increased antioxidant activity was also observed after baking and this was most likely due the increase of the soluble phenols, as well as the production of Maillard reaction compounds.

Gelinas and McKinnon (2006) found baking to significantly increase the concentration of phenolic compounds in white wheat breads but this was not the case for wholemeal breads where the concentration remained unchanged. The authors suggested that the increase observed was due to Mailard reaction products and it was more pronounced in white wheat breads where the concentration of phenolics was lower than that in wholemeal breads.

Vogrincic et al. (2010) found that polyphenol content decreased as a result of the heat treatment during baking breads containing tartary buckwheat. As it was discussed earlier, the authors showed that rutin was degraded during mixing and approximately 85% was transformed to quercetin. Quercetin was more stable during baking and there was no difference detected between crust and crumb for both quercetin and rutin.

The stability of phenolic acids and flavonoid compounds in amaranth, quinoa and buckwheat grains during the bread making process has been evaluated by Alvarez-Jubete et al. (2010). Polyphenol content was generally found to be reduced in the bread samples when compared to the grains. The phenolic acids content in quinoa and buckwheat decreased significantly from grain to bread. Furthermore, the contents of flavonoid compounds such as quercetin and kaempferol glycosides in 100% quinoa breads was 17.1 and 19.2 μmol/100 g, compared with 43.4 and 36.7 μmol/100 g in quinoa seeds. In the case of buckwheat, quercetin glycosides content decreased significantly with bread making, resulting in an increase in quercetin content through hydrolysis.

The effect of baking on carotenoid levels has been studied by Leenhardt et al. (2006) and Hidalgo et al. (2010). Both studies reported carotenoid losses after baking. Leenhardt et al. (2006) reported losses ranging from 35% to 45% depending on the wheat species used. Hidalgo et al. (2010) reported losses in the bread crust of einkorn and wheat of approximately 29%, whereas smaller losses were noted for the bread crumb (0% and 5% for einkorn and wheat respectively) and for water biscuits (5% and 25% for einkorn and wheat respectively). Carotenoids are known to be susceptible to heat (Mercadante, 2007). Therefore, these results highlight the effect of baking temperature and time, as the highest losses occurred in the crust which experiences higher temperatures than the crumb or the biscuits. The authors also reported changes in the profile of the carotenoids during baking. β-cryptoxanthin levels increased after baking in bread crust and water biscuits whereas the levels of lutein, zeaxanthin and α- and βcarotene decreased. It was suggested that these changes could be due to isomerisation and hydroxylation of carotenes at high temperatures.

Using the same flours and processes as the above study, Hidalgo and Brandolini, (2010) investigated the effect of baking on tocols. They found that baking did not affect total content of tocol compounds significantly for breads or water biscuits containing either einkorn or wheat, and no differences were detected between crumb and crust. Wennermark and Jagerstad (1992), studied the effect of breadmaking on vitamin E. They reported losses between 24-47% in white breadmaking and 10-15% in wheat/rye baking during the baking stage. They also found that major losses occur during doughmaking (mixing stage) and baking losses occur mainly due to changes in the extractability of vitamin E . When the saponification method was used the vitamin E yield was higher than the yield when the Soxhlet method was used. The same was not observed for the vitamin E yield after doughmaking. The authors hypothesised that saponification could break up complexes formed during baking between vitamin E and maybe starch or protein.

The fate of tocols in gluten-free baking has also been investigated recently by Alvarez-Jubete et al. (2009). In particular, the authors examined the tocopherol content of amaranth, quinoa and buckwheat as affected by baking. It was found that vitamin E losses for gluten-free control (no pseudocereals), amaranth (50%), and buckwheat (50%) breads were ≈30%, whereas in quinoa (50%) bread losses were 13.6%. Lower losses of vitamin E were observed when 100% pseudobreads (buckwheat and quinoa) were studied compared to the respective 50% counterparts. Lowest losses amongst all of the breads studied were recorded for the 100% quinoa bread (7.5%). Also, a significant negative correlation (R^2 = –0.84) between the initial vitamin E content of breads and the degree of loss was found, thus indicating that higher initial content of vitamin E results in lower degree of loss (%). Significant negative correlations were also observed between the degree of vitamin E loss (%) and α-, and γ-tocopherol initial content (R^2 = –0.79 and –0.84, respectively), whereas no correlations were obtained for the other tocol compounds. According to the authors, the observed differences in vitamin E recovery could be partly explained on the basis of differences in the initial α-, and γ-tocopherol content, thus highlighting the importance of γ-tocopherol activity as an antioxidant in addition to that of α-tocopherol. Another likely factor responsible for some of the variation observed in the degree of loss among the different grain samples may be related to the presence of compounds with antioxidant capacity other than tocols such as flavonoid compounds (Alvarez-Jubete et al., 2009). Flavonoid compounds spare liposoluble antioxidants such as vitamin E from oxidation when present in the same sample matrix (Scalbert et al., 2005).

Other bioactive compounds such as folates have been found to be affected by baking. Kariluoto et al. (2004) reported an approximate 25% decrease in folate content after baking rye and wheat breads. These changes were accompanied by folate vitamer redistribution. Liukkonen et al. (2003) also found losses in the folate content as well as in the content of tocopherols and tocotrienols in whole-grain rye breads (however they were reportedly low). In the same study, the levels of sterols, lignans, and alk(en)ylresorcinols remained the same after baking.

Storage

The end of baking and subsequent cooling of baked products is followed by the process of staling. This consists of changes that take place simultaneously, and affect sensory attributes such as taste and texture of the baked product. The shelf-life of bread is limited by bread firming, microbial growth and the development of flavours, which are often described as ‘rancid’, ‘acidic’, and ‘off’ (Jensen et al., 2011). Auto-oxidation or lipid peroxidation of food products is known to deteriorate the product and affect its stability during storage, and is associated with the taste attributes described above (Jensen et al., 2011; Nanditha and Prabhasankar, 2009). Antioxidant compounds present in baked products are known to possess antimicrobial activities as well as antioxidative properties (Nanditha and Prabhasankar, 2009). Information on the stability of phytochemicals during the storage period of baked products is limited.

Abdel-Aal et al. (2010) monitored the stability of lutein in pan breads, flat breads (no proofing involved), cookies, and muffins, made from einkorn, khorosan, durum, durum/corn, commercial pastry flour and commercial whole wheat. They observed that storage for 5 days decreased lutein levels in pan breads, and the degree of degradation was found to be dependant on the base flour. On the other hand, storage did not significantly affect flat breads, cookies or muffins.

Jensen et al. (2011) studied the oxidative stability of whole wheat breads during storage, and the changes which took place due to oxidation. Oxidation products such as lipid hydroperoxides were higher in the crumb than the crust of stored breads, and increased after storage of 2-3 weeks. A decrease was then observed in the following 4-5 weeks, possibly as a result of primary oxidation products decomposing into secondary oxidation products. The content of tocopherols and the antioxidant capacity were also determined and used as indicators of the changes induced due to oxidation. The antioxidant activity, measured using the TEAC and ORAC assays was significantly higher in bread crust compared to bread crumb or dough possibly due to the formation of Maillard reaction compounds and decreased significantly during storage. On the other hand the antioxidant activity of the bread crumb remained stable during the same storage period. The content of tocopherols remained stable during storage which indicates that other compounds such as phenolic compounds, are most likely involved in the oxidative stability of baked products, rather than tocopherols.

Main outcomes and recommendations

Table 1 summarises the main phytochemical compounds that are affected during milling and the breadmaking process as they have been reported in the literature.

The review on the effect of breadmaking processes on phytochemical substances in cereal grains indicated that the greatest losses of significant bioactive compounds occur during kneading. The losses are mainly attributed to oxidation facilitated by the incorporation of oxygen and water, and by the activation of oxidative enzymes (such as lipoxygenase).

Heat induced losses were also significant, particularly as a result of the known susceptibility of many compounds to heat. However, the antioxidant capacity of baked products seemed to increase after baking in some cases, possibly due to the formation of Maillard reaction compounds.

However significant the impact of breadmaking on the phytochemical compounds, bread and baked products still present an important medium to deliver a variety of compounds (e.g. fibre, minerals, vitamins, and bioactive compounds such as tocopherols, carotenoids, polyphenols and phytosterols), with the potential to beneficially affect human health. In most of the studies reviewed, it was shown that in spite of the losses that may occur during breadmaking, substantial amounts of phytochemicals remained in the end product. This could have a positive impact by contributing towards increasing the nutritional value of the product. To achieve the development of baked products of high nutritional content various different factors must be considered. The use of flours which are naturally high in bioactive compounds is a starting point that can be achieved as well as flours of higher extraction rate. A breadmaking process which is suitable for the end product, but also with a view to limit oxidation should be adapted. This can be achieved by limiting the mixing intensity and time, and by increasing fermentation time. Both baking temperature and time will have a major impact on the level of bioactive compounds present and optimising these conditions can aid in minimising the losses induced due to heat. The selection of the correct or most appropriate additives to potentially decrease the susceptibility of vital compounds to oxidation is also important and should be taken into consideration.

Acknowledgements

The authors would like to acknowledge the support of the Irish Department of Agriculture, Food and the Marine under the Food Institutional Research Measure (FIRM).

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References

Abdel-Aal, E. S. M., Young, J. C., Wood, P. J., Rabalski, I., Hucl, P., Falk, D. and Frégeau-Reid, J. (2002). Einkorn: A Potential Candidate for Developing High Lutein Wheat. *Cereal Chem.* **79**:455-457.

Abdel-Aal, E. S. M., Young, J. C., Akhtar, H. and Rabalski, I. (2010). Stability of Lutein in Wholegrain Bakery Products Naturally High in Lutein or Fortified with Free Lutein. *J. Agric. Food Chem.* **58**:10109-10117.

Adom, K. K., Sorrells, M. E. and Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. *J. Agric. Food Chem.* **51**:7825-7834.

Adom, K. K., Sorrells, M. E. and Liu, R. H. (2005). Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *J. Agric. Food Chem.* **53**:2297-2306.

Alvarez-Jubete, L., Holse, M., Hansen, A., Arendt, E. K. and Gallagher, E. (2009). Impact of baking on the vitamin E content of the pseudocereals amaranth, quinoa and buckwheat. *Cereal Chem.* **86**:511-515.

Alvarez-Jubete, L., Wijngaard, H., Arendt, E. K. and Gallagher, E. (2010). Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chem.* **119**:770-778.

Ameille, V., Davidou, S., Drapron, R., Potus, J. and Nicolas, J. (2000). Continuous measurement of oxygen consumption during mixing of unyeasted wheat flour dough. *Sci. Alim.* **20**:221-236.

Andreasen, M. F., Christensen, L. P., Meyer, A. S. and Hansen, A. (2000). Content of phenolic acids and ferulic acid dehydrodimers in 17 rye (Secale cereale L.) varieties. *J. Agric. Food Chem.* **48**:2837–2842.

Angioloni, A. and Collar, C. (2010). Polyphenol composition and "in vitro" antiradical activity of single and multigrain breads. *J. Cereal Sci.* **53**:90-96.

Bloksma, A. H. (1990). Dough structure, dough rheology, and baking quality. *Cereal Food. World,* **35***:* 237-244.

Drapron, R., Beauc, Y., Cormier, M. and Geffroy, J. (1971). Enzymic oxidation of tocopherols and carotenoid pigments during breadmaking. *C. R. Acad. Agric.Fr.* **57**:2445-251.

Finocchiaro, F., Ferrari, B., Gianinetti, A., Dall'Asta, C., Galaverna, G., Scazzina, F. and Pellegrini, N. (2007). Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Mol. Nutr. Food Res.* **51**:1006-1019.

Gelinas, P. and McKinnon, C. M. (2006). Effect of wheat variety, farming site, and bread-baking on total phenolics. *Int. J. Food Sci. Technol.* **41**:329-332.

Graveland, A. (1970). Enzymatic oxidations of linoleic acid and glycerol-1-monolinoleate in doughs and flour-water suspensions. *J. Am. Oil Chem. Soc.* **47**:352-361.

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. *Eur. Food Res. Technol.* **214**:33-42.

 ACCEPTED MANUSCRIPT

Hidalgo, A. and Brandolini, A. (2008). Protein, ash, lutein and tocols distribution in einkorn (Triticum monococcum L. subsp monococcum) seed fractions. *Food Chem.* **107**:444-448.

Hidalgo, A. and Brandolini, A. (2010). Tocols stability during bread, water biscuit and pasta processing from wheat flours. *J. Cereal Sci.* **52**:254-259.

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

Hidalgo, A., Brandolini, A. and Ratti, S. (2009). Influence of Genetic and Environmental Factors on Selected Nutritional Traits of Triticum monococcum. *J. Agric. Food Chem.* **57**:6342-6348.

Jensen, S., Oestdal, H., Clausen, M. R., Andersen, M. L. and Skibsted, L. H. (2011). Oxidative stability of whole wheat bread during storage. *LWT - Food Sci. Technol.* **44**:637-642.

Kariluoto, S., Liukkonen, K.-H., Myllymaki, O., Vahteristo, L., Kaukovirta-Norja, A. and Piironen, V. (2006). Effect of germination and thermal treatments on folates in rye. *J. Agric. Food Chem.* **54**:9522-9528.

Kariluoto, S., Vahteristo, L., Salovaara, H., Katina, K., Liukkonen, K. H. and Piironen, V. (2004). Effect of baking method and fermentation on folate content of rye and wheat breads. *Cereal Chem.* **81**:134-139.

Katina, K., Liukkonen, K. H., Kaukovirta-Norja, A., Adlercreutz, H., Heinonen, S. M., Lampi, A. M., Pihlava, J. M. and Poutanen, K. (2007). Fermentation-induced changes in the nutritional value of native or germinated rye. *J. Cereal Sci.* **46**:348-355.

Katina, K., Salmenkallio-Marttila, M., Partanen, R., Forssell, P. and Autio, K. (2006). Effects of sourdough and enzymes on staling of high-fibre wheat bread. *LWT - Food Sci. Technol.* **39**:479-491.

Leenhardt, F., Lyan, B., Rock, E., Boussard, A., Potus, J., Chanliaud, E. and Remesy, C. (2006). Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *J. Agric. Food Chem.* **54**:1710-1715.

Lindenmeier, M. and Hofmann, T. (2004). Influence of baking conditions and precursor supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. *J. Agric. Food Chem.* **52**:350-354.

Liu, R. H. (2007). Whole grain phytochemicals and health. *J. Cereal Sci.* **46**: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., Hietaniemi, V. and Poutanen, K. (2003). Process-induced changes on bioactive compounds in whole grain rye. *Proc. Nutr. Soc.* **62**:117-122.

Liyana-Pathirana, C. M. and Shahidi, F. (2007). The antioxidant potential of milling fractions from breadwheat and durum. *Journal of Cereal Science* **45**:238-247.

Lopez, H. W., Adam, A., Leenhardt, F., Scalbert, A. and Remesy, C. (2001). Control of the nutritional value of bread. *Ind. Cereales* **124**:15-20.

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

Mercadante, A. Z. (2007). Carotenoids in foods: sources and stability during processing and storage. Pp. 213-240. **In**: Food colorants chemical and functional properties Socaciu, C., Eds. CRC Press (Taylor and Francis Group), FL, USA.

Michalska, A., Ceglinska, A., Amarowicz, R., Piskula, M. K., Szawara-Nowak, D. and Zielinski, H. (2007). Antioxidant contents and antioxidative properties of traditional rye breads. *J. Agric. Food Chem.* **55**:734-740.

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

Nanditha, B. and Prabhasankar, P. (2009). Antioxidants in Bakery Products: A Review. *Crit. Rev. Food Sci. Nutr.* **49**:1-27.

Nicolas, J. (1978). Effets de différents paramètres sur la destruction des pigments caroténoïdes de la farine de blé tendre au cours du pétrissage. *Ann. Technol. Agric.* **27**:695-713.

Pinzino, C., Nanni, B. and Zandomeneghi, M. (1999). Aging, free radicals, and antioxidants in wheat seeds. *J. Agric. Food Chem.* **47**:1333-1339.

Poutanen, K., Flander, L. and Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiol.* **26**:693-699.

Scalbert, A., Manach, C., Morand, C., Remesy, C., & Jimenez, L. (2005). Dietary Polyphenols and the Prevention of Diseases. *Crit. Rev. Food Sci. Nutr.* **45:** 287 –306.

Siebenhandl, S., Grausgruber, H., Pellegrini, N., Del Rio, D., Fogliano, V., Pernice, R. and Berghofer, E. (2007). Phytochemical profile of main antioxidants in different fractions of purple and blue wheat, and black barley. *J. Agric. Food Chem.* **55**:8541-8547.

Slavin, J. L., Jacobs, D. and Marquart, L. (2000). Grain processing and nutrition. *Crit. Rev. Food Sci. Nutr.* **40**:309-326.

Sluimer, P. (2005). Principles of breadmaking: functionality of raw materials and process steps. American Association of Cereal Chemists, St. Paul, Minessota.

Tiwari, U. and Cummins, E. (2009). Nutritional importance and effect of processing on tocols in cereals. *Trends Food Sci. Technol.* **20**:511-520.

Van Hung, P. and Morita, N. (2008). Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacities. *Food Chem.* **109**:325-331.

Vitali, D., Dragojevic, I. V. and Sebecic, B. (2009). Effects of incorporation of integral raw materials and dietary fibre on the selected nutritional and functional properties of biscuits. *Food Chem.* **114**:1462-1469.

Vogrincic, M., Timoracka, M., Melichacova, S., Vollmannova, A. and Kreft, I. (2010). Degradation of Rutin and Polyphenols during the Preparation of Tartary Buckwheat Bread. *J. Agric. Food Chem.* **58**:4883-4887.

Wennermark, B. and Jagerstad, M. (1992). Breadmaking and storage of various wheat fractions affect vitamin E. *J. Food Sci.* **57**:1205-1209.

Table 1 The effect of milling and breadmaking on phytochemical compounds in grains

Process	Compounds present	Cereal used	Product	Effect on original compounds	Reference
Milling	Phenolic acids	Rye Wheat Durum wheat Buckwheat Rice	Flours of varying extraction rates	Decrease	Adom et al., 2005 Andreasen et al., 2000 Liukkonen et al., 2003 Liyana-Pathirana and Shahidi, 2007
	Flavonoids	Rice		Decrease	Finocchiaro et al., 2007
	Tocols	Rye Rice		Decrease	Finocchiaro et al., 2007 Liukkonen et al., 2003
	Carotenoids	Wheat Barley		Decrease or no effect	Abdel-Aal et al., 2002 Adom et al., 2005 Leenhardt et al., 2006 Pinzino et al., 1999 Siebenhandl et al., 2007
	Sterols	Rye		Decrease	Liukkonen et al., 2003
Mixing/Kneading	Phenolic acids	Rye Pseudocereals	Bread	Decrease	Angioloni and Collar, 2010 Hansen et al., 2002
	Tocols	Wheat species	Bread Pasta Biscuits	Decrease	Hidalgo and Brandolini, 2010 Leenhardt et al., 2006
	Carotenoids	Wheat species	Bread Pasta Biscuits	Decrease	Hidalgo et al., 2010 Hidalgo and Brandolini, 2010 Leenhardt et al., 2006
Fermentation	Phenolic acids	Wheat Rye	Pizza dough Bread	Increase free phenolics	Moore et al., 2009 Liukkonen et al., 2003
	Carotenoids	Wheat	Bread	Slight or no effect	Leenhardt et al., 2006 Hidalgo et al., 2010
	Folates	Rye	Bread	Increase	Kariluoto et al., 2004 Kariluoto et al., 2006
	Tocols	Rye Wheat	Bread	Decrease No effect	Katina et al., 2007 Liukkonen et al., 2003 Hidalgo and Brandolini, 2010
	Lignans	Rye	Bread	Slight effect	Katina et al., 2007 Liukkonen et al., 2003

	Phenolic acids	Wheat Rye Fibres	Pizza Bread Biscuits	No effect or slight increase in free phenolics	Hansen et al., 2002 Moore et al., 2009 Vitali et al., 2009
		Pseudocereals	Bread	Decrease	Alvarez-Jubete et al., 2010
Baking	Carotenoids	Wheat species	Bread Pasta Biscuits	Decrease	Hidalgo et al., 2010 Leenhardt et al., 2006
	Tocols	Wheat species	Bread Pasta Biscuits	No effect	Hidalgo and Brandolini, 2010
		Pseudocereals	Breads	Decrease	Alvarez-Jubete et al., 2009
	Folate	Rye Wheat species	Breads	Decrease	Kariluoto et al., 2004
	Flavonoids	Pseudocereals	Bread	Decrease	Alvarez-Jubete et al., 2010 Vogrincic et al., 2010
Storage	Carotenoids	Wheat species	Bread	Decrease	Abdel-Aal et al., 2010 Jensen et al., 2011
	Tocols	Wheat	Bread	No effect	Jensen et al., 2011