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REVIEW

Optimizing the bioactive potential of wheat bran by processing

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1. What is the wheat bran?

Wheat bran is the outermost fraction of the wheat kernel. It consists of multiple layers and altogether is around 10–15% of the kernel weight (Fig. 1). From the inside out the bran is composed of: the aleurone layer, the hyaline layer (nucellar epidermis), the testa or seed coat, the inner pericarp (cross and tube cells), and the outer pericarp.[1] The aleurone layer represents 50% of the bran and it is composed of thick non-lignified cell walls enclosing bioactive intracellular compounds (phytate and niacin inclusions) and protein.[2–4] The hyaline layer is composed of arabinoxylan with high amounts of monomer ferulic acid and low amount of dimers, which indicates few crosslinks.[5] The testa is a hydrophobic tissue rich in lignin and lipidic bioactives, such as the alkylresorcinols.[6] The inner and outer pericarp are composed of empty cells, the cytoplasmic degeneration of cross and tube cells is preceded by a thickening and lignification of the cell walls.[7] Their cell walls have a high content in branched heteroxylans, cellulose and lignin and dimers of ferulic acid cross-linking the polysaccharides.[8,9] All these layers constitute the bran fraction, of which the main physiological function is the protection of the seed.

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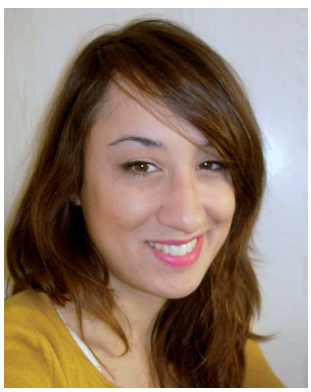
2. Bioactive compounds in wheat bran

The term bioactivity actually refers to a modulating effect on any particular biological process in a living cell or organism, however, it is often used in terms of human health. Bioactivity is no longer merely restricted to drugs but also used for food components with health benefits. The bioactive compounds present in wheat bran are reviewed below in relation to their antioxidant and anti-inflammatory activities. By definition, an antioxidant is a molecule that protects a biological target against oxidative damage.[10] Free radicals are any species containing one or more unpaired electrons (electrons singly occupying a molecular or atomic orbital).[10] The term of reactive species is more general since it includes free radicals and nonradicals. For instance, reactive oxygen species (ROS) include oxygen radicals and some nonradical derivatives that are generated during the



Nuria Mateo Anson

Nuria Mateo Ansón (9/6/1982, Zaragoza, Spain) graduated in Food Science, Public Health, and Food Technology from Zaragoza University in 2005. Thereafter, her first research experience abroad started with a Leonardo da Vinci grant, which first was 6 months in the Netherlands and became 6 years. During those years, she embarked together with the Maastricht University and TNO Zeist on her PhD entitled "Bioactive Compounds in Whole Grain Wheat" within the European project HealthGrain. Besides obtaining her Doctoral degree in 2010 she received several awards: the Exxentia International Award, and Kootstra Fellowship for talented Researchers. Her career is still ongoing as a postdoctoral researcher.

Youna M Hemery

Youna Hemery has been a research fellow at the Institute of Research for Development (IRD, research unit NUTRIPASS, Montpellier, France) since 2011. She obtained her Ph.D in Food Science and Technology in 2009. During her Ph.D. she worked at INRA (research unit IATE) and participated in the European HEALTHGRAIN project. Her thesis focused on the physico-chemical properties of wheat grain peripheral layers, novel bran fractionation technologies, and nutritional potential of bran fractions. She carried out a post-doctoral stay at IRD in Madagascar, to study the nutritional quality of local plant foods, with the aim of improving micro-nutrients intake in children.

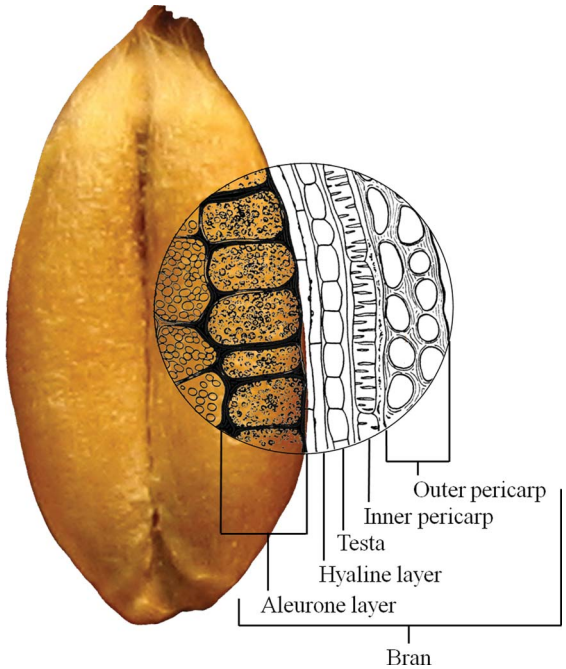


Fig. 1 A histological representation of the main constitutive layers of the wheat bran.

reduction of oxygen. ROS are free radicals, such as superoxide ($O_2^{\cdot-}$) and hydroxyl ($^{\cdot}OH$), and nonradicals, such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), and lipid hydroperoxides (LOOH).[11] Other reactive species are reactive nitrogen species, such as nitric oxide ($^{\cdot}NO$), and reactive chlorine and bromine species.[12] These reactive species are produced in the inflammatory process and in other physiological cellular processes. However, their uncontrolled production is pathological, leading to oxidative stress and the consequent oxidative damage of biologically important molecules, such as lipids, proteins and DNA.[11,13–16]

The content of bioactive compounds in wheat grain and bran are given in Table 1. The wide ranges found in the contents of some compounds are the result of a difference in environmental conditions, geographical areas of cultivation, genetic factors and evolution, varieties, or extraction and quantification methods. The largest and most diverse group of bioactives in wheat bran is the group of the phenolic compounds. They are considered as secondary metabolites in the plant physiology. Some of these compounds are potent antioxidants by multi-faceted mechanisms. The free radical scavenging activity is the best documented. The hydroxyl group of the phenolic ring donates one

Table 1 Content of bioactive compounds per 100 g of wheat grain and wheat bran[a]

Bioactive	Wheat grain	Wheat bran	Ref.
Phytic acid	910–1930 mg	2180–5220 mg	46,173
Ferulic acid	10–200 mg	500–1500 mg	5,26,46,174
Alkylresorcinols	28–140 mg	220–400 mg	6,46,175
Vitamin E	1.4–2.2 mg	1.4 mg	46,176
Betaine	6.9–290 mg	1000–1300 mg	46,177,178
Choline	1.6–14 mg	47–100 mg	46,63,177
Niacin	4.0–9.3 mg	14–18 mg	176,179–181
Pantothenic acid	0.7–1.1 mg	2.2–3.9 mg	176,182
Riboflavin	0.19–0.37 mg	0.39–0.75 mg	113,176,181
Biotin	4.6–11.6 mg	0.048	183
Thiamin	0.26–0.61 mg	0.54 mg	113,129
Pyridoxine	0.15–0.32 mg	1–1.3 mg	63,184
Folate	20–87 μg	79–200 μg	46,176,185
Lutein	37–560 μg	97–140 μg	51,186–188
Glutathione	82–670 μg		189,190
Iron	3.2 mg	11 mg	46,176
Manganese	3.1 mg	12 mg	46,176
Zinc	2.6 mg	7.3 mg	46,176
Selenium	0.5–75 μg	78 μg	46,176

[a] Assuming that 13% of the grain is water, the dry weights have been converted to wet matter.

Aalt Bast

Prof. Dr. Aalt Bast is chairman of the Department of Toxicology at the Maastricht University. He studied chemistry in Amsterdam and did his PhD in Rotterdam (Medical Faculty) and Utrecht (Faculty of Pharmacy). In 1988 he became professor in Molecular Pharmacology at the Free University in Amsterdam, which was followed by a chair in Human Toxicology in Maastricht at the Faculty of Health, Medicine and Life Sciences. His research focuses on the interface between food and pharma in cardiovascular, pulmonary and liver disease. A special interest is the therapeutic modulation of redox processes. He (co-)authored over 450 papers and book chapters.

Guido R. M. M. Haenen

Guido Rembertus Michiel Marie Haenen (17/6/1959, Axel, The Netherlands) was registered as a Pharmacist in 1985 (with honors), and he obtained his Ph.D. in Medicinal Chemistry in 1989. He was awarded the 'Shell Prize' for his thesis entitled 'Thiols in Oxidative Stress'. In 1998 he joined the Department of Toxicology of Maastricht University. This year he received the 'Onderwijsprijs' for being the best teacher at Maastricht University. He is registered as a Toxicologist, Clinical Pharmacologist and Pharmacist. His research activities focus on the role of free radical processes in disease and the modulation of free radical toxicity by drugs and nutrients.

electron to the radical molecule, which is followed by a rapid proton transfer. The net result is the equivalent to one hydrogen atom transfer to the free radical. In turn, the phenol is oxidized. However, the phenol radical does not propagate the radical reaction, since it is relatively stable due to resonance, in which the unpaired electron is delocalized to the *ortho* or *para* position of the phenyl ring. Finally, the oxidized antioxidant can be converted back to its reduced form by enzymatic and non enzymatic antioxidants.[17] The phenolic compounds found in wheat grain are basically phenols containing one aromatic ring: phenolic acids, such as ferulic acid, sinapic acid, and *p*-coumaric acid, alkylresorcinols, and vitamin E. The polyphenols found in wheat grain are mainly lignins and lignans.

2.1. Phenolic acids

Ferulic acid is the most abundant phenolic acid in wheat bran (Table 1). Ferulic acid is the common name for 3-(4-hydroxy-3-methoxyphenyl) propionic acid (Fig. 2). It is mostly located in the bran of the wheat grain, where it occurs linked by ester binding to cell wall polysaccharides.[18,19] The antioxidant potential of ferulic acid is mainly attributed to the electron donation and hydrogen atom transfer to free radicals.[17] Its ability to inhibit lipid peroxidation by superoxide ($O_2^{\cdot-}$) scavenging is of greater magnitude than that of cinnamic acid and *p*-coumaric acid but less than that of caffeic acid.[20,21] The presence of a hydroxyl group instead of the methoxy group at the C3 substantially increases the radical-scavenging activity.[22] Its ability to inhibit oxidation of low-density lipoprotein (LDL), the main cholesterol carrier in blood, is greater than that of ascorbic acid.[23] Also ferulic acid was more effective than vanillic, coumaric and cinnamic acids in protecting ˙OH induced protein peroxidation in the synaptosomes.[24] The ferulic acid radical (phenoxy radical) that is formed from its oxidation is very stable and does not initiate an oxidative chain reaction in its own,[25] the presence of the methoxy group enhances the resonance stabilization.[22,26] A recent study using model systems has shown that ferulic acid reduces the formation of certain Maillard reaction products (MRPs), such as advanced glycation end products (AGEs),[27] which are associated with diabetic complications.[28] In traditional Chinese medicine ferulic acid has been used for years,

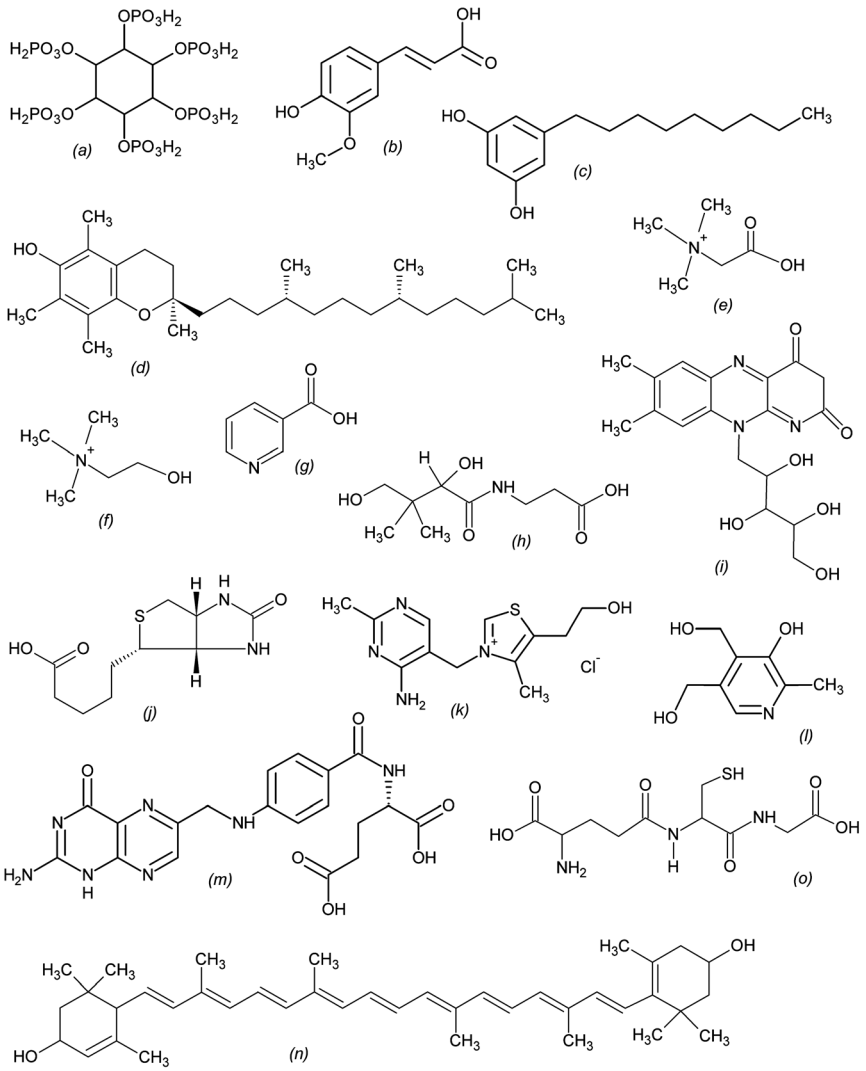


Fig. 2 Chemical structures of phytic acid (a), ferulic acid (b), alkylresorcinol (c), vitamin E (d), betaine (e), choline (f), niacin (g), pantothenic acid (h), riboflavin (i), biotin (j), thiamin (k), pyridoxine (l), folate (m), lutein (n), glutathione (o).

and it is approved by the State Drugs Adminstration of China as a drug for the treatment of cardiovascular and cerebrovascular diseases.[29] Ferulic acid has numerous anti-inflammatory effects, as documented in the Table 2 and Table 3. The main anti-inflammatory action of ferulic acid seems to be mediated by the regulation of cyclooxygenase (COX) and the MAP kinase/NFκB pathway. Although recent investigations suggest that ferulic acid may act as a hormetic agent interfering in the Nrf-2/ARE pathway and the expression of protective genes.[30]

2.2. Alkylresorcinols

Alkylresorcinols are also very abundant in wheat bran and they are specifically located in the testa (Table 1).[6] They are amphiphilic molecules consisting of 2-hydroxyphenol and an alkyl side chain of different length at position 5, the most common are C15:0, C17:0, C19:0, C21:0, C23:0, and C25:0 (Fig. 2). They have little hydrogen donation and peroxyl scavenging activities,[31] but they protect against lipid peroxidation of membranes.[32] This is due to the lipophilic nature of the alkyl chain in alkylresorcinols, which confers membrane modulating effects by interactions with phospholipids or proteins in the embranes.[31,33] Additionally, alkylresorcinols can prevent *in vitro* oxidation of fatty acids[34] and LDL oxidation.[35] Alkylresorcinols are phytochemicals rather specifically from grain sources and they are, therefore, commonly used as biomarkers of whole-grain consumption.[36]

2.3. Vitamin E

Vitamin E is a lipophilic vitamin and is primarily found in the germ of the wheat grain. Vitamin E is the collective name for a set of eight related compounds or vitamers: α-, β-, γ-, and δ-tocopherols and the corresponding four tocotrienols. Despite the fact that all the different forms of vitamin E have antioxidant activity, α-tocopherol is preferentially maintained in plasma. This is due to (i) the specific binding to the α-tocopherol transfer protein and (ii) the extensive hepatic metabolism of the other vitamers.[37] The α-tocopherol molecule consists of a chroman head, which is responsible for the antioxidant function, and a phytyl side chain that intercalates with the phospholipids of the cell membrane. The free hydroxyl group on the aromatic ring is responsible for the antioxidant properties (Fig. 2). The hydrogen from this group is donated to the free radical, resulting in a relatively stable free radical form of vitamin E.[38] In this way, vitamin E molecules can interrupt free radical chain reactions. Vitamin E has also protective effects on glutathione-dependent enzymes.[39] The function of vitamin E in the human body has been recently reviewed, the major function appeared to be as radical scavenger protecting polyunsaturated fatty acids from oxidation, hereby maintaining the integrity of the cell membrane.[37]

2.4. Lignin and lignans

Lignin biopolymers have a heterogeneous structure; they constitute 30% of plant biomass and belong to the most abundant organic polymers on earth. Lignins are a major component of whole-grain cereals, and may account for 3–7% of the bran fraction.[40,41] Their polyphenolic structure confers potential antioxidant capacities,[42] such as protection of DNA oxidative damage in cells.[43,44] Lignins can be metabolized into mammalian lignans.[45]

Table 2 Effects of ferulic acid on the reduction (↓) or increase (↑) of inflammatory messengers, inducible nitric oxide synthase (iNOS) activity, reactive oxygen species (ROS) production and superoxide dismutase (SOD) activity in several *in vitro* and *in vivo* models of inflammation

In vitro model of inflammation	Inflammatory messengers	iNOS activity	ROS	Ref.
LPS-BV2 microglial cells	↓↓↓↓	↓		191
Glutamate toxicity in cortical neurons	↓[a]			192
LPS/INF-γ-RAW macrophages	No effect	No effect		193
Aβ stimulated histocyte from rats	↓	↓		194
Human PBMC	↑			195
PHA-splenocytes	↓			196
LPS/INF-γ-RAW macrophages	No effect			197
PMA-adenocarcinoma cells (MTLN)	↓[a]			198
LPS-RAW macrophages	↓			199
Influenza virus-RAW macrophages	↓			200
Respiratory burst in polymorphonuclear cell			↓	201

In vivo model of inflammation	Inflammatory messengers	iNOS activity	SOD activity	Ref.
Osteoarthritis in rats		↓		202
Aged rats	↓↓↓[a] ↓[b]	↓		203
Acetic acid induced colitis in rats	↓↓[a] ↓[b]	↓		204
Nicotine toxicity in rats	↓[a] ↓[b]			205
Hemorrhagic shock after reperfusion in rabbits	↓		↑	206
Aβ induced toxicity in hippocampus in rats	↓[a]			207
Aβ induced Alzheimer in rats	↓↓[a]			208
Aβ toxicity in hippocampus in rats	↓			209
Aβ toxicity on astrocytes of mice	↓	↓		210

[a] Transcription factors or kinases involved in the production of cytokines and prostaglandins. [b] Enzyme cyclooxygenase (COX).

Table 3 Effects of ferulic acid (FA) derivatives on the reduction (↓) or increase (↑) of inflammatory messengers, inducible nitric oxide synthase (iNOS) activity, and superoxide dismutase (SOD) activity in several *in vitro* models of inflammation

FA derivative	*In vitro* model of inflammation	Inflammatory messengers	iNOS activity	SOD activity	Ref
FA dehydromer	PHA-splenocytes	↓			196
NO-releasing derivative of FA	Carrageenan-RAW macrophages	↓↓[b]			211
NO-releasing derivative of FA	LPS/INF-γ-RAW macrophages	↓[a]	↓		212
Phytosteryl ferulate	LPS-macrophages	↓[b]	↓	↑	213
FA ethyl ester	Hippocampal cultures			↑	214
2-methyl-1-butyl ferulic acid	LPS/INF-γ-RAW macrophages	↓[a]↓[b]	↓		197
Phenethyl FA in extract of Qianghuo	COX assay	↓[b]			215
FA containing ethyl acetate extract from adlay testa	LPS-RAW macrophages	↓[b]	↓		216
Colonic metabolites of FA	Colonic HT-29 cells	↓[b]			217
Colonic metabolites of FA	IL-1β-fibroblasts	↓			218
Colonic metabolites of FA	LPS-PBMC	↓↓↓			219

[a] Transcription factors or kinases involved in the production of cytokines and prostaglandins. [b] Enzyme cyclooxygenase (COX).

Lignans are dietary phytoestrogens that are present in a wide variety of plant foods, including whole grain wheat. The group includes secoisolariciresinol, matairesinol, lariciresinol, pinoresinol and syringaresinol. They all have a polyphenolic structure and have antioxidant effects.[40,46,47] Lignans and their metabolites, the mammalian lignans enterodiol and enterolactone, have antioxidant activity in different lipid and aqueous *in vitro* model systems and decrease lipid oxidation.[48] An antioxidant mechanism of lignans may be metal chelation.[49] Lignans have less marked effects than lignins upon oxidative genetic damage.[50]

2.5. Carotenoids

Carotenoids are another group of bioactive compounds in bran that contribute to the pigments of wheat grain. Lutein is the most predominant carotenoid (70–80% of all carotenoids), followed by zeaxanthin and β-carotene.[51] They are lipophilic compounds and they contain a system of conjugated double bonds that allow them to interact efficiently with reactive oxygen species. The antioxidant activity of the carotenoids is characterised by (i) their relative ability to scavenge the 2,2′ azino-bis(3-ethylbenzthiazoline) 6-sulfonic acid (ABTS) radical cation, reflected by the so-called trolox equivalent antioxidant capacity (TEAC), (ii) their relative rate of oxidation by a range of free radicals, and (iii) their capacity to inhibit lipid peroxidation, leading to a decrease in the formation of thiobarbituric acid reactive substances (TBARS).[52,53]

2.6. Methyl donors

Wheat bran also contains substantial amounts of the methyl donors: betaine, choline and folate (Fig. 2). They participate in recycling the potentially toxic amino acid homocysteine to methionine and, ultimately, to the methyl donor *S*-adenosylmethionine (SAM). Betaine (trimethylglycine) is present in wheat grain, mainly in the bran (1%) (Table 1), but it can also be formed from oxidation of choline in liver and kidney. The two principal biological functions of betaine are as osmolyte and as methyl donor.[54] Choline is also contained in wheat grain, although in lower amounts than betaine (Table 1). Choline can also be synthesized in the liver.[55]

2.7. B-vitamins

Folates are classified as B-vitamins, namely vitamin B_9. They are present in the grain mainly as reduced forms (tetrahydrofolates) rather than as folic acid (pteroylmonoglutamic acid). Tetrahydrofolates have a varying number of glutamyl residues (1–7) and can be methylated or formylated at N5 and N10 (Fig. 2). Among all these possible structures, 5-methyltetrahydrofolate is biologically the main active form (Table 1).[56] Some forms of folate have radical scavenging properties *in vitro*[57] and prevent mitochondrial dysfunction and apoptosis *via* intracellular superoxide scavenging ($O_2^{\cdot-}$).[58] However, the main mechanism of folate in antioxidant protection has been reported to be indirect, by lowering homocysteine[59,60] and as an electron and hydrogen donor to tetrahydrobiopterin (H_4B), an essential cofactor for the endothelial nitric oxide synthase (eNOS) to form nitric oxide.[59,61]

Wheat grain also contains several other B-vitamins, mainly niacin, pantothenic acid and riboflavin. They are mainly contained in the bran (Table 1), especially in the aleurone layer. Cereals and cereal products contribute around 30% of the daily intake of these vitamins in the diet.[62] Niacin or vitamin B_3 is a water soluble vitamin abundant in wheat grain (Fig. 2). Besides the dietary source, niacin can be formed from tryptophan in liver. Niacin is the generic term to refer to nicotinic acid (pyridine-3-carboxylic acid), nicotinamide, and derivatives exhibiting the biological activity of nicotinamide.[63] Nicotinamide is used to form the coenzymes nicotinamide adenine dinucleotide [NAD(H)] and nicotinamide adenine dinucleotide phosphate [NADP(H)]. NAD+/NAD(H) and NADP+/NADP(H) are required by as many as 200 enzymes to accept/donate electrons in redox reactions, as well as for the activity of the enzyme poly(-ADP-ribose) polymerase-1, involved in DNA synthesis and repair.[64] Niacin is used for the treatment of dyslipidemia and atherosclerosis for years. This vitamin has also been reported to increase the redox state (glutathione reductase needs NADPH for regeneration of GSH) that leads to a decrease in ROS and LDL oxidation,[65] and at the same time to inhibit redox-sensitive genes in aortic endothelial cells.[66] Pantothenic acid or 3-[(2,4-dihydroxy-3,3-dimethylbutanoyl)amino]propanoic acid is also known as vitamin B_5 (Fig. 2). Pantothenic acid is a water soluble vitamin that cannot be synthesized in the human body, but it is

widely available in the diet, especially in wheat grain and bran. Pantothenic acid and its reduced derivative pantothenol are precursors of two important enzyme cofactors: coenzyme A (CoA) and acyl carrier protein (ACP). Both cofactors contain a sulfhydryl group (–SH), which reacts with activated carboxylic acids to form thioesters, such as with acetic acid to form acetyl-CoA.[67] CoA and ACP have important functions in oxidative metabolism, in transfer reactions in the citric acid cycle. Pantothenic acid could increase the glutathione (GSH) cellular levels in cell experiments, which was explained by an increased ATP production, which is necessary for GSH synthesis, associated to higher mitochondrial CoA.[68,69]

Riboflavin or 7,8-dimethyl-10-ribityl-isoalloxazine is also known as vitamin B_2 (Fig. 2). In wheat grain only a small amount of riboflavin is present as such, while the most of it is present as flavin adenine dinucleotide (FAD) and a smaller amount as flavin mononucleotide (FMN). Upon digestion, FAD and FMN need to be hydrolyzed to riboflavin in order to be absorbed. FAD and FMN act as intermediate hydrogen acceptors in the mitochondrial electron transport chain and pass on electrons to the cytochrome system in the cellular respiration.[70] Riboflavin does not have inherent antioxidant action, but FAD forms the catalytic centre of glutathione reductase, an enzyme that converts glutathione disulfide (GSSG) into GSH.[70]

Other B-vitamins present in wheat bran are biotin, thiamin and pyridoxine (Table 1). Biotin is the trivial designation of the compound hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid (Fig. 2). Biotin is present in wheat grain in two forms: free and bound to protein lysyl residues (biocytin). Biotin functions metabolically as a coenzyme for carboxylases. Biotin deprivation has been shown to increase the nuclear translocation, binding and transcriptional activity of the pro-inflammatory molecule NF-κB.[63] Thiamin or vitamin B_1 is the compound 3-(4-amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium (Fig. 2). Grain and grain products are good sources of this vitamin in the human diet providing around one third of the dietary thiamin. In the wheat grain, thiamin is typically found as free thiamin in the scutellum (a thin layer between the endosperm and the germ). Baker's yeast is also a source of thiamin. Thiamin is essential in carbohydrate metabolism and neural function.[63] Pyridoxine or vitamin B_6 is the generic name for all 3-hydroxy-2-methylpyridine derivatives with similar biological activity of pyridoxine [3-hydroxy-4,5-*bis*(hydroxymethyl)-2-methylpyridine] (Fig. 2). Pyridoxine is widely distributed in foods, occurring in the greatest concentrations in wheat grain products. Bread accounts for the 16% of daily requirements of pyridoxine.[71] In the wheat grain, this vitamin is mainly located in the aleurone and in the germ. The metabolic active form of vitamin B_6 is pyridoxal phosphate, which functions as a coenzyme for reactions involving aminoacids.[63]

2.8. Glutathione

Wheat flour contains substantial amount of thiols in the form of cysteine and glutathione (GSH), which are reported to have some weakening effects on the dough by the formation of disulfide bonds with gluten proteins.[72,73] GSH is a tripeptide of the amino acids cysteine, glycine, and glutamic acid (L-gammaglutamyl-L-cysteinyl-glycine) (Fig. 2). It is produced in the liver and other organs in the human body and it is present in all cells. GSH is found free or bound to proteins in the cell.[74] GSH participates directly in the neutralization of free radicals and reactive oxygen species. GSH also participates in indirect antioxidant mechanisms; GSH acts as an electron donor in enzymatic reactions, such as that by GSH-dependent dehydroascorbate reductase to regenerate ascorbate (vitamin C) from its oxidation product dehydroascorbate.[75,76] GSH is also used by glutathione peroxidases and glutathione-*S*-transferases in the detoxification of peroxides. In both reactions GSH acts as an electron donor, which leads to its oxidation to glutathione disulfide (GSSG). As mentioned above, the cellular GSH pool can be regenerated from GSSG *via* the NADPH-dependent enzyme glutathione reductase. Therefore, GSH is considered an important endogenous antioxidant.[77]

2.9. Phytates

The phytates is the generic term for myo-inositol tri- (IP3), tetra- (IP4), penta- (IP5) and hexakis- (IP6) phosphate (Fig. 2). Phytic acid (IP6) constitutes the main storage of phosphate in the seed, it is mainly contained in the bran (Table 1), which function was believed to be protection against oxidative damage during storage. The antioxidant activity of phytic acid is mainly attributed to iron chelating properties, which interrupts the reactions of the Haber–Weiss cycle. As a consequence, the formation of hydroxyl radicals (˙OH) is prevented, which in turn can prevent lipid peroxidation.[78] Phytic acid has also been shown to inhibit xanthine oxidase mediated $O_2^{\cdot-}$ generation.[79] However, phytic acid forms insoluble complexes with dietary cations (Mg, Ca, Fe, Zn) that impairs mineral absorption in humans.[80] Although dietary phytates are substantially hydrolyzed during digestion by phytases, which cleave the phosphate groups from the inositol ring, reducing the chelating activity.[81,82] Nevertheless, the nutritional effects of phytate seem more detrimental than beneficial.

2.10. Minerals

Wheat bran contains considerable amounts of iron, magnesium and zinc, as well as lower levels of many trace elements, *e.g.* selenium and manganese. They are highly concentrated in the aleurone.[4] Their contents vary greatly depending on the location due to the soil characteristics.

Iron (Fe) is the most abundant trace element in the body, and almost all iron is bound to proteins. Free iron concentrations are particularly low for two reasons: Fe^{3+} is not water soluble, and Fe^{2+} participates in the generation of free radicals, such as ˙OH production by the Fenton reaction in the Haber–Weiss cycle. An increase in free iron concentrations can result from dietary protein deficiency, dietary iron loading, low concentrations of iron-binding proteins, or cell injury. This will result in production of reactive oxygen species, lipid peroxidation, and oxidative stress. Increasing the extracellular concentration of non-heme iron also enhances inducible nitric oxide synthase (iNOS) protein expression and inducible NO synthesis in many cell types, which can further exacerbate oxidative damage *via* peroxynitrite generation.[83]

Manganese (Mn) is essential for many ubiquitous enzymatic reactions, such as the manganese superoxide dismutase (Mn-SOD), which catalyzes the dismutation of $O_2^{\cdot-}$ into O_2 and H_2O_2. Consequently, a deficiency of this mineral markedly decreases the Mn-SOD activity and results in peroxidative damage and mitochondrial dysfunction.[83]

The main function of zinc (Zn) is in a structural role as the zinc finger involved in the DNA domains of many proteins, peptides, enzymes, hormones, transcriptional factors and growth factors, including cytokines, relevant to the maintenance of body homeostatic mechanisms. A zinc finger is made up of a short stretch of 28–40 amino acids containing a characteristic Cys2His2 (cysteine, histidine) motif that are stabilized by one or more zinc ions.[84] Zinc also plays a critical role in the structure, function, stabilization and fluidity of biomembranes because of zinc binding to thiol groups.[85] The antioxidant action of zinc is as cofactor for the activities of Cu/Zn-superoxide dismutase in the conversion of superoxide to oxygen and hydrogen peroxide.[86]

Selenium (Se) is an essential trace mineral that occurs mainly as selenomethionine (Se-Met) in cereal grains. Se-Met can be non-specifically incorporated into proteins as a substitution for methionine. Se-Met can also be converted into selenocysteine (Se-Cys) and into inorganic selenium by demethylation. Se-Cys is an important component of selenoproteins, such as selenoprotein P (main plasma carrier of Se), iodothyronine deiodinases, thioredoxin reductase and the selenium-dependent glutathione peroxidases. These selenoproteins are all Se dependent, and generally have Se-Cys at their active sites. In these enzymes Se functions as a redox centre.[87] The best-known example of this redox function is the reduction of hydroperoxides by the family of Se-dependent glutathione peroxidases.[88]

3. Bioaccessibility and bioavailability

The concept of bioavailability originates from the pharmacological term referring to the fraction of an oral dose that reaches the systemic circulation. In nutritional sciences, however, the bioavailability rather refers to the efficient use of nutrients by the body. First factors involved in the bioavailability of food compounds are the intake as well as the bioaccessibility, *i.e.* the proportion of a compound that is released from the food matrix and becomes free for possible intestinal absorption.[89] The intake of bioactive compounds depends on their content in the bran-rich food. The bioaccessibility from the product is restrained by compound-food matrix interactions. Both the content in bioactive compounds of wheat bran and their bioaccessibility can be affected by different wheat processing techniques as it is reviewed hereafter.

4. Effects of processing on the bioactivity, bioaccessibility and bioavailability of wheat bran

4.1. Processes before the milling

4.1.1. Pretreatments. Wheat bran and, in general, grain can be pretreated before the milling process for the purpose of improving the tissue dissociation. The conditions of some frequent pretreatments can also modify the biochemical properties and biological activity of the bran. The most common pretreatment is tempering, during which the moisture content is increased. This results in an increase in the extensibility of the bran, which facilitates the separation of the bran from the starchy endosperm during the milling.[90] The water used during tempering may include chemical agents (sodium chloride) or enzymes (cellulose, xylanase, beta-glucanase) in the tempering water. These processes can induce changes in the physical and biochemical properties of wheat bran.[91–93] For instance, in the study of Desvignes *et al.* the addition of sodium chloride in the tempering water increased the concentration of extractable *p*-coumaric acid, which was suggested to be the result of modifications in the cell-wall polymers that allow a higher accessibility to the phenolic acids of the bran.[94] In addition to tempering, physical pretreatments may be performed. Possible physical pretreatments are: ozone treatment, IR radiation and UV radiation. The effects of UV radiation on the composition of wheat bran have been investigated by Peyron *et al.*[95] UV exposure for 48 h resulted in a 25% decrease in ferulic acid monomer and a 44% decrease in dehydrodiferulic acid ester-linked to the cell-wall arabinoxylans. This reduction partly explained the significant increase of ferulic acid (30%) and dehydrodiferulic acid (36%) engaged in hot alkali-labile linkages. The results of this study suggest that UV irradiation induced the formation of new cross-links between feruloylated arabinoxylan and lignin in the pericarp. The induction of these links might consequently affect the bioaccessibility of ferulic acid from the wheat bran matrix. Although these processes can affect the bioaccessibility of bioactive compounds from the bran matrix, they have been mainly studied with the aim at improving the flour yield, thus the extraction of flour from the bran during the milling.

4.1.2. Germination. Germination is the process in which the plant emerges from the seed. During germination important nutrients, such as dietary fiber, minerals and phytochemicals, located in the bran and germ are reported to increase.[96,97] Also phenolic compounds are increased in germinated or sprouted wheat. The content of phenolic compounds and antioxidant activity were increased in alcohol extracts from flour of sprouted wheat, which were proportional to the length of germination. Caffeic acid and syringic acid increased from 1 to 3.8 μg g^{-1} and from 194 to 369 μg g^{-1}, respectively.[98] Also, other phenolic acids, such as ferulic acid and vanillic acid, increased their concentrations with increasing germination time from approximately 600 to 900 μg g^{-1} and from 6 to 14 μg g^{-1}, respectively, as well as β-carotene from undetectable levels to 3 μg g^{-1}, and α-tocopherol from 4.4 to 10.9 μg g^{-1} and γ-tocopherol from 0.9 to 1.5 μg g^{-1}.[99] Also, total folate rose during germination, reaching a maximum 3.6-fold increase.[96] In germinated rye, besides the higher levels of folates, plant sterols and benzoxazinoids were also increased from 88 to 114 μg per 100 g and from 6.4 to 53 mg per 100 g, respectively.[100] Wheat sprout extracts were able to effectively inhibit DNA oxidative damage *in vitro*, which was attributed to the presence of glycosylated antioxidants.[101]

During germination an increased activity of hydrolytic enzymes, such as xylanases, arabinofuranosidases, β-glucanases, proteases and xylosidases, has been reported.[96,102–104] This will result in hydrolytic transformations of the bran matrix to mobilize nutrients and bioactive compounds needed for the plant growth. This might also result in a higher bioaccessibility and

a higher bioavailability. A higher bioavailability of phenolic compounds was indeed observed from bread enriched with wheat sprouts. Consumption of the enriched bread for 9 days had a glucose lowering effect and enhancement of the plasma antioxidant capacity, which was associated with a higher bioavailability of the phenolic compounds from the wheat sprouts.[105]

4.1.3. Debranning. Debranning is a process in which the bran layers are removed by sequential friction and abrasion. Originally it was used with a cleaning purpose to remove the outermost layers of the grain that have high levels of contaminants, such as microorganisms and heavy metals, as well as a poor nutritional value. Debranning to a removal of 4% of the wheat grain weight reduces the total microbial contamination by more than 80%.[106,107] Recently, debranning processes have gained interest in grain fractionation. The two most common debranning techniques are peeling and pearling. In peeling, the bran removal is by friction of the wheat kernels through the machine and against each other, while in pearling the removal of the bran is by abrasion of the wheat kernels against an abrasive stone. The degree of removal can be controlled by adjusting the time of debranning. The pearling fractions from a bran removal of 5 and 10% of the wheat kernel have the highest phenolic content and antioxidant capacity.[108,109] Therefore, minimizing the bran removal will reduce the loss in bioactive compounds. Following this reasoning, the peeling process may be more interesting than the pearling process, since the bran removal is up to approximately 3–4% of the grain weight compared to the 6–10% of the pearling process.[1] The flours obtained from peeled wheat kernels were indeed higher in antioxidant capacity and ferulic acid concentration than those from pearled wheat kernels.[110] This is attributed to the removal of less of the outer-layers of the kernel by the peeling debranning, which allows the aleurone layer to remain substantially intact.[1,110,111] The aleurone layer, which is overlying the endosperm of the grain and is usually removed with the bran fraction during the milling, is the richest fraction in antioxidant capacity of the wheat grain.[110] The aleurone content of different wheat fractions was strongly correlated with the ferulic acid content and antioxidant capacity of the fraction.[110] Therefore, it is important to preferably preserve the aleurone content during the manufacturing of healthy cereal products.[4]

4.2. Grain milling

In simple words, milling is the process of removing the bran and grinding wheat into flour or semolina. The first step in the milling process is the cleaning of the grains with the aim of removing foreign material (straw, dust, soil, sand), molds and bacteria. After this cleaning step, normally done by polishing, a debranning pretreatment can take place before the tempering. Afterwards, the grinding of the grain is performed in a roller mill. Milling produces the fragmentation of the grain into smaller particles. During this process, a separation normally based on particle size is done, frequently by sieving, that removes the bran and germ from the white flour (mainly endosperm). The study of Borrelli *et al.* shows that the content of α-tocopherol in flour decreased by 60% when increasing the time from 30 to 180 s of milling of the wheat kernel.[112] After milling, 43%, 67% and 20% of thiamin, riboflavin and pyridoxine were recovered in white flour, compared to 80%, 100% and 95% in reconstituted whole wheat flour, respectively.[113] Milling of wheat grain also leads to a decrease in the antioxidant capacity of semolina[114] and to a factor 10 reduction in phenolic acids.[115] Accordingly, the content in other bioactive compounds that are mainly located in the bran will be remarkably reduced by the conventional milling process.

4.3. Bran fractionation

The bran fractionation comprises two steps; fragmentation and separation. In the fragmentation step, the bran tissues are broken down and/or dissociated by grinding. Depending on the fragmentation process, the stress imposed on the bran is of different type (impact, shearing, compression, crushing, attrition, *etc.*), resulting in particles with different properties. In the separation step, the bran particles are sorted out according to certain properties, such as their size, shape, mass, density, or dielectric properties. There are various separation methods, the widely used sieving and air-classification, and other more innovative methods, such as electrostatic separation, which will be further discussed.[1] Another method worth mentioning is the fragmentation process of cryogenic ultra-fine grinding, which has been investigated by Hemery *et al.*[115]

4.3.1. Ultra-fine grinding. Ultra-fine grinding aims at reducing the particle size of the bran fragments. When this process is performed at low temperatures below 0 °C (−10 °C to −140 °C) it is termed cryogenic grinding. In this process liquid nitrogen is used for the cooling. It is suggested that cryogenic grinding makes the particles fragmentation easier and may avoid the degradation of thermo-labile compounds.[116] A decrease in particle size may facilitate solvent–compound interactions and their extractability and bioaccessibility. In this manner, the ultra-fine grinding may increase the bioaccessibility and bioavailability of bioactive compounds from the wheat bran. Hemery *et al.* showed that the decrease in wheat bran particle size results indeed in an increase in the bioaccessibility of ferulic acid and sinapic acid, as well as the total antioxidant capacity of the bioaccessible fraction from bran-rich breads in a gastrointestinal model. In that study, a correlation was found between the quantities of fine particles (diameter of 10–20 μm) and the amount of bioaccessible ferulic acid and sinapic acid. It was observed that ultra-fine grinding of the bran produces the disruption of the cell walls and the aleurone cells.[115,117] This results in smaller fragments of the cell walls and the release of intracellular contents, which can explain the higher bioaccessibility of certain compounds during their gastrointestinal transit.

4.3.2. Electrostatic separation. Electrostatic separation is a separation method based on charging the bran particles with an electric charge, followed by a second step in which the charged particles are separated in an electric field depending on the acquired charge. The effects of electrostatic separation after ultra-fine grinding of bran have been recently investigated by Hemery *et al.*[9,118] By the process of electrostatic separation, the bran particles could be classified into three fractions: a negatively charged fraction, a positively charged fraction and a less charged

fraction. These fractions have different histological and biochemical composition due to the different charging behaviors of pericarp cell-walls and aleurone cell-walls. The negatively charged fraction, richer in outer pericarp, has higher contents of arabinose and thus arabinoxylan, galactose, dimers and trimers of ferulic acid, which are known to be characteristic of the complex heteroxylans of the pericarp.[8] The less charged fraction is characterized by a higher proportion of intermediate layers (including inner pericarp, testa, and hyaline layer), and was therefore the richest in alkylresorcinols, which are known to be located in the testa.[6] The positively charged fraction exhibited higher β-glucans and ferulic acid monomer contents, due to its high content in aleurone cell walls, and it was richer in folates and phytates, because of its high proportion of aleurone intracellular content.[9]

The size of the particles was the lowest in the positively charged fraction, rich in free intracellular aleurone contents and small cell walls fragments, with a median particle diameter of 26.5 μm and 77% of the particles below 50 μm. Besides the variation in the particle size distribution among the fractions, a factor that influences the bioaccessibility of compounds as discussed above, the biochemical composition also differs among the fractions. The biochemical composition of the fraction could be related to their charging behavior. The charge of the particles was positively correlated with the content of ferulic acid. For instance, the most positively charged fraction obtained with this method by Hemery *et al.* represents 34% of the bran and contains 62% of the ferulic acid content in bran.[9] Moreover, the bioaccessibility of ferulic acid, sinapic acid and *p*-coumaric acid were higher from the breads made with the positively charged fraction (3%, 40% and 12%, respectively) than from the breads made with bran (2.5%, 27% and 6%, respectively) when bran-rich breads were investigated in an *in vitro* gastrointestinal model.[115] Thus, this processing technology can be used to obtain fractions of the bran that concentrate specific bioactive compounds with potential use as healthy ingredients or starting material for a follow-up processing.

4.4. Processes after the milling

4.4.1 Bread making. Bread has been a popular staple food for ages. The process of bread making is thus one of the most-well documented processing techniques in cereal technology. It consists of three major stages: mixing, fermentation and baking.[119] Heat processing, such as in the bread baking, and kneading (mixing), in which oxygen comes in contact with labile compounds, can reduce the stability of some bioactives, such as the phenolic compounds.[120,121] The kneading process during bread and pasta manufacturing has been reported to produce significant losses of tocopherols and tocotrienols.[122–125] The loss of tocopherols is reported to be mainly due to direct oxidation or enzymatic oxidation catalysed by lipoxygenase.[123] A reduction in kneading time and intensity associated with a longer period of dough fermentation may retain carotenoids and vitamin E by limiting oxygen incorporation.[124] Baking of bread produces a significant reduction (37–41%) of the lutein content.[126] Antioxidant activity of purple wheat bran, heat-treated purple wheat bran, and purple wheat bran muffins was evaluated to determine the impact of thermal processing on potential health benefits by Li *et al.* The conditions selected for heat treatment did not markedly change the antioxidant activity of purple wheat bran. However, there was a significant reduction in total phenolic content, oxygen radical absorbance capacity (ORAC) values and total anthocyanins during processing of purple wheat bran- or heat-treated purple wheat bran-enriched muffins. On the contrary, muffin extracts still remained excellent in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.[127] Whereas processes involving boiling may lead to losses of around 40% for most B-vitamins and slightly higher losses of folate, losses observed during baking are generally lower.[128] In bread making 31% of thiamin[113,129,130] and 37% of pyridoxine were lost in whole bread and 37% and 62% in white bread respectively due to their instability to heat (baking) and oxygen (kneading).[113] Also, folate losses of around 25% occur during the bread baking process.[131] During the bread making process, the content of phytic acid decreases due to the action of phytases in the dough and the heat.[120] Phytate-degrading enzymes exist in cereals, yeast and lactic acid bacteria isolated from sourdough.[132–134] This hydrolytic effect of bread baking has some advantage, as it may improve the bioavailability of minerals, such as iron, calcium and zinc that is normally hindered by phytate.[62] Remarkably, increasing the baking time from 7 to 14 min and the temperature from 205 to 288 °C of the wheat dough resulted in an increase by 60 and 82%, respectively, of the antioxidant properties, evaluated by different radical scavenging methods. This increase, however, could not be explained by an increase in the concentrations of soluble free or conjugated ferulic acid.[135,136] It might therefore be a result of the formation of Maillard reactive compounds that have been reported to show radical scavenging activities.[137,138] Similarly, increasing the temperature of the baking conditions enhances the antioxidant properties of whole-wheat pizza crust[136] and the phenolic antioxidant content (determined as gallic acid equivalents) of the crust in white bread,[139] probably due to the formation of Maillard reaction products.[137]

4.4.2. Fermentation. Fermentation in food is basically the conversion of carbohydrates into alcohols and carbon dioxide or organic acids by microorganisms under anaerobic conditions. There are two main systems for fermentation processes: submerged fermentation (SmF), which is based on the cultivation of the microorganisms in a liquid medium containing nutrients, and solid-state fermentation (SSF), which refers to any fermentation process performed on a non-soluble material that acts both as physical support and source of nutrients in the absence of free flowing liquid. The substrate has a moisture content that varies between 40–80% to allow microorganism growth and metabolism.[140,141] In this review the focus is on SSF as it has become the most commonly used fermentation process to increase the content of phenolic compounds in food products, enhancing their antioxidant activity.[142] By solid state fermentation bioactive compounds are obtained as secondary metabolites produced by microorganisms after the microbial growth is completed. For instance; two different filamentous fungi (*Aspergillus oryzae* and *Aspergillus awamori*) used in SSF were very effective for the improvement of phenolic content and antioxidant properties of wheat grains. In this study, fermented wheat grains were considered to be antioxidant richer and healthier food supplement compared to non-fermented wheat

grains.[143] The most common fermentation processes in bread making are yeast fermentation and sourdough. During the fermentation process in bread, carbon dioxide is produced by yeast, which makes the dough rise and it increases its volume, a process also known as proofing. In addition to yeast, lactic acid bacteria are used in the production of sourdough bread, which makes the end product more acidic.[119]

4.4.2.1. Yeast fermentation. Yeast fermentation is a regular treatment in the standard process of bread making. The most commonly used ferment is Baker's yeast consisting of *Saccharomyces cerevisiae*.[119] Long yeast fermentation of 360 min instead of a standard classic fermentation of 90 min in whole bread making increased the riboflavin concentration by 30% in wheat bread, suggesting a potential synthesis or a gain from the ferments used.[129] Similarly, yeast compensates for the losses of folate that occur in the bread baking, by its high intrinsic folate content but also by synthesizing folates.[131,144] On the other hand, the fermentation step decreases the content of some other B vitamins. A depletion of pyridoxine by 47%[129] and thiamin by 2–35% were encountered during the bread making.[113,130] However, using longer fermentation times the thiamin levels became similar to the original ones.[129] In the study by Moore *et al.* solid-state yeast treatments increased the phenolic acids, total phenols and the antioxidant capacity of the wheat bran extracts determined by various methods (ORAC, TEAC, DPPH, and hydroxyl radical scavenging).[135] In a following study by the same authors, longer fermentation times of 18–48 h increased further the antioxidant capacity of wheat dough. For instance, the hydroxyl scavenging activity was increased by 25–27%, which was associated with an increase in soluble free ferulic acid by 75–130% for 48 h fermentation.[136]

4.4.2.2. Sourdough. Sourdough bread baking consists of a fermentation process by a mixture of yeasts and lactic acid bacteria. This fermentation is usually at ambient/moderate temperatures for around 16–24 h.[145] The changes in the cereal matrix potentially leading to an improved nutritional quality are numerous. They include acid production, suggested to retard starch digestibility, and to adjust pH to a range which favours the action of certain endogenous enzymes. The action of enzymes during fermentation causes hydrolysis and solubilisation of proteins and fiber, which may affect the bioavailability of minerals and phytochemicals from the food matrix. This is especially beneficial in products rich in bran to deliver bioactive and potentially protective compounds in the blood circulation.[145,146]

The influence of fermentation by two types of lactic acid bacteria *Lactobacillus rhamnosus*, and the yeast *Saccharomyces cerevisiae* on the antioxidant activities and total phenolics of buckwheat was determined and compared with those of their unfermented counterparts. The total phenolic content and antioxidant activities (DPPH, FRAP, TBA) were enhanced. Thus fermentation offers a tool to further increase the bioactive potential of cereal products.[147] On the other hand, during sourdough baking the levels of phytate are reduced by the action of phytases, and as a consequence, the bioavailability of minerals is increased.[148–153] Sourdough in bread making has, however, some undesired effects on the contents of some bioactive compounds. Tocopherols and tocotrienols are reduced by 20–60%.[123,154] Also pyridoxine contents are depleted by about 20% in sourdough bread baking compared to the initial flour. In contrast, the levels of B-vitamins thiamin and riboflavin remain similar to original levels, while in normal baking losses of thiamin are observed.[129] Also sourdough fermentation increases the levels of folates and easily extractable total phenolics and free ferulic acid.[154,155]

Besides the effects of sourdough on the contents of bioactives described above, as well as on their bioaccessibility from the wheat bran, some health effects related to sourdough bread processing have already been suggested. For instance, sourdough wheat bread ingestion could lower the postprandial glucose and insulin responses in insulin resistant subjects.[156,157]

4.4.3. Enzyme technology. Many of the wheat bran bioactives occur bound to fiber or protein, and trapped in the aleurone cells. This is the case of the phenolic acids that are covalently bound to cell-wall polysaccharides, mainly to arabinoxylans (AX). The backbone of AX is composed of β-(1,4)-linked xylose residues, which can be bound to arabinose residues on the C(O)-2 and/or C(O)-3 position. Ferulic acid can be esterified on the C(O)-5 position of arabinose (Fig. 3). Most of the ferulic acid in wheat bran is bound to AX, which limits its bioaccessibility and bioavailability from bran rich products.[26,158–161] There are numerous enzymes targeting specific linkages of the arabinoxylan structure. Endo-β-(1,4)-D-xylanases cleave the xylan backbone internally, β-D-xylosidases remove xylose monomers from the non-reducing end of xylo-oligosaccharides, α-L-arabinofuranosidases remove arabinose substituents from the xylan backbone, and ferulic acid esterases remove ferulic acid groups from arabinose substituents.[104,162] Therefore strategies that involve the use of these and other enzymes are likely to cause a food matrix restructure that facilitates the release of the embedded compounds, such as ferulic acid. Furthermore, a synergy in the enzymatic release of ferulic acid from wheat bran has been reported for ferulic acid esterase and xylanases, which makes the combination of these enzymes an interesting approach to improve the ferulic acid bioaccessibility.[163–166] It has been observed that treatment of wheat bran insoluble dietary fibre with xylanases released feruloylated oligosaccharides from the bran[167] and feruloyl oligosaccharides have been reported to protect against oxidative DNA damage in normal human peripheral blood lymphocytes.[168]

Treatment of wheat fiber with the hydrolytic enzymes (mainly 1,3-β-glucanase and xylanase activites) of Tricoderma produced an increase in the soluble fiber as well as a 4-times increase of the water extractable ferulic acid.[169] In another study with wheat grain treated with the fungi Aspergillus, a positive correlation was found between the phenolic content of the wheat extracts and the activities of the hydrolyzing enzymes α-amylase, β-glucosidase and xylanase.[143]

Moore *et al.* tested five commercial food-grade enzyme preparations with reported enzyme activities, such as β-glucanase, cellulose, polygalacturonase, aminopeptidase, and other side activites, on wheat bran. The most efficient enzyme preparation (mainly β-glucanase activity) released 50% of the insoluble bound ferulic acid to its soluble free form, which is the bioaccessible form. Other bound phenolic acids were also released by the enzyme treatment, such as *p*-coumaric acid, syringic acid

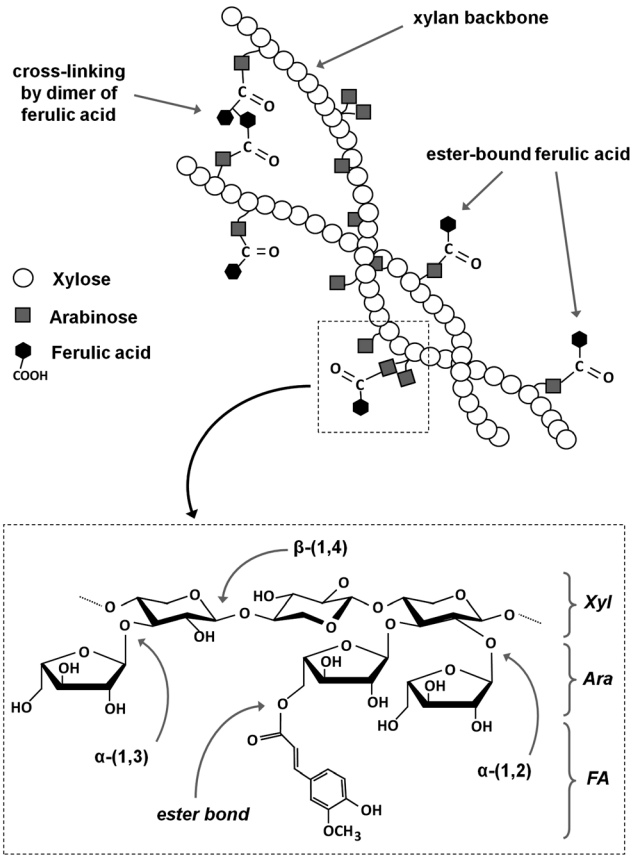


Fig. 3 Ferulic acid bound to the arabinoxylan structure in wheat bran.

and vanillic acid. The increase in free phenolic acids was in line with an increase in the antioxidant capacity, measured by different assays: hydroxyl radical scavenging, DPPH scavenging, ABTS scavenging and oxygen radical absorbing capacity.[170]

In a study by the authors, wheat bran was treated with an enzyme mixture, consisting of xylanase, β-glucanase, cellulose, α-amylase and ferulic acid esterase, before adding it to wholemeal flour for bread making. The main enzyme activities of this treatment were xylanase and β-glucanase activities. The enzyme treatment was applied on yeast fermented bran to enrich wholemeal bread. The bioaccessibility of phenolic acids from wholemeal bread with enzyme treated and fermented bran was compared to that with just fermented bran in the TNO Intestinal Model (TIM) of the gastrointestinal tract. The bioaccessibity of ferulic acid, *p*-coumaric acid, and sinapic acid from the wholemeal bread with enzyme treated and fermented bran was higher than that of the wholemeal bread with fermented bran. The highest increase was in the bioaccessibility of ferulic acid, which was 2.5-fold that of the wholemeal bread with just fermented bran. Despite this increase, the bioaccessibility of ferulic acid was merely 5.5%, meaning that even with the enzyme treatment most of the ferulic acid in wheat bran is not released in the small intestine. Most of it remains bound to the cell walls that constitute the indigestible fraction that enters the large intestine. Further, colonic experiments by the authors revealed that ferulic acid is mostly bioconverted by the colonic microbiota into other compounds of different chemical structure and bioactivities, mainly phenylpropionic acids with different grades of hydroxylation. These colonic metabolites of ferulic acid were found at higher concentrations when the bran was treated by fermentation and hydrolytic enzymes.[171]

The *in vitro* results obtained with the intestinal models described above have been confirmed *in vivo* by a follow-up study in humans by the authors. The human trial was a cross-over postprandial study, in which the participants had to consume 300 g of wholegrain bread with wheat bran or wholegrain bread with bioprocessed wheat bran. The bioprocessing of the bran was a combined treatment of yeast fermentation and hydrolytic enzymes, the same as in the *in vitro* studies described above. The wheat bran bioprocessing increased the bioavailability of ferulic acid and other phenolic compounds by a factor of 2.5 from the bread. This could be further associated with an increase in the anti-inflammatory capacity in an *ex vivo* LPS-induced inflammatory response.[172]

5. Conclusions

Processing of food has often received a negative perception, as numerous reports show losses in bioactive compounds during commonly used processing techniques. However, the positive effects of processing have not received enough attention. Positive effects of processing are related to modifications in the structure of the food matrix and compound interactions that enhance the bioaccessibility or intestinal release of bioactive compounds. Innovative processing techniques such as those involving microorganisms and enzyme technology appear to be promising tools to optimize the bioactive potential of wheat bran. But conventionally applied traditional processing methods also have a substantial effect on the bioactive content of bran products that could be optimized. The several described processing methods show the extensive possibilities of production of flours with tailored bran tissue composition and thus controlled content of bioactive compounds, as well as an improved bioaccessibility of these compounds from the food matrix, which can be related to a higher bioavailability and expected health effects.

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