The impact of processing on wheat grain components associated with health benefits

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Abstract

Wheat based foods, mainly in the form of bread and pasta, are staples in many countries around the world and as such contribute substantially to nutrient intake of human beings. The mature wheat grain is composed for over 70-75% of its dry weight of starch and for around 10-14 % of protein, which has led to the widely spread perception of wheat foods as providers mainly of energy and protein to the human diet. Nonetheless, whole grains are also an important source of dietary fibers, vitamins and minerals and contain notable levels of bioactive compounds of health benefit as for examples lignans, phenolic acids, alkylresorcinols, phytosterols, folates and tocols.

Processing is a pre-requisite for using cereal grains as food and obtaining a safe to consume and appealing final product for the consumer. It can help reducing potential hazardous molecules as pesticides, mycotoxins and heavy metals and allows to obtain products with varied and unique properties. Indeed, most of the importance of wheat grain in the human diet is due to its versatility to be processed into various end products like flour, semolina, and other bakery products. Processing of wheat grain involves different regulated steps each impacting either or both on the composition and physical-chemical properties of its different components, which in turn define the technological quality and the nutritional and health promoting properties of the end product.

While the unique textural properties of wheat foods are largely determined by starch and gluten proteins present in the starchy endosperm, and therefore associated with white flour or semolina, health effects of wheat-based products are mainly associated with their dietary fibers and bioactive compounds. These compounds are enriched in the grain peripheral layers, mainly in the aleurone layer which is generally driven in the bran fraction upon milling. Milling fractionation and the way the different milling streams are subsequently recombined has, therefore, a profound impact on the relative abundance of the different grain components in the wheat flour/semolina and, consequently, in the end products. Further processing steps, as dough making, microbial fermentation, extrusion, and baking can also have an impact on relative amount of grain components, and are also known to affect their bioavailability.

Some examples of the effect of grain processing procedures on bioavailability of important grain components in the wheat products will be presented in this chapter. Suggestions of how to improve these processes in light of their implication for human health will also be discussed.

Introduction

Accounting for one-third of total worldwide, grain production (FAOSTAT) wheat not only provides over 20 % of the daily protein and of the food calories for a large portion of the world population but it is also an important source of micronutrients, dietary fibre and other compounds promoting better health. All wheat grains consumed are subjected to some type of processing aimed at increasing their palatability, digestibility, and safeness for consumption, as well as providing shelf stable products. However, processing can also impact, positively or negatively, on the nutritive value and health benefits associated with the specific wheat product by determining changes in the relative content of bioactive components or their physical-chemical properties.

Since only a minority of wheat grain is consumed as wholemeal, primary transformation generally takes place in the form of milling aimed at isolating the starchy endosperm from the other tissues (Posner 2009). While wheat macronutrients, *i.e.* starch and storage proteins (gliadins and glutenins), are the main components of the starchy endosperm, from which white flour and semolina derives, micronutrients and bioactive components are mainly located in the embryo and outer layers of the grain (Evers et al. 1999; Hemery et al. 2007). Therefore, the nutritional and health promoting potential of wheat flour or semolina are inversely associated with their degree of refinement which is determined through milling and assembling of the different milling fractions. Nevertheless, milling generally impacts positively on the product safety by removal of potentially toxic compounds as pesticides, mycotoxins and heavy metals.

Secondary processing, where flour or semolina are mixed to prepare a dough which is further processed according to the specific requirements of the different end products (bread, pasta, biscuits, breakfast cereals or snacks…), also impacts on the nutritional value of wheat-based foods. The extent of the impact will depend both on the treatments applied and on the addition to the dough of compounds which may interfere on the stability and release of wheat grain components from the formulated matrix.

Preparation of leavened products, for example, requires microbial fermentation of the dough, while pasta preparation steps include extrusion and drying of the extruded product and its subsequent cooking by boiling before consumption. Commercial cereals and snacks may be extruded, puffed, or flaked to improve product quality.

These last years have seen a number of studies linking the consumption of products made from whole grains to better health outcomes (Cooper et al. 2017; Kristensen et al. 2012; Nelson et al. 2016;). However, in many of the studies the whole grain products are poorly described in terms of their composition and although a definition of whole grain was recently proposed by the American

Association of Cereal Chemists (AACC) but still appears under discussion (http://www.aaccnet.org/initiatives/definitions/Pages/WholeGrain.aspx).

In this review, we try to inventory what is known about the effect of the different processing procedures on the amount and properties of wheat grain compounds which have been associated with health benefits.

Alkylresorcinols (AR)

AR are phenolic lipids, more precisely 1,3-dihydroxy-5-n-alkylbenzenes with an odd number of carbon atoms comprised between 17 and 25, giving a mixture of AR homologues in specific proportions depending on the cereal, wheat being enriched in 19 and 21 carbon homologues (Ross et al. 2003).

AR were absorbed by the small intestine and distributed through the blood plasma or incorporated into the erythrocyte membrane, as recently reviewed in Landberg et al. (2014). A number of *in vitro* activities identified for AR such as antioxidant properties (Kozubek and Nienartowicz 1995) or inhibition against cancer cell growth (Zhu et al. 2012) or inhibition of glycerol-3 phosphate deshydrogenase activity, a key enzyme in the triacylglycerol synthesis in adipocytes (Rejman and Kozubek 2004) was reviewed in Kozubek and Tyman, (1999) and in Landberg et al. (2014) . However, identification of *in vivo* efficiency remains difficult as AR consumption in cereal products was associated with other potentially active molecules.

Total AR content in wheat grains varies depending on the species, cultivars and growing environment with contents between 54 and 1489 μg/g (d.m.) with a mean content around 500-700 μg/g (d.m.) (Andersson et al.2008, 2010a; Ross et al. 2003; Tluscik et al. 1981).

In grain, AR are only present at the frontier between the outer cuticule of the testa and the inner cuticule of the pericarp (Landberg et al. 2014, 2008). Therefore, their determination along fractionation (Hemery et al. 2009) helped to monitor fate of the tissues between the aleurone layer and the outer pericarp, which represent less than 4% of the grain mass (Barron et al. 2011, 2007).

Since during milling external tissues including the aleurone layer are separated from the starchy endosperm, AR become concentrated in the bran and shorts fractions (3 to 5 fold higher AR content than in grains) with only low amounts recovered in refined flours or semolina and thus in the corresponding final products produced from these (Ross et al. 2003). The AR content in flour can therefore be increased by adding shorts or bran fractions, possibly after further grinding, or by addition of fractions recovered from grain by pearling and equivalent to a cumulative weight between 5 and 10 % of the original grain mass (Bordiga et al. 2016). AR content remained stable through the overall transformation chain as good correlation were found between the amount in final products (bread or pasta) and the sum of the different ingredient amounts, if appropriate solvent able to release AR molecules complexed to proteins or starch was used for extraction (Andersson. 2010b; Chen et al. 2004,

Landberg et al. 2006, Ross et al. 2003). Therefore, ARs in plasma or their metabolites in urine are used as markers of whole grain products intake even if a same concentration of these compounds in products can result from a very diverse content in the different composing fractions. Depending on the particle size, amount and composition of these fractions containing peripheral tissues, storage, cooking, texture, color, sensory and safety properties have to be evaluated to define if acceptable for the consumers. For example, Blandino et al. (2013) demonstrated that refined flour can be replaced up to 10 % with a fraction from grain debranning (between 8 and 16 %) without modifying too drastically the bread technological properties and safety (mycotoxin DON level), allowing in this case to increase by a more than ten fold the level of alkylresorcinols in comparison with the control bread.

Benzoxazinoids (BX)

They are categorized into different groups based on their structural differences: benzoxazolinones, lactams and hydroxamic acids. The main compound in wheat grains appears to belong to the third group and corresponds to the glucoside form of a di-hexoses of 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA-glc-hexose, around 3 □g/g). BX are absorbed and metabolized in mammals and found to display health protecting properties including anticancer and anti-inflammatory properties and also satiety properties, as reviewed in Adhikari et al. (2015). However, potential aneugenic and mutagenic effects of the agluconic hydroxamic acids DIBOA and DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3) were also reported (Buchmann et al. 2007; Hashimoto and Shudo 1996). Like alkylresorcinol metabolites, benzoxazinoid derivatives were recognized as specific biomarkers in a metabolomic approach comparing urinary compounds after human consumption of either refined or whole grains (Zhu et al. 2016). They were found mainly concentrated in the milling fractions (Tanwir et al. 2013) enriched in the peripheral tissues and more specifically fractions containing germ, even if no other markers were used to clearly relate this fraction with the embryo or scutellum tissues. Only one fifth of DIBOA amount was therefore recovered into flour after milling. Pedersen et al. (2011) identified changes in the BX amount and in their relative proportion due to hydrothermal processing of grains or baking. These changes appeared to reflect the synthesis of hydroxamic acid glucosides, the enzymatic liberation of their aglucone forms and their subsequent degradation to benzoxazolinones.

Betaine-Choline

Wheat grains can be considered as a source of glycine betaine (or N,N,N-trimethyl glycine) and its precursor choline even if their concentration, respectively around 1000-2940 µg/g and 170-280 µg/g (d.m.), varied due to genotype, environmental conditions and interactions between these two factors (Corol et al. 2012). Graham et al. (2009) analyzed fractions from a single wheat cultivar and found a higher concentration of both betaine and choline (respectively around 15 mg/g and 2 mg/g of the dry mass) in an enriched aleurone fraction suggesting that these compounds were located into this specific tissue. Bran was shown to contain around 9 mg/g and 1 mg/g (d.m.) of betaine and choline respectively.

Therefore, refined wheat products obtained after milling necessarily contained less amounts of these compounds. Indeed, Graham et al. (2009) found only 0.2 and 0.3 mg/g (d.m.) of betaine and choline respectively into white flour. Betaine and choline are both water-soluble and thus 40-50 % of their content was lost by pasta cooking (Ross et al. 2014).

Keaveney et al. (2015) and Price et al. (2010, 2012) demonstrated a significant increase in the plasma betaine content after consumption of minimally processed bran, aleurone or aleurone enriched cereal products by healthy humans. Moreover the decrease of one of the inflammatory marker, the C-reactive protein in the plasma was attributed at least partly to the increase in betaine following aleurone consumption.

Betaine serves as an osmolyte protecting cells from osmotic stress and as a methyl donor in the human body reducing circulating homocysteine by its remethylation to methionine and preventing excess of hepatic fat deposits. Because elevated homocysteine was recognized as a biomarker of elevated vascular disease risk, increased consumption of betaine was proposed to help preventing cardiovascular diseases (Ross et al. 2014). However, recent data indicates that dietary intake and plasma concentrations of choline, but not betaine, are associated with higher risk of atrial fibrillation (Zuo et al. 2018).

Lignans

Identification of lignans in mammals and of their precursor in plants was first made in 1982 by Axelson and coauthors (Axelson et al. 1982). Lignans display a complex diphenolic structure which can be converted by intestinal microflora in the proximal colon of mammals into phytoestrogens (enterodiol and enterolactone) making them act as agonist or antagonist of the endogenous estrogen molecules (Aehle et al. 2011). Plant lignans have been implicated in a number of health effects although their mechanisms of action are not completely understood (Kiyama 2016; Landete. 2012). Recently, 7-hydroxymatairesinol (HMR) lignan and its isomer were found to display strong anti-inflammatory properties in human aortic endothelial cells (Spilioti et al.. 2014) putting some credit to their potential effect on atherosclerosis.

In grains lignans are present either in aglycone or glycoside form (Smeds et al. 2007) therefore their quantification strongly depends on the method of extraction and the sensibility of the detection method. Using less destructive method of extraction, HMR, was found as the major lignan compound in wheat grain, followed by syringaresinol (Smeds et al. 2007). Total lignan content in wheat samples collected in Finland in eight different locations showed variations between 3-23 µg/g (Smeds et al. 2009). Wheat bran displays the highest lignan content with a four to five fold higher content than in grains (Durazzo et al. 2009; Smeds et al. 2007), therefore wholemeal flour is richer in lignans than refined flours.

There are only a limited number of studies reporting on the stability of lignans upon processing, but they suggest that lignans, such as secoisolariciresinol diglucoside (SDG isolated from flaxseed, would be able to withstand the breadmaking process (Muir and Westcott 2000). A comparison study of

different lignan-containing cereal products showed that the effet of cooking depends on the different lignan profile, the chemical structure of each lignan and of the nature of the food matrix (Durazzo et al. 2013).

Tocols

Tocols included two types of amphipathic and lipo-soluble molecules, tocopherols and tocotrienols, which display a polar chromanol ring and a hydrophobic 16-carbon side chain corresponding respectively to a phytyl or an isoprenoid chain (Tiwari and Cummins 2009). This side chain is fully saturated in tocopherols whereas it presents three double bonds in tocotrienols. Differing in the number and position of methyl groups in the chromanol ring, four forms of these molecules can be distinguished α-, β-, γ-, and δ-. Tocols were found to act as antioxidants by scavenging lipid peroxyl radicals and quenching or reacting with singlet oxygen and other reactive oxygen and nitrogen species. *In vivo* vitamin E activity however seems to be related to the lipophilicity of the molecule which is affected by the number of methyl groups on the phenolic ring and the length and unsaturation in the carbon side chain playing a role in the transport and absorption by the human body (Kamal-Eldin and Appelqvist 1996). Consequently β-tocotrienol was found to display a vitamin E activity corresponding to 5 % of those of α-Tocopherol.

Total tocol amount in wheat grains varied between around 30-88 µg/g d.m, depending both on genotypes and environment, with β -tocotrienol followed by α -tocopherol as the main components (Lampi et al. 2008). Low significance of the genotype was on the contrary reported by Beleggia and coauthors (2013) for durum wheat. Wheat milling fractions enriched in germ were found to mainly contain α-tocopherol whereas β-tocotrienol was enriched in bran and flours (Piironen et al. 1986). Total tocol content was also found to decrease in the following order germ>bran>flour.

Tocols have low stability on exposure to light and temperature, which lead to losses along processing (Andersson et al. 2014; Tiwari and Cummins 2009). The level of vitamin E in milled wheat products depends mainly on the extraction rate of the flour (about 50% reduction from wholegrain to white flour). Presence of part of the germ in flour depending on the processing diagram can also influence the extent of vitamin E loss during storage (Nielsen and Hansen 2008). The total loss of vitamin E during storage was 24% for stone-milled wheat flour (which contain significant amount of germ) but 50% for roller-milled wheat flour (devoid of germ and bran) because it plays a role as an antioxidant. Vitamin E oxidation is also an important cause of losses during the further processing steps: Preparation of gruels and porridges with processes such as extrusion cooking and drum-drying destroyed a major portion of the vitamin E in white flour (Håkansson et al. 1987; Håkansson and Jägerstad 1990). The ratio of tocotrienols to tocopherols was reported to increase after extrusion cooking indicating that tocotrienols are the main residual isomers of vitamin E remaining (Zielinsky et al. 2001) and there is evidence that a higher ratio of tocotrienols to tocopherols in the diet may be important in metabolic regulation.

Subtantial losses of vitamin E activity have also been reported in breadmaking and were found to mainly occur at the stage of dough making (20 to 40 % loss of vitamin E), since mixing of flour with water facilitates lipid oxidation (Galliard 1989), and affects mainly α-tocopherol and α-tocotrienol isomers (Wennemark and Jagerstad 1992).

Losses of vitamin E have been also reported following baking particularly at the kneading step (Leenhardt et al. 2006) due to the incorporation of oxygen in the dough. Vitamin E losses result both by tocols own oxidation (Slover and Lehmann 1972), and as a secondary effect of fatty acid oxidation *ie*. lipoxygenase-catalyzed oxidation of tocols (Galliard 1989; Nicolas and Drapron 1983). The dough-making technique from Chorleywood (UK), which requires particularly high-speed mixing, emphasizes these effects Fermentation of the dough, on the contrary, does generally have only minor effect on vitamin E content since the oxygen is rapidly consumed by the baker's yeast. (Leenhardt et al. 2006; Wennemark and Jagerstad 1992).

During pasta making, the degradation of tocols would be mostly limited to the kneading step (Fratianni et al. 2012) with several physical-chemical and enzymatic factors contributing to their degradation. Significant losses during pasta drying treatments were only observed at high temperatures (Beleggia et al. 2011), the average loss of total tocols amounting to about 30%, with the highest loss reported for β –tocopherol (50%,) and the lowest for α-tocopherol and α-tocotrienol (20%). Important differences in tocopherol changes during pasta making were also observed for pasta made with refined semolinas versus wholemeal pasta: tocopherols in the refined samples showed a progressive decrease during the pasta generation steps, whereas for wholemeal, after a significant decrease during the extrusion step (-52% of tocopherols), the total content of tocopherols increased significantly in dried pasta.

Extrusion caused a significant decrease in both tocopherols and tocotrienols (from 63 to 94%, decrease depending on the original tocol content of the cultivar used), the least resistant to hydrothermal processing being α-tocopherol and α-tocotrienol.

Short chain carbohydrates

The low molecular mass carbohydrate fraction in wheat flour is represented mainly by fructans (fructooligosaccharides or FOS), short-chain carbohydrates of between 3 to 5 fructose units displaying different structures and including sometimes a single glucose unit (Ritsema and Smeekens 2003; Roberfroid 2005). Low amount of galactooligosaccharides (GOS), like raffinose and stachyose (Huynh et al. 2008; Lineback and Rasper 1988) are also present. Fructan content in wheat grains was found to vary depending on genotypes (7-29 mg/g) and it is therefore a character susceptible to be controlled by selection (Huynh et al. 2008). Bran and shorts fractions obtained from milling of wheat grains contain higher concentrations of fructans (34-40 mg/g) compared to white flour and germ (between 14 and 25 mg/g, depending on wheat cultivars and environmental conditions), (Haska et al. 2008; Knudsen 1997). However, in account of flour extraction level (79 % in this paper), the endosperm appeared to contribute to around half of the total fructan amount. FOS and GOS belong to the so-called FODMAPs

(Fermentable Oligo-, Di- and Mono-saccharides and Polyols), a term regrouping molecule which are not digestible to humans, due to lack of the corresponding hydrolytic enzymes, and are therefore poorly absorbed in the intestinal lumen while being highly fermentescible by beneficial bacteria (Gibson and Shepherd 2005) in the gut, and thus considered as prebiotics. However, FODMAPs have also been clearly involved in non-celiac gluten sensitivity (Biesiekierski et al. 2013; Skodje et al. 2018), a condition with symptoms similar to those of celiac disease that improve when wheat and other gluten containing cereals are eliminated from the diet.

The content of fructans in wheat-based products is impacted by milling and further processing of flour. Due to the higher concentration of fructans in the outer layer of the grain, white flour has a lower content of fructans compared to the corresponding whole grain flour, although the difference is likely to be significant only in the case of low extraction flours. Breadmaking has been showed to have a major impact on fructans content, but none on their structure (Gelinas et al. 2015). Dough mixing, with or without baker's yeast, was showed to cause reduction of fructans of about 20%. Dough fermentation lead to even greater reduction, with up to 80% of wheat grain fructans degraded by the action of yeast invertase over a 180 min period, while neither standard baking or overbaking were observed to have an impact. Furthermore, chain length of fructans did not change during breadmaking. The impact of specific processing steps in pasta making on fructan content have also been investigated. Gelinas et al. (2015) compared the fructans content of pastas produced from the same batch of semolina but using drying temperature of 40 or 80 °C and could not detect significant differences. Boiling of pasta on the contrary had a major impact, with 40-50 % of fructans being lost in boiling water during pasta cooking, irrespective of the semolina used for pasta making, pasta's cooking time or cooking loss.

Sterols

Sterols and their saturated forms, stanols, are a class of four-cyclic compounds with a cyclopentane perhydrophenanthrene nucleus, a hydroxyl group at position 3 of the A-ring, and a side chain located at carbon 17. Dietary intake of phytosterols from wheat bran and germ was demonstrated to lower levels of total and low density lipoprotein in the serum (Andersson et al. 2004), however those fractions were also found to be enriched with long chain (C20-C30) aliphatic primary alcohols, policosanols, which were also reported to display similar health effects (Irmak et al. 2006).

Total phytosterol content in wheat grains representing 26 genotypes, 3 growing seasons and four locations was found to vary from 700 to 928 µg/g (Nurmi et al. 2010a). Both genetic and environmental factors were found to impact the grain phytosterols content. Low significance of the genotype was on the contrary reported by Beleggia and coauthors (2013) for durum wheat. Main sterols in wheat are represented by β- sitosterol and campesterol and their corresponding saturated forms (Nurmi et al. 2010a, 2010b). Around 10 % of wheat grain sterols were found esterified to a phenolic acid, mainly ferulic acid, in the form of steryl –ferulate (Nurmi et al. 2010b, Nyström et al. 2007a). Another 10 %

were found glycosidically linked to a mono-, di- or oligosaccharide creating a steryl glycoside which may be further esterified with a fatty acid forming an acylated steryl glycoside (Nyström et al. 2007a). Analysis of commercial milling fractions by Nyström et al. (2007a) found the highest total sterol concentration in a fraction enriched in germ followed by fine and coarse brans. The same authors also observed a similar enrichment of the bran fractions (but not of the germ) with steryl ferulate forms. Moreover, they observed preferential esterification of campestanol, while sitosterol was preferentially found in glycoside forms and appeared in highest concentration in germ and flours enriched in fibers and ash as well as in coarse bran. Nurmi et al. (2012) further characterised the sterols and steryl ferulates content in fractions from wheat grain obtained by pearling, grounded bran separated with electrostatic process and pure aleurone fractions, with each grain outer layer proportion estimated using specific biological markers. Based on their differential concentration they concluded that different phytosterol forms are differentially distributed among the external grain tissues. However no marker for germ was used in this study and neither a mass balance of the different fractions was given.

Processing affects the release of sterols from the food matrix and therefore their bioaccessibility. Nystrom et al. (2007b) reported that the reduction of particle size of wheat bran from an average of 97 μm to an average of 47 μm, using centrifugal milling, increased only minimally the apparent amount of sterols (about 5% increase), while thermal treatments (roasting or microwave heating) decreased their apparent content. The sole addition of water (without enzymes) may also dramatically decrease the availability of sterols from cereal products. Soaking wheat bran in water resulted in an apparent decrease in the sterol content, which was suggested to be a consequence of the formation of arabinoxylan hydrates, whose viscous structure blocks the hydrophobic sterols inside. Subsequent addition of enzymes, such as xylanases or β-glucanases resulted to only partial release of bound sterols, so that their apparent content in the treated samples remained lower than in the untreated bran.

Phytic acid and Minerals (Fe, Zn and Mg)

Phytic acid (IP6) is a myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate which constitutes the main storage of phosphate in the seed and in cereal grains. Its concentration varies between 12 and 18 mg/g in wheat grains (Barron et al. 2011) and it is mainly present in phytin globoids inside the protein storage vacuoles in aleurone cells, where most of the magnesium (Mg) and iron (Fe) of the grain can also be found (O'Dell et al. 1972). Phytic acid content in the aleurone layer varies between 95 and 190 mg/g (Barron et al. 2011) and phytic acid concentration can therefore be used as an efficient marker to monitor the aleurone fate along milling (Greffeuille et al. 2005; Hemery et al. 2009), which is of great practical relevance since the redistribution of aleurone cells into flour or semolina is largely affected by grain hardness (Greffeuille et al. 2005). Being a polyvalent anion, phytic acid is able to chelate minerals present in the grain in the form of mixed salt, called phytates. Its iron chelating properties, by interrupting the reactions of the Haber–Weiss cycle, and consequently the formation of hydroxyl radicals ($OH^{\cdot}$), would prevent lipid peroxidation and therefore underpin phytic acid reported antioxidant

activity. Furthermore, phytic acid has been shown to inhibit xanthine oxidase mediated O_2^- generation (Muraoka and Miura 2004). Recent studies showed a beneficial impact of phytic acid consumption on health in the form of anti-carcinogenic effects, prevention of coronary disease, and boosting of the immune system (Silva and Bracarense 2016). On the other hand, phytic acid has long been considered an antinutritional factor, since by forming insoluble complexes with dietary cations, particularly Mg, Fe, zinc (Zn) and calcium (Ca), impairs mineral absorption in humans (Zimmermann and Hurrell 2007). Mg, Fe and Zn are essential for good preventive nutrition and wheat grain contains considerable amounts of these minerals as well as lower levels of many trace elements, such as selenium and manganese. Magnesium is among the most abundant intracellular cations in the body, being a cofactor in over three hundred biochemical reactions, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation. There is experimental and clinical evidence showing that the amount of magnesium (Mg) in typical Western diets is often insufficient to meet individual needs and low intakes of Mg have been associated with etiologic factors in cardiovascular and nervous diseases, bone deterioration, spasmophilia and stress (reviewed by DiNicolantonio et al. 2018).

Fe also plays an essential role in human physiology. The body requires Fe for the synthesis of its oxygen transport proteins, in particular hemoglobin and myoglobin, and for the formation of heme enzymes and other iron-containing enzymes, involved in electron transfer and oxidation-reductions. Iron deficiency can affect resistance to infection and, cognitive development (Ryan 1997). However, as it can form free radicals, its concentration in the body must be finely controlled (Abbaspour et al. 2014).

Diet is the only source of Zn and Zn deficiency is widespread in human populations. The main biological function of Zn is in the form of Zn finger motifs, which are among the most abundant small proteins structural motifs in eukaryotic and play a role in diverse cellular processes (Matthews and Sunde 2002), including DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly and lipid binding. Zn is therefore found in several system and biological reactions, and is essential for immuno-function, cell division, cell growth, wound healing, blood clotting, thyroid function and the breakdown of carbohydrates (reviewed in Roohani et al. 2013). In addition, Zn is also needed for the senses of smell and taste (Henkin 1984).

Screening of modern bread wheat cultivars, showed concentrations varying from 600 to 1400 ppm, 20-60 ppm and 15-35 ppm for Mg, Fe, and Zn, respectively, with negative correlations with grain yield being reported for all three minerals (Oury et al. 2006).. Cereal grain minerals are mainly present in the aleurone and therefore enriched in the bran fraction after milling. Cubadda et al. (2009) reported various degrees of mineral loss upon milling of durum wheat grains. Selenium had the highest retention with concentrations in semolina equal to 77–85% of that in grain (dry weight basis), followed by calcium (54–60%), copper (49–53%), potassium, phosphorous (42–47%), Fe (36–38%), Mg, and Zn (32–36%). In wheat grain, Fe mainly accumulates in aleurone cells as phytate salts and in this form Fe can be either soluble or insoluble, depending on the nature of the bonding. Insoluble forms are not accessible to iron

transporters in the human gut, but there is evidence that soluble salts in the form of monoferric phytate (MFP) may be a bioavailable source of iron (Sandberg et al. 1999). Balmer et al. (2006) showed that some Fe is also present as ferritin in amyloplast isolated from developing wheat endosperm, and the presence of Fe in the cytoplasm of endosperm cells was subsequently confirmed by NanoSIMS imaging of wheat grains (Moore et al. 2012). Eagling et al. (2014a) showed that the presence/content in the grain of metal chelators may also play a role in iron bioavailability. The chelator nicotianamine (NA), which is involved in the intra- and intercellular transport of metal cations, was reported to enhance iron uptake in a cellular model and so was, although at a lower level with respect to NA, 2′-deoxymugineic acid (DMA), which is involved in the solubilization and acquisition of Fe(III) by the plant from the rhizosphere. Iron speciation studies carried out in white flour and whole grain by the same group (Eagling et al. 2014b) showed that the content of Fe complexed with NA/ DMA in white flour was 4 to 5-fold higher than in the whole grain. Taking also in consideration the lower phytic acid content of the endosperm, it is therefore possible that this tissue, despite having a significant lower content of iron than the bran, may be a better source of bioavailable iron.

Mg and Zn are also largely present in the form of phytate salts in the aleurone, with Mg having been co- localized within globoid crystal with P and K, However, speciation and localization analysis of Zn in wheat grain, showed that this cation can also be found in the endosperm in association with small cysteine-rich proteins (apparent size 10–30 kDa) (Persson et al. 2016).

Phytic acid chelating action, by hindering bioaccessibility of Fe, Mg and Zn, drastically reduce their bioavailability in wheatbased foods (Das et al. 2011). Furthermore, phytic acid also interact with protein, reducing their digestibility (Kumar et al. 2010) and it was also shown to promote Maillard reactions in certain conditions and thus acrylamide formation (Wang et al., 2013). Although reduction of phytic acid in the grain by breeding remains a viable option (Gupta et al. 2015; Magallanes-Lopez et al. 2017), processing represents at the moment the most effective strategy for solubilization of Fe, Mg and Zn from phytate salts in wheat flour.

The sourdough process results in drastic degradation of phytate due mainly to activation of wheat endogenous phytase by microbial acidification of the dough. Leenhardt et al. (2005) observed a 70% reduction in phytic acid after 4 h sourdough fermentation which was accompanied by a 5 fold increase in soluble Mg, while Rodriguez-Ramiro et al. (2017), reported that a 36 h sourdough fermentation, reduced the content of phytic acid from wholegrain flour to a level that does not allow its detection, bringing the IP6:Fe molar ratio to below 1, and resulting in a significantly higher amount of Fe in bread becoming bioavailable.

Substantial increase in phytase activity has also been reported during germination of wheat (Bartnik and Szafranska 1987) and germination has been shown to drastically increase the availability of Zn, but not that of Fe from wheat grain (Luo et al. 2014). High-temperature short-time extrusion cooking, the process used for the production of a variety of breakfast cereals and salty and sweet cereal snacks, was also shown to efficiently enhance mineral availability in cereal products. Minerals are considered stable

during heat treatment but extrusion may hydrolyze the complex between phytic acid and minerals to release phosphate molecules with 13–35% reduction in phytate content from a wheat bran-starch-gluten extruded mix having been reported (Andersson et al. 1981)

There is also evidence that absorption of minerals from wheat grain may also be impaired by the presence of tannins and fibres, which can form insoluble complexes with divalent ions in the gastrointestinal tract. Shear forces and high temperatures during extrusion cooking are very effective in the destruction of polyphenols (Singh et al. 2007) and may cause modification of fibre components (Wang et al. 1993) and their chelating properties, which could also contribute to improve bioavailability of minerals in extruded foods.

Phenolic acids

Phenolic acids are a complex group of secondary metabolites exhibiting large diversity in structure but all containing at least one benzene ring with one or more hydroxyl groups.

They are the most abundant phytochemicals in cereals and have been shown to function as free-radical scavengers, reducing agents and quenchers of singlet oxygen formation Their antioxidant properties (reviewed in Laddomada et al. 2015) are mainly attributed to the electron donation and hydrogen atom transfer to free radicals, but it has also been suggested they may partly derive from their ability to modify some cellular signalling processes. Their ability to act as antioxidants may prevent heart disease and lower the incidence of colon cancer and they have also been shown to exert anti-inflammatory action in the gut which may be significant in maintaining gastrointestinal health (reviewed in Laddomada et al. 2015).

Ferulic acid (FA), a derivative of hydroxycinnamic acid, has the highest antioxidant activity and account for 70-90% of phenolic acids in the grain, which include also other hydroxycinnamates, such as caffeic acid, chlorogenic acid, sinapic acid, and p-coumaric, and derivatives of hydroxybenzoic acid (Klepacka and Fornal 2006). Through reactions involving their carboxylic and hydroxyl groups, phenolic acids may form both ester and ether linkages which allow them to cross-link with cell wall macromolecules (Bunzel et al. 2004). Wheat FA is found both as a free compound, as a soluble conjugate bound to low molecular weight compounds such as sugars, and as bound forms in association with the fibre fraction, mainly as dimeric esters bound to arabinoxylan but also to lignin. The biological properties and physiological effect of dietary polyhenols, and notably their antioxidant properties, depends upon their availability for absorption and subsequent interaction with target tissue, which in turn will depend greatly on their degree of polymerization. Early studies highlighted an inherently low bioavailability of FA in wheat grain and suggested it would reflect the fact that it is present for a large proportion (up to 80%) in the bound forms (Anson et al. 2009a). They reported that while free forms of FA were efficiently absorbed in the intestine, only a small portion of bounds phenolics appeared to be metabolised in the stomach and small intestine and could therefore be considered as bioavailable, However, the bound phenolic fraction demonstrated a significantly higher antioxidant capacity *in vitro*

in comparison with free and esterified phenolic acids, which suggested their inclusion as essential when aiming at evaluating the antioxidant activity in grains in relation to their phenolic acid content. Subsequent studies showed that intestinal microbes are able to cleave the ester- or ether-bonds responsible for crosslinking of wheat phenolic acids to cell wall polymers, making the phenolic acids nutritionally available (Vitaglione et al. 2008). Furthermore, the structural complexity of bound phenolics, by allowing them to reach the colon mostly undigested, would be functional to their ability to exert unique antioxidant and anti-inflammatory activity locally and therefore contribute to reduce the risk for colorectal cancer (Andreasen et al. 2001; Drankham et al. 2003).

Barron et al. (2007) reported that the content and nature of phenolics acids differ among different grain tissues and so do also the amount and molecular composition of cell wall polymers present in the different layers, particularly the relative amount of arabinose (Ara) and xylose (Xyl). The outer layers of the wheat grain were reported to contain the highest amount and broader array of phenolic acids, including ferulic acid (FA), dehydrodimers (DHD) and dehydrotrimers (DHT) of ferulic acid, sinapic acid (SA), and p-coumaric acid (p-CA) (Barron et al. 2007; Parker et al. 2005). The embryo tissues (the scutellum in particular) are relatively rich in FA and DHD, while FA and sinapic acid were the main phenolic acids in the starchy endosperm, although the content of FA in concentrations several fold lower than in the cell walls of the seed coat and aleurone layer. The outer pericarp displays the highest concentration of a trimeric form of FA alloing its use as a marker to monitor this tissue behavior along fractionation (Hemery et al. 2009). Furthermore, the aleurone tissue has the highest antioxidant capacity among the wheat grain layers and it has been suggested that this would result mainly (over 60 % of the antioxidant capacity) from its high content of FA (Anson et al. 2008). Among the tissues constituting the bran fraction, the outer pericarp and tissues in the crease region contain the highest proportion of strongly bound (ether-linked) phenolic acids, while the aleurone and the hyaline layer present the highest proportion of weakly cross-linked FA (Barron et al. 2007). The aleurone and hyaline layer also display a lower level of substitution with a lower level of Ara/Xyl ratio (<0.5) in the arabinoxylan polymers of their cell walls.

Using debranning, Beta et al. (2005) showed that total phenolic content and total antioxidant activity are strongly correlated, present similar values in the very first pearling fractions (up to 10% removal), which correspond almost exclusively to pericarp tissues and part of the aleurone layer (Rios et al. 2009), to then progressively decrease in fractions with increased endosperm content.

Processing, both industrial and domestic can affect the content, composition, and stability of phenolic compounds in wheat-based products. Modern milling, which is based on the separation of different tissues of the grain into milling streams which are then recombined to give flours of different extraction rate, has a major impact on the content and composition of phenolic acids of final flours: the relative content of aleurone and pericarp tissues in the flour increases with its extraction rate, and since these tissues are rich in phenolic acids so does the content of this components. The practice of debranning

before roller milling (usually removing about 5% of initial grain weight), would instead impact specifically on the ratio of soluble to bound phenolic acids in the flours, since the outer pericarp removed by debranning contain a higher proportion of bound phenolics compared to the other grain tissues (Barron et al. 2007).

Bakery and pasta-making processes can also result in large modifications of content, composition, and bioaccessibility of phenolic acids, with respect to the original flour ingredient.

The bioavailability of FA appeared to be determined by the percentage of free FA, and since this has been reported to be extensively absorbed in the intestine (Adam et al. 2002), absorption itself is unlikely to represent the limiting factor for this compound. On the contrary, bioaccessibility *i.e.*, release from the food matrix, appears to be a determinant factor in FA bioavailability in wheat-based products, since most of FA in the grain is bound to arabinoxylans and other indigestible polysaccharides, restricting its release in the small intestine. (Anson et al. 2009 a; Kern et al. 2003).

Bioprocessing techniques to release bound phenolic compounds from wheat bran have been applied with success to increase the content of free phenolic acids in bran containing breads (Anson et al. 2009b). Bran bioprocessing involve fermentation with baker's yeast or a combination of fermentation with enzymatic treatments using hydrolytic enzymes (mainly xylanase, β-glucanase, α-amylase, cellulase and ferulic acid esterase), whose combined action enables the degradation of different wheat polymers, thus improving the solubility of the complex cell wall structure in the bran. Bran fermentation alone was reported to increase the amount of free FA in the bread by approximately 3-fold, while the combination of fermentation and enzymatic treatment brought this increase to 8-fold, which corresponded to an increased in FA bioaccessibility of 5 folds, as measure by an *in vitro* system. These same bioprocessing techniques also increased the free form of p-coumaric acid and sinapic acid.

Increases in free phenolic acid as a result of dough fermentation have been reported also for wholemeal and white breads, the increase being higher when sourdough rather than only yeast fermentation was used (Konopka et al. 2014; Moore et al, 2007). The lowering of pH during sourdough fermentation favours the activity of hydrolases (native flour enzymes and/or enzymes of microbiological origin) and can contribute to chemical disintegration of arabinoxylans, and hydrolysis of both esters and glycosides of phenolic acids leading to structural breakdown of the cell wall matrix and the release of free phenolic acids. However, souring of dough to approximately pH 4 has been reported to have an inhibitory effect on wheat flour native cinnamoyl esterase activity (Konopka et al. 2014) Cinnamoyl esterase activity observed in wheat flour has been s ascribed to microflora on the grain surface (Dornez et al. 2006) and normally contribute to degradation of the arabinoxylans in cell walls.

Baking has also been reported to significantly increase the concentration of free ferulic acid, particularly in the crumb of sourdough fermented bread (Konopka et al. 2014).

However, reductions in the amount of free phenolic acids during the bread production process have also been reported. One possible explanation for the reduction in total phenolic acid content in breadmaking is their decomposition by microflora: *Saccharomyces cerevisiae* is able to convert trans-ferulic acid into

4-hydroxy-3-methoxystyrene with a 96% yield (Huang et al. 1993). Furthermore, temperature at 230ºC during baking has been suggested to cause either re-binding of ferulic acid released by fermentation, or its degradation (Han and Koh 2011) a phenomenon that would be at the basis of the lower content of free ferulic acid reported in bread crust compared to bread crumb (Konopka et al. 2014).

Of great interest are also recently developed more sustainable, non-toxic techniques for the extraction of plant phenolics based on microwave and ultrasound-assisted technologies (Tiwari 2015; Wang and Weller 2006) which make possible to extract the phenolic compounds from bran for their incorporation in functional foods without requiring any chemical preliminary hydrolysis and eliminating the use of organic solvents.

Dietary Fibre

Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine, and promoting physiological effect or benefit to health (according to the AACC definition, Cereal Foods World 46: 112-126, 2001).

Mature wheat grain DF is represented by: cell wall polysaccharides, which are ubiquitous in the grain; lignin, a phenolic polymer which is only in the pericarp/seed coat (Antoine et al. 2003; Stone and Morell 2009); fructans (described in a previous paragraph), enriched in bran tissues, but also present in endosperm cells and therefore white flour; small amount of resistant starch (RS), which derive solely from the endosperm cells.

The non-starch polysaccharides (NSP) present in cell walls account for about 11% of the mature wheat grain dry weight (Andersson et al. 2013) and are the major components of the dietary fibre fraction in wheat, The major cell wall polysaccharides of wheat grain are arabinoxylan (AX) and (1→3, 1→4)-β-D-glucan (β-glucan), with smaller amounts of cellulose ((1→4)-β-D-glucan), glucomannan and pectin. AX comprises a backbone of xylose residues with some residues being substituted with arabinose residues at either one or two positions. Some of the arabinose residues present as single substitutions on xylose may also be substituted with ferulic acid, allowing the formation of diferulate cross-links by oxidation of ferulate present on adjacent AX chains. The extent of diferulate cross-linking is important as it affects physico-chemical properties of AX such as solubility and viscosity, which govern its behaviour in food processing and its health benefits. AX is therefore often divided into two classes, according to its extractability in water: water extractable (WE-AX) or unextractable (WU-AX). Branching and the presence of ionic groups increases the solubility and so do also changes in monosaccharide units and their molecular form (α- or β-form). β-glucan comprises glucose residues joined by (1→3) linkages usually separated by two or three (1→4) linkages, but longer stretches of up to 14 (1→4) linked glucan units have been reported for wheat bran β-glucan (Li et al. 2006). Only 10–20% of the total β-glucan in wheat wholemeal flour was found soluble (Nemeth et al. 2010).

The content and composition of cell wall NSP vary between grain tissues: compared to the starchy endosperm, the outer layers of the mature wheat grains comprises higher content of cell wall material (at least 50%) (Shewry and Hey 2015), mainly in the form of arabinoxylan (60%), cellulose (25%), and lignin (~10%) (reviewed by Stone and Morell 2009); cell walls account for about 35–40% of the aleurone dry weight and comprise mainly arabinoxylan (AX) (65%) and β-glucan (30%) (Bacic and Stone 1981), while starchy endosperm cells show thinner walls (about 2–3% dry weight), which also consist mainly of AX (70%) and β-glucan (20%) (Mares and Stone 1973; Stone and Morell 2009). Differences in the proportions of AX and β-glucan exist also between regions of the starchy endosperm, with β-glucan having been reported as more abundant in the region close to the germ (Saulnier et al. 2009). Depending on their location in central or prismatic cells, AX structure was also found to be different (Saulnier et al. 2009) and the content of arabinose in AX was found to increase from the outside to the inside of the endosperm (Toole et al. 2010). AX from the starchy endosperm contains low levels of ferulic acid, ie 0.2–0.4% (w/w) in WE-AX and 0.6–0.9% (w/w) in WU-AX (as reported in Shewry and Hey2015), while AX from the aleurone are more esterified and cross-linked, (Antoine et al. 2003; Parker et al. 2005). In this aleuronic AX polymer, additional esterification with p-coumaric acid and acetyl groups were also reported (Rhodes and Stone 2002). A complex highly branched structure is also characteristic of pericarp AX which often comprises galactose and glucuronic acid residues in addition to high contents of ferulic acid and di-or tri-ferulic acid (Hemery et al 2009; Parker et al. 2005) and acetylation (Mandalari et al. 2005) with also significant amounts of ferulic acid trimer (Barron et al. 2007)

Lignin, is a complex polymer of aromatic alcohols tightly associated to cell wall polysaccharides either directly through covalent links with sugar residues and indirectly via ferulic acid esterified to polysaccharides (Davin et al. 2008; Iiyama et al. 1994). Lignin, which is insoluble and largely resistant to bacterial degradation, cements and anchor the cellulose microfibrils and other matrix polysaccharides, stiffening the cell walls and making it very rigid and difficult to degrade by the microorganisms in the large intestine.

RS is the term given to starch escaping digestion in the small intestine and therefore becoming available as substrate for fermentation by colonic microorganisms, with production of short chain fatty acids (SCFA) which have positive effect on health (Topping and Clifton 2001, Topping et al. 2008). Cereals generally contain about 3% RS comprising of starch entrapped in the food matrix, and therefore physically inaccessible to digestive enzymes, native (undamaged and/or uncooked) starch granules and retrograded starch formed after starch granules gelatinization, with the amount of native and retrograded starch strongly influenced both by amylose content and processing conditions (Eerlingen et al. 1993, 1994; Hallström et al. 2011).

A number of mechanisms probably contribute to the beneficial action of dietary fibre on health (Brownlee 2011; Buttriss and Stokes 2008; Theuwissen and Mensink 2008; Topping 2007), including physical properties (faecal bulk, water solubility, water-holding capacity, swelling power and viscosity)

and fermentation in the colon. The insoluble fraction of dietary fibre activates intestinal peristalsis and is capable of binding bile acids and water. Soluble fibre reduces the blood cholesterol level, the risk of ischemic heart disease and postprandial glycemia. Fermentation of dietary fibre by gut bacteria produces SCFAs which have physiological effects on the colon and other tissues. There is also increasing evidence that mixed-linkage β-glucans are able to regulate the immune responses that are involved in fighting infection, attacking tumors and various inflammatory conditions (Brown and Gordon, 2001; Rice et al. 2005). More recently, immune stimulatory effects have also been proposed for arabinoxylan (Capek and Matulova 2013; Mendis et al. 2016).

Processing can lead to the modification of fibre composition and microstructure impacting on physicochemical properties and nutritional effect of fibre (Zhang et al. 2011).

Due to the uneven composition of dietary fibre components within the different grain tissues, milling can have a dramatic effect on dietary fibre amount and composition of flour. Wholemeal wheat flour contains on average about 13% dry weight of total dietary fibre, half of which is represented by total AX, but only in minimal part being water soluble (~0.57% WE-AX). By comparison white flour has an average total dietary fibre content of 3.5%, about 75% of which total AX and the same amount of WE-AX than wholemeal flour (Shewry and Hey 2015).

Therefore, milling affects flour 's dietary fibre composition via the extraction rate and the effectiveness of the separation between bran and the endosperm tissues. Furthermore, milling parameters determine the particle size production which was also found to play a role in the fibre properties. Studies on the effect of ultrafine grinding on the physicochemical properties of wheat bran dietary fibre (Zhu et al. 2010) showed that as particle size decreased, the hydration properties (water holding capacity, water retention capacity and swelling capacity) of wheat bran dietary fibre significantly decreased and a redistribution of fibre components from insoluble to soluble fractions was observed. Ultra-fine grinding was also shown to increase the antioxidant capacity of wheat bran probably due to a greater exposure/accessibility of the phenolic acids linked to fibres (Rosa et al. 2013): The effect persisted in gastric conditions, showing that ultra-fine grinding can be used to produce wheat bran fractions with higher nutritional value. Physicochemical properties of wheat dietary fibre are also significantly affected by the combination of high temperature, pressure and shear force characteristic of extrusion cooking technology, increasingly used to produce highly expanded and low-density products such as ready-to-eat breakfast cereals and snacks. Extrusion-cooking of white wheat flour (T = 161-171 °C, water content = 15-20%; screw speed = 100-200 RPM) was found to cause a redistribution of insoluble to soluble dietary fibre (Björck et al. 1984) with 50-75% of total fibre (depending on process conditions) being soluble in the extruded flour, versus 40% in the raw flour. Extruded white flour had also increased fermentability, as determined by faecal recovery in rat balance experiments, and the authors suggested it would be the consequence of its higher solubility. Extrusion, particularly at highest screw speed has been successfully used to increase solubility of dietary fibre in wheat bran (Rashid et al. 2015; Wang et al. 1993) although relative fibre solubilisation is significantly lower compared to white flour and it does

not seem to have an effect on *in vivo* fermentability (Björck et al. 1984). Rashid et al. (2015) examined the suitability of wheat bran for extrusion cooking and checked the effect of different extrusion parameters on the dietary fibre profile, as well as on the water solubility index.

Several studies have reported on the impact on flour endogenous fibre of the breadmaking process and of its different processing steps and results suggest that both mechanical effects and enzymatic reactions would be involved in determining the observed changes. Rouau et al. (1994) monitored the amount of water extractable arabinoxylans during breadmaking and reported increases that they attributed to solubilisation of some of the water-unextractable arabinoxylans: more than 10% had become extractable by the end of kneading and solubilisation increased to reach 25% at the end of fermentation. Cleemput et al. (1997) also observed substantial increase (7 to 12%) in water extractable NSP during the mixing and baking (14 to 15%) phases but only very low levels (0 to 5%) of water-unextractable NSP solubilised during fermentation. Furthermore, clear changes in molecular weight distribution of AX during fermentation were observed with no modification in the A/X ratio of the AX fractions.

A more recent study on the impact of breadmaking on endosperm flour dietary fibre (Comino et al. 2016) reported an increase of approximately 18.5% for the total solubilised NSP (+12.5% WE-AX and +6% β-glucan). This increase results from a 7% yield decrease in wheat flour unextractable NSP during dough fermentation and 19% decrease during baking, with only relatively smaller increases ascribable to dough preparation. The contribution of the dough mixing step in determining the final amount of water extractable AX was instead substantial, in a similar study carried out by Gelinas et al. (2015). It is likely that differences in specific parameters used during dough mixing, dough fermentation and baking are at the basis of the above reported differences. A decrease in insoluble AX of 35% from wholemeal flour to the baked bread product has also been reported by Hansen et al. (2002).

The breadmaking process, and in particular the type of fermenting inoculum and parameter of fermentation, has also a significant impact on the content of other types of dietary fibre, namely fructans and RS. Yeast leavening results in major reduction of flour fructans in dough and bread (Gelinas et al. 2015), while sourdough breadmaking results in comparative higher amounts of RS (Scazzina et al. 2009) likely as a consequence of the presence of organic acids produced during fermentation, which could facilitate debranching of the amylopectin moiety during baking. It has been shown, in fact, that debranched amylopectin may form a high level of RS on heat treatments (Berry 1986).

Pasta and noodles making and cooking pasta also have an effect on dietary fibre amount and composition. Pasta extrusion is known to result in products eliciting reduced glycaemic responses (Monge et al. 1990; Wolever et al. 1986), and thus to induce the metabolic advantages of low glycaemic index food (Jenkins et al. 1987). Available data suggest that pasta (both dried and fresh egg pasta) is a comparatively rich source of RS (Brighenti et al. 1998) with respect to other conventional wheat-based foods. This slow-release features of starch in pasta probably relates to the continuous viscoelastic network formed during pasta making, which surrounds the starch granules restricting starch swelling and leaching during boiling and likely also reducing its accessibility for enzymatic digestion. However,

the pasta surface area does not relate to the glycaemic response (Wolever et al. 1986) and, similarly, the shape of pasta does not seem important in relation to the RS content.

Processing methods based on wet heat and extrusion cooking are also being assessed for their potential to induce the formation of amylose-lipid complexes (ALCs), which represent a novel form of RS, in cereal based products (reviewed in Panyoo and Emmambux, 2017).

Dietary fibre (arabinoxylan and β-glucan) amounts were not significantly affected by the alkaline and/ or boiling (100°C) conditions used for the production of yellow alkaline noodles (Comino et al. 2016).

Anthocyanins

Anthocyanins are abundant secondary metabolites responsible for most blue to blue-black, and red to purple colours of many plant organs. They have antioxidant, photo-protective and defence roles in the plant, and play an important role in the reproductive mechanisms (Escribano-Bailòn et al. 2004). Their molecular structure consists in an anthocyanidin (aglycone), with saccharide residues bound at different hydroxylated positions. The differences between individual anthocyanins are related to the number of hydroxyl groups, to the nature, number and position of sugars attached to the molecule, and to the aliphatic or aromatic acids attached to the sugars (Kong et al. 2003). In colour-grained wheats, six anthocyanidins have been observed: cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin.

Garg et al. (2016) identified 22 different anthocyanins in blue, 23 in purple and 26 in black wheats; the same authors determined that the anthocyanin content was highest in black, followed by blue, purple and amber wheat lines. The concentration and composition of total anthocyanins in wheat kernels is influenced by the cropping environment (Abdel-Aal and Hucl. 2003; Varga et al. 2013), but Ficco et al. (2014) found high heritability for anthocyanins, and only minor genotype×year effects.

In the food field, anthocyanins are a viable alternative to artificial colors. Furthermore, they are valued for their bioactive properties against oxidative stress, cancer, inflammation, diabetes, obesity, reduction of postprandial glycaemic levels (Kruger and Morlock 2018). Anthocyanin content in wheat kernels can be assessed either spectrophometrically or by HPLC (high performance liquid chromatography) and LC-MS (liquid chromatography-mass spectrometry). Using the quicker and cheaper spectrophotometer approach, Abdel-Aal and Hucl (1999) found 155.6, 103.8 and 5.2 mg cyanidin 3-O-glucoside/kg in the wholemeal of blue, purple and red bread wheat lines. Similarly, higher values in blue wheats than in purple wheats were observed by Varga et al. (2013) (21.4-157.6 vs. 6.7-30.7 mg/kg), and by Jaafar et al. (2013) (111.5-251.8 vs. 50.1-171.7 mg/kg). Meanwhile, Liu et al. (2010) recorded 234.5 mg/kg in purple wheat and 9.6 mg/kg in red wheat. Anthocyanins are also present in wild and ancient wheats. Brandolini et al. (2015) analysed samples of four diploid wheats, i.e. *T. monococcum* ssp. *thaoudar* (wild einkorn), *T. monococcum* ssp. *aegilopoides* (feral einkorn), *T. urartu* (red wild einkorn) and *T. monococcum* ssp. *monococcum* (domesticated einkorn), and found significant anthocyanins concentrations: 43.0±4.66, 17.4±3.01, 15.3±0.85 and 11.6±1.59 mg/kg d.m., respectively.

Anthocyanins are mainly located in the outer layers of the caryopsis. Abdel-Aal and Hucl (1999) observed that blue, purple and red wheats display different anthocyanins concentration in flour (22.5, 5.2 and 1.7 mg/kg) and in bran (458.3, 250.7 and 10.4 mg/kg). Furthermore, the distribution of anthocyanins in blue and purple cultivars is different: in the former they are concentrated in the aleurone layer, while in the latter they are rather located in the pericarp (Knievel et al. 2009; Kruger and Morlock 2018). Böhmdorfer et al. (2018) analysed bran fraction from 17 purple pericarp, 10 blue aleurone, and 13 deep purple grained genotypes reporting 47.5-502.5, 117.6-879.2, and 359.9-1289.6 mg/kg d.m. contents.

The impact of breadmaking on anthocyanin content of purple wheat bread was investigated by Yu and Beta (2015): total anthocyanin content (TAC) was found to decrease by 21% after mixing, then gradually increased to 90% of the original level after fermentation, and finally decrease by 55% during baking, with the lowest value for TAC observed in the bread crust.

Substantial losses of anthocyanin have also been reported (Escalante-Aburto et al. 2013) as resulting from high-temperature short-time extrusion process of cereal flour, widely used for the preparation of cereal based snacks (55 to 80% loss depending to the particular product).

Carotenoids

The carotenoids are lipid-soluble antioxidants formed by most photosynthetic organisms that give the typical yellow, orange and red colours to many flowers, fruits and bird feathers. Two classes of carotenoids exist: the carotenes, which are tetraterpenoid hydrocarbons, and the xanthophylls, which have one or more oxygenated functions in the molecule (Van den Berg et al. 2000). The single and double bonds in the polyenic chain shape their antioxidant properties, while the presence of polar groups influences their interaction with cellular membranes (Britton 1995). In plants, they behave as light collectors and protectors against photosensitization in chloroplasts. The carotenoids are not produced by the animals who must obtain them from the food.

The main carotenoids found in wheat species are, in decreasing concentrations, lutein, zeaxanthin, α- and β-carotenes and cryptoxanthin (Abdel-Aal et al. 2007; Hidalgo et al. 2006; Hidalgo et al. 2010). Lutein accounts for 90-100% of the total carotenoids (Abdel-Aal et al. 2007) and may occur in both the esterified and non-esterified forms. Ziegler et al. (2015) identified six lutein monoesters and nine diesters, representing 22.2%, 29.7%, and 7.6% of the total lutein in bread wheat, spelt, and einkorn, respectively; lutein esters are absent in durum and emmer wheats.

All the carotenoids are appreciated for their antioxidant activity, which protects cells and tissues from free radicals. The α- and β-carotenes are involved in the biosynthesis of vitamin A, which is essential for cellular reproduction, embryo development, visual functions, etc. (Zile 1998), while lutein and zeaxanthin protect the macula region of the retina, prevent the cataracts, enhance the immune response, shield against solar radiation, inhibit some type of cancers and contribute to the prevention of degenerative and cardiovascular diseases (Krinsky 1994; Van den Berg et al. 2000).

The carotenoid content of the seeds is linked to wheat species and variety (Brandolini et al. 2008; Hidalgo et al. 2006; Paznocht et al. 2018; Ziegler et al. 2015), environmental conditions and stresses (Hidalgo et al. 2009; Lachman et al. 2013), fertilisation (Hidalgo and Brandolini 2017) as well as post-harvest storage and milling (Hidalgo and Brandolini 2008a, 2008b; Mellado-Ortega and Hornero-Méndez 2016). The carotenoids are easily degraded by oxygen, with a strong influence of heat, light end exposure to hydroperoxides. During processing, some enzymes (mainly lipoxygenase) catalyse the hydroperoxidation of polyunsaturated fatty acids, creating conjugate hydroperoxides; the radicals formed during this reaction are responsible for the oxidative degradation of the carotenoids (Gardner 1988; Hidalgo and Brandolini 2012; Leenhardt et al. 2006). Flour particles of different size, the result of distinct wheat species, grain hardness and moisture content at milling (Posner et al. 2009), present different carotenoids concentration and colour (Hidalgo et al. 2014; Symons and Dexter 1991).

Carotenoids are scarce in bread wheat, where they range from 0.1 to 2.5 mg/kg d.m., but are more abundant in durum wheat, spanning from 1.5 to 4.8 mg/kg d.m. (Panfili, et al. 2004; Zandomeneghi et al. 2000), where the yellow colour of the semolina is perceived as an important quality trait. Recently, Paznocht et al. (2018) reported average total carotenoid contents of 3.60 and 2.41 mg/kg d.m. in purple- and blue-grained wheats, respectively, but observed peaks of 7.46 and 7.04 mg/kg d.m. in other blue- and yellow-grained accessions, respectively. The highest carotenoid content among cultivated wheats was found in einkorn, with an average of 8.5 mg/kg d.m. and a range of 5.3-13.6 mg/kg d.m. (Abdel-Aal et al. 2007; Brandolini et al. 2008; Hidalgo et al. 2006).

Lutein is particularly concentrated in wheat germ but relevant concentration is also found in the endosperm (Hidalgo and Brandolini 2008b, Masisi et al. 2015, Ndolo and Beta 2013). As the endosperm represents between 75-85% of the total kernel weight in different wheat species (Hidalgo and Brandolini 2008b; Pomeranz 1988), most of the overall lutein content is retained in the refined flour.

Carotenoid losses during pasta processing have been reported and vary widely according to the extent of lipoxygenase activity in the durum wheat kernel (Borrelli et al. 2003). Relevant losses (up to 48%) were observed during the kneading-extrusion phase while the drying step does not appear to induce significant changes (Hidalgo et al. 2010). Processing, by determining structural changes in the food structure, also affects the bioaccessibility of carotenoids, with reported values of about 70% in durum wheat pasta versus 57% in pasta containing 10% egg (Werner and Böhm 2011).

Vitamin B complex

Vitamins are essential organic micronutrients that are not synthesized by the human body, but by plants and microorganisms. The water-soluble B vitamins in wheat grains include thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folate (B9). They act *in vivo* in the human body as coenzymes or their precursors, and/or as factors involved in genetic regulation and genomic stability. Wheat grain is an important source of B vitamins, and a high genetic variation in their content among and within wheat species was reported. Additionally, the presence of B vitamins is not only

influenced by the genotype but also by the environment and by the genotype x environment interactions (Davis et al. 1981; Shewry et al. 2011).

Batifoulier et al. (2006) observed concentrations of 2.59-5.41, 0.53-1.07 and 1.44-3.05 mg/kg d.m. vitamin B1, B2 and B6, respectively, in 46 bread wheat cultivars, values similar to those (3.6-5.2, 1.1-1.4 and 2.6-5.7 mg/kg d.m.) found in 378 bread wheat accessions by Davis et al. (1981). Shewry et al. (2011) instead, reported higher ranges for B1 and B2 (5.53-13.55 and 0.77-1.40 mg/kg d.m. respectively) in 26 bread wheat lines. Lower levels of B1 vitamin were observed by Parveen et al. (2015) in eight wheat cultivars (1.22-1.95 mg/kg) and by Witten and Aulrich (2018) in 151 wheat genotypes (1.58-2.96 mg/kg). B2 and B6 vitamins, instead, were in the same range i.e. 0.62-1.19 mg/kg (Witten and Aulrich 2018) and 2.23-2.86 mg/kg (Parveen et al. 2015), respectively. In durum wheat, Batifoulier et al. (2006) reported concentrations of 4.73, 0.70 and 1.91 mg/kg d.m. for B1, B2 and B6; these results are similar to those found by Tekin et al. (2018) in three durum wheat lines (4.95-5.66, 0.46-0.92 and 2.46-4.07 mg/kg, respectively) and by Davis et al. (1981) in 28 durum genotypes (3.9-4.8, 1.3-1.4 and 3.7-5.1 mg/kg d.m., respectively), except for B2 and B6 which were found in higher amount.

Davis et al. (1981) reported higher levels of vitamin B3 in durum wheat accessions (65.3-75.9 mg/kg d.m.) than in bread wheat (43.3-67.0 mg/kg d.m.), but very low levels of B3 (0.16-1.74 mg/kg d.m.) were observed by Shewry et al. (2011) analysing 26 bread wheats. Vitamin B5 content was 0.88-4.04 mg/kg in three durum wheats (Tekin et al. 2018), while mean B9 concentration was 0.323-0.774 mg/kg d.m. in 150 bread wheat genotypes (Piironen et al. 2008).

B vitamins in wheat are mostly concentrated in bran and germ. The aleurone layer is particular rich in vitamin B3 (171-741 mg/kg d.m.; Ndolo et al. 2015; Pomeranz 1988) and B9 (4.0-6.0 mg/kg fresh weight; Fenech et al. 1999). In white flour, the vitamin B content is significantly lower, i.e. 1.46-2.19 (B1), 0.43-0.58 (B2) and 0.28-0.52 mg/kg d.m. (B6) than those in whole meal (2.24-4.16, B1; 0.75-0.96, B2; and 1.31-2.58 mg/kg d.m., B6) (Batifoulier et al. 2005, 2006). Lebiedzińska et al. (2018) reported a total B6 vitamin concentration of 1.85 mg/kg in spelt flour and 3.27 mg/kg in spelt whole meal, while Keagy et al. (1980) in white flours observed only 32% (B1), 35-42% (B2) and 15% (B6) of the whole wheat content. Therefore, a relevant vitamins loss occurs during milling; consequently, the intake of whole meal products is highly recommended to fulfill the recommended dietary allowance for the B vitamins.

In general, during the classical breadmaking process the loss of B vitamins is significant. According to Nurit et al. (2016), breadmaking elicited significant losses of vitamins B1, B5, and B6, but a significant increase of vitamin B2; the B3 vitamers, instead, showed contrasting variations: while nicotinic acid decreased, nicotinamide increased. As a way to limit the loss of some B-group vitamins, Batifoulier et al. (2005) proposed a long yeast fermentation, which leads to an increase of B1 and B2 concentrations as the result of yeast metabolism.

The impact of processing on B9, one of the most important vitamins for normal human metabolic function, has also been studied. Germination of grain seems to have the highest impact on folate content of flours: Koehler et al. (2007) reported a folate content of 0.58 mg/kg d. m. while values of 0.14 mg/kg where reported by Hefni and Witthoft (2012) but both groups observed increases of 3-6 folds of total folate upon grain germination. The breadmaking process does not seem to impact on the content of flour native folates (B9): Gujska and Majewska (2005) reported that although total content of flour native folate increased from flour to proofed dough as the result of the action of fermenting yeast, it decreased upon baking to return in bread to values similar to those recorded in flour; however, significant losses (between 12 to 21 %, depending on the specific processing parameters) were observed for folic acid added to flour for fortification purposes.

Conclusions

Wheat is largely consumed in the form of breads, pasta and other processed products and the content and properties of specific wheat grain components in these products compared to the native grain can greatly differ. Studies reported in this review underlay how the major nutritional losses occur at the milling step, with the refinement of the flour, which is carried out mainly to improve its processing properties, storability and safety through the removal of undesirable molecules (mycotoxins, pesticides and some heavy metals). It is therefore essential to develop milling methods that minimize the loss of bioactive components in flour while optimizing also its commercial and processing quality. Further processing technologies have more specific effects on the different micronutrients and bioactive components. In particular: sourdough fermentation decreases the content of phytic acid and increase the bioavailability of minerals and the content of soluble cell wall polysaccharides and phenolics; yeast fermentation decreases the amount of dietary fibre in the form of fructans but help maintaining or increasing the content of native folates from flour with varying effect on other B group vitamins; high heat and high pressure extrusion used in breakfast cereals and snacks has a major impact on cell wall polysaccharides, increasing their extractability in water, but reducing the content of tocols; extrusion used in pasta making increases the content of resistant starch while pasta cooking drastically reduces the content of most soluble components, including fructans, betaine, choline, lignans and sterols.

It is clear therefore, that improved/targeted processing technologies could bring to substantial gain in nutritional value and health benefits of both wholegrain and refined wheat-based products.

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