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Chemical composition and nutritional quality of wheat grain

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Review

Abstract

Deficiencies of micronutrients such as iron, zinc, and vitamin A ("hidden hunger") afflict over three billion people. Currently there is an increasing preference among consumers for foods that contain not only traditional nutrients but also provide other compounds that are beneficial to health and well-being. Food systems that feed the world must be changed in ways that will insure that balanced nutrient supplies are available continuously to all people in adequate, affordable amounts. This paper reviews about the most important wheat grain components and their nutritional value. The opportunities of plant breeding and other technologies to improve the nutritional quality of wheat are also discussed.

Keywords: fibre, micronutrients, proteins, starch, wheat grain

Introduction

The nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources. The importance of wheat is mainly due to the fact that its seed can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pastas, and thus it presents the main source of nutrients to the most of the world population.

A huge increase in demand for cereals is predicted if the food needs for the estimated world population growth are to be met. But there is another potentially great benefit to these communities and that is the possibility to ensure such staple crops are nutritionally-balanced

and help remove the millions of cases of nutritionally-related deficiency disease that afflict them. It should be emphasised that in the past there has not been a single instance where plants have been bred to improve their nutritional content. If this has occurred it is purely by accident not design (Lindsay 2002; Welch and Graham 2002).

Over three billion people are currently micronutrient (i.e. micronutrient elements and vitamins) malnourished. This global crisis in nutritional health is the result of dysfunctional food systems that do not consistently supply enough of these essential nutrients to meet the nutritional requirements of high-risk groups (Welch 2005). One sustainable agricultural approach to reducing micronutrient malnutrition among people at highest risk (i.e. resource-poor women, infants and children) globally is to enrich major staple food crops with micronutrients through plant-breeding strategies. Available research has demonstrated that micronutrient-enrichment traits are available within the genome of wheat (as well as other food crops) that could allow for substantial increases in the levels of minerals, vitamins and other nutrients and health-promoting factors without negatively impacting crop yield. Importantly, micronutrient bioavailability issues must be addressed when using a plant-breeding approach to eliminate micronutrient malnutrition. Enhancing substances (e.g. ascorbic acid, S-containing amino acids, etc) that promote micronutrient bioavailability or decreasing antinutrient substances (e.g. phytate, polyphenolics, etc) that inhibit micronutrient bioavailability, are both options that could be pursued in breeding programs (Welch 2002; Welch and Graham 2004; Welch 2005)

Grain anatomy

The fruits of most plants contain one or more seeds, which, at ripeness, can be easily separated from rest of the fruit tissue. For *Germineae* this is different: fruit wall (pericarp) and seed coat are united. As a result the seed and fruit cannot be separated. This type of fruit, which is characteristic for all grasses, including cereals, is given the botanical term of *caryopsis*.

Wheat grains are generally oval shaped, although different wheats have grains that range from almost spherical to long, narrow and flattened shapes. The grain is usually between 5 and 9mm in length, weighs between 35 and 50mg and has a crease down one side where it was originally connected to the wheat flower. The wheat grain (Fig 1) contains 2-3%

germ, 13-17% bran and 80-85% mealy endosperm (all constituents converted to a dry matter basis) (Belderok et al., 2000).

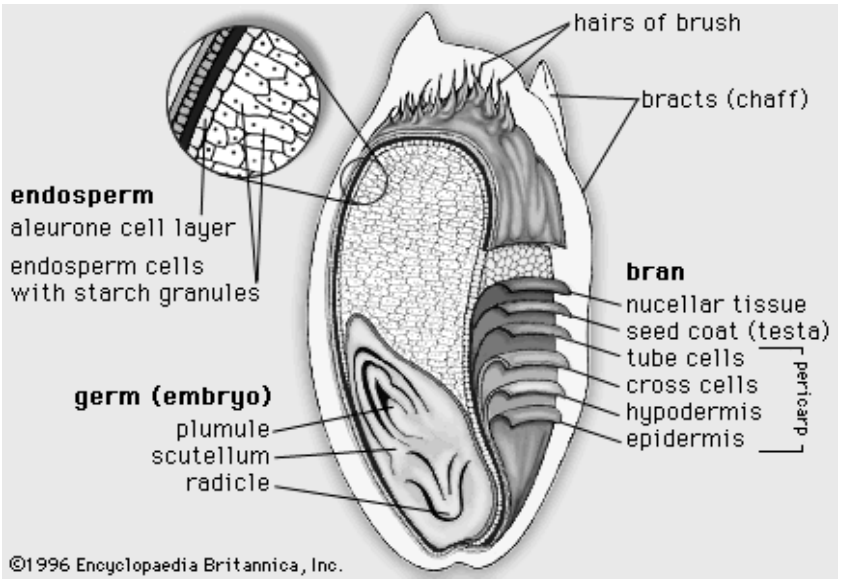

Fig. 1 Wheat grain (from Encyclopaedia Britannica, *http://www.britannica.com*)

The bran (outer layers of wheat grain) is made up of several layers, which protect the main part of the grain. Bran is rich in B vitamins and minerals; it is separated from the starchy endosperm during the first stage of milling. In order to protect the grain and endosperm material, the bran comprises water-insoluble fibre. More than half the bran consists of fibre components (53%). Chemical composition of wheat bran fibre is complex, but it contains, essentially, cellulose and pentosans, polymers based on xylose and arabinose, which are tightly bound to proteins. These substances are typical polymers present in the cell walls of wheat and layers of cells such as aleurone layer. Proteins and carbohydrates each represent 16% of total dry matter of bran. The mineral content is rather high (7,2%). The two external layers of the grain (pericarp and seed coat) are made up of dead empty cells. The cells of the inner bran layer- aleurone layer are filled with living protoplasts. This explains the rather high levels of protein and carbohydrate in the bran. There are large differences between the levels of certain amino acids in the aleurone layer and those in flour. Glutamine and proline levels are only about one half, while arginine is treble and alanine, asparagine, glycine, histidine and lysine are double those in wheat flour (Cornell 2003).

The endosperm is surrounded by the fused *pericarp* and *seed coat.* The outer endosperm, the aleurone layer, has a special structure: it consists of single layer of cubic shaped cells. The aleurone layer is rich in proteins and enzymes, which play a vital role in the germination process. The inner endosperm, i.e. the endosperm without the aleurone layer, is referred to as mealy or starchy endosperm. The endosperm mainly contains food reserves, which are needed for growth of the seedling, it is rich in energy-yielding starch. Apart from carbohydrates, the mealy endosperm contains fats (1,5%) and proteins (13%): albumins, glubulins and the major proteins of the gluten complex- glutenins and gliadins.- proteins that will form the gluten at dough making. The contents of minerals (ash) and of dietary fibers are low; 0,5% and 1,5%, respectively (Belderok et al., 2000).

The germ lies at one end of the grain. It is rich in proteins (25%) and lipids (8-13%). The mineral level is also rather high (4,5%). Wheat germ is available as a separate entity because it is an important source of vitamin E. Wheat germ has only one half the glutamine and proline of flour, but the levels of alanine, arginine, asparagine, glycine, lysine and threonine are double (Cornell 2003).

Proteins

Classification and function

Protein is considered the most important nutrient for humans and animals, as manifested by the origin of its name, from the Greek *proteios* for primary. The protein content of wheat grains may vary between 10% - 18% of the total dry matter. Wheat proteins are classified according to their extrability and solubility in various solvents. Classification is based on the classic work of T.D. Osborne at the turn of the last century. In his procedure, sequential extraction of ground wheat grain results in the following protein fractions:

- albumins, which are soluble in water;
- globulins, which are insoluble in pure water, but soluble in dilute NaCl solutions, and insoluble at high NaCl concentrations;
- gliadins, which are soluble in 70% ethyl alcohol, and;
- glutenins, which are soluble in dilute acid or sodium hydroxide solutions.

Albumins are the smallest wheat proteins, followed in size by globulins. The separation of albumins and globulins turned out to be not as clear as initially suggested by Osborne. Gliadins and glutenins are complicated high-molecular weight proteins. Most of

physiologically active proteins (enzymes) in wheat grains are found in the albumin and globulin groups. In cereals, the albumins and globulins are concentrated in the seed coats, the aleurone cells and the germ, with a somewhat lower concentration in the mealy endosperm. The albumin and globulin fraction cover about 25% of the total grain proteins (Belderok et al., 2000).

Gliadins and glutenins are storage proteins and cover about 75% of the total protein content. The wheat plant stores proteins in this form for future use by the seedling. Gliadins and glutenins are mainly located in the in the mealy endosperm and are not found in the seed coat layers nor in the germ. Storage proteins in wheat are unique because they are technologically active. They have no enzyme activity, but they have a function in the formation of dough as they retain gas, producing spongy baked products (Belderok et al., 2000).

Nutritional quality and improvement strategies

The nutritional quality of a protein can be measured by a variety of criteria, but in essence, it is the relative amounts and the balance of essential amino acids in the dietary protein that determine its nutritional value. In comparison with meat, plant protein is much more economical to produce; but when used as a source of dietary protein for humans and monogastric livestock, most plant proteins are nutritionally incomplete due to their deficiency in several essential amino acids (EAAs). Deficiency in certain amino acids reduces the availability of others present in abundance. In general, cereal proteins are low in Lys (1.5–4.5% vs. 5.5% of WHO recommendation), tryptophan (Trp, 0.8–2.0% vs. 1.0%), and threonine (Thr, 2.7–3.9% vs. 4.0%). Because of this deficiency, these EAAs become the limiting amino acids in cereals. It is thus of economic and nutritional significance to enhance the EAAs in plant proteins (Bicar et al., 2008).

In the past, plant geneticists and breeders have made much effort to improve the quality of plant proteins. Natural mutations such as high-lysine corn and barley have been identified and developed into elite genotypes (Bright and Shewry 1983). Unfortunately, undesirable traits such as greater susceptibility to diseases and pests and lower yields were associated with these mutations. Modern biotechnology now offers alternative approaches to rectify these deficiencies (Sun and Liu 2004; Bicar et al., 2008). Various improvement strategies have been developed to enhance the content of a specific essential amino acid in the protein.

Modification of protein sequence

In principle, sequence modification of a protein for increased EAAs is a straightforward molecular strategy. Since most seeds contain one or a few types of storage protein in abundance, it is thus an obvious idea to modify the codons of a major storage protein for additional and desirable EAA(s). This can be accomplished by site-directed mutagenesis or insertion of EAA-rich sequence(s). For this approach, however, a critical task is to select suitable regions in the protein that can be altered without affecting its overall structure, stability, and function. The variable regions of a protein represent possible target sites for modification. Using this strategy, attempts have been made to modify cereal proteins for more Lys residues. Torrent et al., (1997) modified zein protein by insertion of Lys-rich (Pro- Lys)n residues contiguous to, or in substitution of, the Pro-Xaa region of the γ-zein. The modified Lys-rich γ-zeins were accumulated to high levels in protein bodies and co-localized with the endogenous α- and γ-zeins in the transiently transformed maize endosperms. But these mutated proteins were posttranslationally modified in transgenic *Arabidopsis* plants, resulting in mis-sorting and secretion to the leaf cell wall, while the normal γ-zeins were correctly targeted to the endoplasmic reticulum (ER) of the transgenic *Arabidopsis* leaf cells (Alvarez et al., 1998).

Synthetic protein

Progress in understanding the structure, function, folding, and topology of proteins allows the design and synthesis of a gene encoding a new protein with desirable EAA composition. Based on an α-helical coiled-coil structure, Keeler et al., (1997) designed a de novo protein, CP3-5, containing 31% Lys and 20% Met. This synthetic protein could be expressed in transgenic tobacco seeds and accumulated up to 2% of the total seed protein in some transgenic lines, resulting in a significant increase in the total Lys content of the mature seeds.

Expression of heterologous protein

In this strategy, a gene encoding a protein rich in a desirable EAA is isolated from any source organisms and transferred into a target plant. Proper expression of this heterologous gene can introduce this EAA-rich protein into the host protein pool, resulting in an increase in the desirable EAA content. The key factor contributing to the success of this approach is the availability of candidate EAA-rich protein genes. Considerable efforts have also been directed

to identify Lys-rich proteins (LRPs) for crop improvement, especially cereals, but with limited success. Legume proteins generally contain more Lys residues than cereal proteins, thus expression of a pea seed vicilin in tobacco and alfalfa was tested. The accumulation level of vicilin was not sufficient to increase the content of Lys (Wandelt et al., 1992). Glycinin and phaseolin from legume seeds were successfully expressed and accumulated to high levels in the seeds of transgenic rice (Katsube et al., 1999), but no significant increase in the content of Lys or other EAAs was reported. The gene encoding a LRP from the winged bean seed (WBLRP, contains 10.8 mol% Lys, representing the most abundant amino acid in this protein) was transferred and expressed in the seed of *Arabidopsis*, resulting in a 5% accumulation of this LRP as total seed protein (Cheng 1998). More recently, the WBLRP was transferred into rice and high levels of LRP accumulation (up to 12% of total soluble seed proteins) in the endosperm of transgenic rice seeds was detected, resulting in some 20% increase in protein-bound Lys (Liu, 2002). Another group of high-Lys proteins are legume vegetative storage proteins (VSPs) (Staswich 1994). Two soybean VSPs (S-VSPa and S-VSPb), both containing about 7% Lys residues, were recently expressed in transgenic tobacco, resulting in 2–6% accumulation of the total soluble leaf proteins and up to 40% increase in leaf soluble Lys content. In vitro experiments revealed that S-VSPb was stable to rumen proteolysis (Guenoune et al., 2002), suggesting that this protein is a suitable candidate for improving the nutritional quality of forage crops. Expression of quality animal proteins in plants has also been reported. This was demonstrated in maize expressing milk protein α-lactalbumin. The lysine content was 29–47% greater in endosperm from transgene lines kernels. As a result, transgenic endosperms have an improved amino acid balance relative to non-transgenic endosperms produced on the same ear. Kernel appearance, weight, density and zein content did not exhibit substantial differences in kernels expressing the transgene when compared to non-expressing siblings. Assessment of the antigenicity and impacts on animal health will be required in order to determine the overall value of this technology (Bicar et al., 2008).

Manipulation of homologous protein expression

The overall amino acid composition of a seed is often determined by a few abundant storage proteins. However, some minor proteins may contain elevated levels of a special EAA. Enhancing the synthesis and accumulation of such protein by over-expression can lead to an increase in the concentration of this particular EAA. Lys-rich minor proteins have been identified from Lys-deficient cereal seeds (Singh et al., 2000). Genes encoding Lys-rich

proteins may serve as candidate genes for plant protein improvement, but there have been no reports to date on this application. This strategy has already been applied in maize. Similar approaches may also be used to improve the nutritional quality of rice kernel, as rice endosperm contains abundant Met-rich prolamin. Maruta et al., (2001) generated transgenic rice lines with increased prolamin content by reducing the synthesis of glutelin, which is the most abundant storage protein in rice. Recently, transgenic rice lines with lower amounts of the prolamin fraction were produced through overproducing a glutelin polypeptide. The Lys content in the seeds of these transgenic lines was significantly increased.

Free amino acids

Another approach for enhancing the EAA content in plants is to increase the level of a desirable EAA in the free form (vs. protein-bound) through manipulation of the amino acid biosynthetic pathway. The two key enzymes in the Lys biosynthetic pathway are aspartokinase (AK) and dihydrodipicolinate synthase (DHPS), which are feedback inhibited by Lys. Lys synthesis can be increased by expressing the mutant AK and/or DHPS enzyme(s) that are insensitive to Lys feedback inhibition (Sun and Liu 2004). However, although high lysine levels in seeds are beneficial, increases in the level of this amino acid in vegetative tissues are undesirable, because high levels of lysine cause abnormal vegetative growth and flower development that, in turn, reduces seed yield. Targeting the expression of lysine-insensitive DHPS to seeds of several transgenic model and crop plants using seed-specific promoters, eliminated its undesirable effects in vegetative tissues, resulting in plants with good growth characteristics and high lysine levels in their seeds. This approach has also recently reached commercial application by the approval of a high-lysine maize variety (MaveraTM) for animal feeding (Tang et al., 2007). Lys content in transgenic *Arabidopsis* seeds was synergistically boosted by increasing Lys synthesis coupled with a knockout of Lys catabolism (Zhu and Galili 2003).

Tryptophan, another limiting EAA in cereal crops, is produced by the Trp biosynthetic pathway. The key enzyme, anthranilate synthase (AS), is also feedback inhibited. Over-expression of mutant AS in transgenic crops thus can lead to accumulation of Trp. Tozawa et al., (2001) cloned and modified the rice AS (OASA1) gene to encode a feedback-insensitive mutant OASA1. Expression of this mutant OASA1 in transgenic rice callus and leaves resulted in increased Trp accumulation up to 180- and 35- fold, respectively. Although a significant increase in the free Lys and Trp pools in several important cereal crop plants can be achieved by metabolic engineering, a disadvantage of enhancing free EAAs, as compared

with protein-bound ones, is that free amino acids could be leached from the plant tissues and lost during boiling and other processing.

Although expression of an EAA-rich protein and metabolic engineering of the EAA biosynthetic pathway are the two main approaches used for Lys or Met enhancement, some drawbacks have been reported. In the case of expressing EAA-rich proteins, the over-produced and accumulated foreign proteins often resulted in redistribution of EAAs or down-regulation of endogenous protein(s) in the transgenic seeds. A clear future direction for protein quality improvement emerging from published studies is an integrated approach to enhance the EAA pool (source) through metabolic pathway engineering and, at the same time, to trap the over-produced free EAA into the EAA-rich transgenic protein (sink). With this coupled source and sink strategy, the proper ratio of free and protein-bound EAA in plants can be maintained and the desirable EAA levels can be achieved (Sun and Liu 2004)

Bioactive wheat proteins

Another limitation of plant proteins is that they can be poorly digested by animals or can cause allergic reactions. Wheat allergy is the result of abnormal immunological reactions to certain wheat proteins. It has totally different mechanism from that in coeliac disease and the proteins involved are not gliadins but albumins and globulins. These proteins cause Type 1 hypersensitivity reactions which are mediated by allergen-specific immunoglobulin E (IgE). Bakers' asthma is a typical condition in which water soluble flour proteins bond to serum IgE as a result of inhalation of flour particles (Baldo and Wrighly 1984). Coeliac disease (CD), or gluten-sensitive enteropathy, is a condition that results in damage to the small intestine, resulting in malabsorption. The symptoms are commonly poor growth, diarrhoea and abdominal pain. The major causative agents of CD in wheat were shown to be due the gluten proteins- gliadins and glutenins.

Thioredoxin (Trh) plays a central role in germination and seed development where it appears to facilitate a red-ox change in the seed storage proteins during germination. In wheat nitrogen and carbon are mobilised by the thioredoxin through the reduction of the gliadin and glutenin storage proteins, and the low molecular weight disulphide proteins that inhibit starch-degrading enzymes are inactivated. A notable characteristic of Trh in these systems is to reduce intra- as opposed to inter-molecular disulphide bonds. In gluten-hypersensitive dogs it has been shown that this allergenicity is reduced when the proteins are reduced by Trh

Interestingly reduced glutathione had no effect on the allergenic potential indicating the specific role played by Trh (Lindsay 2002).

Some studies referred that a sufficient genetic variation is present to endeavour the selection of wheat accessions that contain low amounts of T-cell–stimulatory sequences. Such materials may be used to select and breed wheat varieties suitable for consumption by CD patients, contributing to a well-balanced diet and an increase in their quality of life. Such varieties also may be useful for disease prevention in individuals at risk. Spaenij–Dekking et al., 2005; Molberg et al., 2005)

Starch

Cereal grains store energy in the form of starch. The amount of starch contained in a wheat grain may vary between 60% and 75% of the total dry weight of the grain. Starch occur in seed in the form of granules. Wheat has two types of starch granules: large (25-40 um) lenticular and small (5-10um) spherical ones. The lenticular granules are formed during the first 15 days after pollination. The small granules, representating about 88% of the total of granules, appear 10-30 days after pollination (Belderok et al 2000).

Starch is basically a polymer of glucose. Chemically, at least two types of polymers are distinguishable: amylose and amylopectin. The molecular weight of amylose is around 250.000 (1500 glucose molecules) but varies widely. Amylose is a mostly linear α-(1,4)-linked glucose polymer with a degree of polymerization (DP) of 1,000–5,000 glucose units. The structure of this polymer was assumed to be mainly linear, but this appears to be true for only part of the amylose, the remainder is slightly branched.

Amylopectin is branched to a much greater extent than amylose. So much that, on the average, the unit chain in amylopectin is only 20-25 glucose molecules long. Amylopectin has a molecular weight of about 10^8. The ratio of amylose to amylopectin is relatively constant, at about 23. Amylopectin is a much larger glucose polymer (DP 105–106) in which α-(1,4)-linked glucose polymers are connected by 5–6% α-(1,6)-linkages. Normal wheat starch typically contains 20–30% amylose and 70–80% amylopectin (Konik-Rose et al., 2007).

Manipulations with starch composition

Recent advances in the study of wheat starch biosynthesis have been possible with the discovery of wheats with null alleles or mutations for the different starch biosynthesis enzymes (Yamamori et al., 2000; Regina et al., 2006). The generation of modified starches

through genetic engineering has also been reported (Regina et al., 2006). This has extended the variation in wheat starch properties and expanded the possibilities for novel end uses.

Starch is synthesized by a complex pathway, which involves a number of enzymes including ADP-glucose pyrophosphorylase, starch synthases, branching enzymes and de-branching enzymes (Smith 2001; Morell et al., 2006). Five classes of starch synthases have been identified in the cereal endosperm, granule bound starch synthase (GBSS), starch synthase I (SSI), SSII, SSIII and SSIV. The predominant SSII form in wheat starch granule is classified as an SSIIa type (Li et al., 2003). The SSIIa gene in wheat encodes the SGP-1 protein (starch granule bound protein -1) present in the wheat starch granule (Li et al., 1999). Yamamori et al., (2000) reported SGP-1 null wheat line missing activity of all three starch synthase IIa (*ssIIa*) genes (from the three wheat genomes A, B, D). The lack of starch synthase IIa activity in the SGP-1 null lines is caused by an insertion in the B genome and deletions for both A and D genomes in exon sequences. Starch from this wheat line was found to have increased amylose content (31– 37%) (Konik-Rose et al, 2007).

Aiming to increase the relative content of amylose in wheat grains, a RNAi construct designed to silence the genes encoding the two starch-branching isozymes of amylopectin synthesis, were expressed under a seed-specific promoter in wheat. This resulted in increased grain amylose content to over 70% of the total starch content. Starches with elevated amylose contents are of interest because they provide resistant starch with positive impacts on human health (Regina et al., 2006). While insufficient food calories is one of the major causes of malnutrition in developing countries, an excess of digested calories leads to obesity and other diseases in developed countries (Tang et al., 2007).

Resistant starch (RS) is defined as starch that is not absorbed in the small intestine of humans. Three different types of RS were defined by Englyst et al., (1995). Type 1 is defined as physically inaccessible starch, type 2 (RS2) as native starch granules and type 3 (RS3) as retrograded starch. More recently, a fourth type of RS has been classified, comprising chemically modified starches. RS is claimed to be a good substrate for colonic fermentation and to be beneficial because of its high ratio of butyrate production, which may play a major role in the prevention of colon cancer, as shown in several studies on animal models. The fermentation products from RS are known to lower the pH in the colon which leads to less production, and/or accumulation of potentially harmful by-products of protein fermentation, for example, ammonia or phenols which may promote tumorigenesis (Fassler et al., 2006). RS have glycemic index lowering capacity and manage type II diabetes with potential health

benefits (Behall, et al., 2006; Konik-Rose et al., 2007**).** To estimate potential health benefits of RS it is important to be able to predict its behavior in the human gastrointestinal tract, in particular the amount and the structure of starch reaching the large intestine (Fassler et al., 2006).

Lipids

Lipids are present only in a small extent in cereals but they have a significant effect on the quality and the texture of foods because of their ability to associate with proteins due their amphipatic nature and with starch, forming inclusion complexes. In wheat, the maturing seed synthesises fatty acids at different rates. The biosynthesis of lipids depends upon acetyl coenzyme A. this important compound is involved in synthesis of the acyl lipids such as glycerides, phospholipids, waxes, sphingosine lipids as well as the isoprenoid series. Malonyl-CoA is also utilised, together with NADPH, and further dehydration and condensation reactions occur to produce palmitic acid ($C_{16:0}$), which can then be extended to stearic acid by another reaction. Synthesis of linoleic acid occurs in higher plants by two separate pathways in the presence of microsomal enzymes. Isopentenyl pyrophosphate is formed from mevalonic acid which in turn is synthesised from acetyl CoA. It is able to form b-squalene and, from this compound, the characteristic steroid structures, of which cholesterol is a member, are produced, although in very small amounts (Cornell 2003).

The germ has the highest amount of lipids (11%), but significant amounts are also associated with the bran and the starch and proteins of the endosperm. Complex polar lipids extracted by WSB (1-butanol saturated with water) account for about half the total lipids in the endosperm compared with about 23% in the bran and 17% in the germ, but the latter two contain more triglicerides. The bound lipids are mostly phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serin, as well as lysophosphatidyl derivates, where there is one free hydroxyl group on the glycerol moiety. The principal sterols were identified as b-sitosterol, campesterol and C_{28} and C_{29} saturated sterols. Numerous studies have been carried out showing a high level of linoleate ($C_{18:2}$) in both the total lipid and the triglycerides from the three fractions with lower amounts of palmitate ($C_{16:0}$) and oleate ($C_{18:1}$) (Cornell 2003).

Although some attempts have been realized to manipulate the lipid composition in order to improve the nutritional quality of the crops, there is a lack of such research in wheat. (Anai et al., 2003; Murphy 2006)

Fibre

Numerous studies (McKee and Latner 2000; Philippe et al., 2006; Weickert and Pfeiffer 2007; Rave et al., 2008) have demonstrated the beneficial effects of fiber consumption in protection against heart disease and cancer, normalization of blood lipids, regulation of glucose absorption and insulin secretion and prevention of constipation and diverticular disease. Dietary fiber is defined as lignin plus the polysaccharide components of plants which are indigestible by enzymes in the human gastrointestinal tract (Bermink, 1994). These components are typically divided into two categories. Soluble dietary fiber is those components that are soluble in water and includes pectic substances and hydrocolloids. Insoluble dietary fiber is those components that are insoluble in water and includes cellulose, hemicellulose and lignin. Whole grains are good sources of insoluble fiber. Arabinoxylans (AX) and (1→3),(1→4)-β-glucans are major components of wheat endosperm cell walls. Arabinoxylan (insoluble type of fibre) is considered to be an optimal substrate for fermentative generation of short-chain fatty acids (SCFAs)—in particular, of butyrate in the colon. Butyrate at high concentrations in the colon is hypothesized to improve bowel health and lower cancer risk by several possible mechanisms (Philippe et al., 2006).

The increasing awareness of the potential benefits of high-fibre diets has promoted a growing interest for the consumption of whole-grain breads and bran breads. Supplementation has been used to enhance fiber content of foods. Some fiber-fortified baked goods have been available for years. While supplementation has focused on cookies, crackers and other cereal-based products, enhancement of fiber content in snack foods, beverages, spices, imitation cheeses, sauces, frozen foods, canned meats, meat analogues and other foods has also been investigated (Hesser 1994). Traditionally, fiber supplementation has focused on the use of milling by-products of cereal grains. All of the milling by-products of wheat, corn, sorghum and other grains, as well as the by-products from the wet milling of corn and wheat, have been investigated as possible fiber supplements (Matz 1991).

Nevertheless, along with nutritional benefits, whole-wheat flours contain significant amounts of undesirable compounds, such as phytates (myo-inositol hexaphosphate) (Lopez et al., 2000; Lopez et al., 2001). Most of the inorganic phosphorus (Pi) present in mature cereal seeds (40–80%) is stored as phytate that forms complexes with minerals such as Ca^{2+}, Fe^{3+}, Zn^{2+} and Mg^{2+} reducing their bioavailability. Addition of wheat bran significantly reduced the nutritional properties of the cookie samples because of the phytic acid (PA) presence (Bilgiçli et al.,, 2007). The PA content of tarhana (a dried soup base made from yoghurt and wheat

flour) mixture increased as wheat germ/bran amount added to tarhana increased. However, more than 90% of the PA present in the mixtures were inactivated by fermentation (Bilgiçliet al., 2006; Bilgiçli and İbanoğlu 2007) The results reported by Ficco et al., (2009) open the possibility of designing a specific breeding program for improving the nutritional value of durum wheat through the identification of parental lines with low-Pi and high minerals concentration in whole grains.

Soluble fibre such as (1→3,1→4)-*β*-D-glucan (referred to as *β*-glucan), has been shown to have immunostimulating activity as well as effects on the glycaemic, insulin, and cholesterol responses to foods (Dalmo and Bøgwald 2008; Shimizu et al., 2008). Cereals (such as barley and oats) are good sources for these functional ingredients, with studies clearly demonstrating their potential nutritional benefits (Brennan and Cleary 2005). Wheat is not generally thought of as a *β*-glucan source; levels are usually less than 1 %, commonly about 0,6%. However, specific histochemical techniques reveal a distinct localisation in the aleurone and adjacent sub-aleurone region. Although this still constraints with the much greater deposit in oats, new friction/abrasion pre-processing techniques for wheat are able to access this region in a more precise fashion than has been achieved with conventional milling (Wood 1997). Moreover, the *β*-glucan content of cereal grains is genotype-dependent (Ehrenbergerová et al., 2008; Šramková et al., 2008). Thus, *β*-glucans should be regarded as important functional ingredient for the cereal food industry that can be increased by plant breeding methods.

Vitamins

Vitamins are defined as a diverse group of food-based essential organic substances (relatively small molecules but comparable in size to amino acids or sugars) that are not synthesized by the human body, but by plants and microorganisms. Therefore, vitamins are nutritionally essential micronutrient for humans and function *in vivo* in several ways, including: (1) as coenzymes or their precursors (niacin, thiamin, biotin, pantothenic acid, vitamin B6, vitamin B12 and foliate), (2) in specialized function such as vitamin A in vision and ascorbate in distinct hydroxylation reactions; and (3) as components of the antioxidative dafense systems (vitamin C and E and some carotenoids), and as factors involved in human genetic regulation and genomic stability (folic acid, vitamin B12, vitamin B6, niacin, vitamin C, vitamin E and D) (Paredes-López and Osuna–Castro 2006).

Tocols

Tocopherols (T) and tocotrienols (T3), collectively called tocols, are composed of a chromanol ring with an attached phytyl side chain. Tocotrienols differ from tocopherols in that their phytyl side chain is unsaturated, containing three double bonds. There are four isomers in both T and T3 based on the number and positions of methyl groups on the chromanol ring; α-(5,7,8 trimethyl), β-(5,8 dimethyl), γ -(7,8 dimethyl) and δ-(8 methyl). The biological activity of vitamin E has generally been associated with its well defined antioxidant property in biological membranes. α-T has long been considered to be the most active form in the vitamin E complex for preventing destructive oxidation in cell membranes. However, α-T3 has the highest biological activity of all of the tocols and has been shown in some studies to possess novel hypocholesterolemic effects together with the ability to reduce the atherogenic lipoprotein levels in plasma. The hypocholesterolemic effect of α- T3 is due to the suppression of hydroxyl-β-methylglutaryl coenzyme A reductase, the key enzyme of cholesterol synthesis. In addition, T3 isomers collectively have been suggested to have anti-thrombotic and anti-tumor effects, thus may serve as an effective agent in the prevention and/or treatment of cardiovascular disease and cancer (Theriault et al., 1999).

Hidalgoa and Brandolini (2008) studied the distribution of tocopherols and tocotrienols in the germ, bran and endosperm portions in seeds of two einkorn accessions and one bread wheat. The germ fraction showed the highest concentration of α-tocopherol, β-tocopherol and total tocols. α-Tocotrienol and β-tocotrienol levels were highest in the bran fraction, although significant quantities were detected also in the flour.

Ehrenbergerova et al., (2006) presented data indicating significant effects of cropping system, genotype, and year grown on the tocol levels in barley. The results suggest that a selective breeding program using the best genotypes would be beneficial to produce food barleys with higher levels of total tocols and T3.

Carotenoids

Vitamin A (retinol) is a fat-soluble micronutrient and is mainly contained in eggs, liver and butter. Vitamin A precursors such as b-carotene, and other carotenoids, are produced in green and yellow vegetables. After uptake, β-carotene and the other carotenoids are oxidatively cleaved in the intestinal mucosal brush border or liver to form the isoprenoid retinol. Carotenoids from a plant origin have an advantage over retinol from animal sources, because excessive retinol may cause a toxic excess in vitamin A, whereas carotenoids can be

converted as needed to meet metabolic requirements. Vitamin A deficiency (VAD) causes night-blindness, xerophthalmia, keratomalacia, bone growth deficiencies, and weakens the immune system. The clinical effect of VAD is inversely related to the age of patient, and the mortality of children with severe VAD can reach 50% (Yonekura-Sakakibara and Saito, 2006).

In attempting to enhance micronutrient levels in wheat through conventional plant breeding, it is important to identify genetic resources with high levels of the targeted compound, to consider the heritability of the targeted traits, to explore the availability of high throughput screening tools and to gain a better understanding of genotype by environment interactions (Ortiz-Monasterio et al., 2007). The nutritional properties of einkorn and its potential as a donor of useful traits to cultivated wheat prompted the survey of carotenoid content of 54 accessions of einkorn originating from different eco-geographical areas. Einkorns from different geographical areas have been found to have diverse average total carotenoid values; two geographic gradients were observed, possibly reflecting the original routes of spread of einkorn into Europe from the Middle East (Hidalgo et al., 2006)

Research on carotenoid metabolic engineering in plants has made it possible to: (1) introduce variation in carotenoid products (tomato); (2) produce higher levels of preexisting carotenoids (canola); and (3) accumulate carotenoids in normally carotenoid-free tissues (rice endosperm). (Paredes-López and Osuna–Castro 2006)

The carotenoid biosynthetic pathway has been well-studied in bacteria and plants. In plants, carotenoids are synthesized from geranylgeranyldiphosphate (GGPP) in plastids. Phytoene synthases (PSY) catalyze the formation of phytoene (15-cis-phytoene) from two molecules of GGPP. Phytoene is converted to f-carotene by phytoene desaturase and further to prolycopene by f-carotene desaturase. Carotene cistrans- isomerase catalyzes the trans-isomerization of prolycopene to all-trans-lycopene and lycopene cyclase (LCY) converts trans-lycopene to α-carotene or β-carotene. α- and β-carotene are converted to zeaxanthin and lutein, respectively by hydroxylases (Yonekura-Sakakibara and Saito 2006).

A well-known success of genetic transformation in cereals represents the development of the golden rice. It was first engineered with the insertion of the PSY gene from daffodil (*Narcissus pseudonarcissus*) and the bacterial phytoene desaturase (CrtI) gene from *Erwinia uredovora* (Ye et al., 2000). Bacterial CrtI can catalyze three enzymatic steps from phytoene to all-trans-lycopene. The PSY gene is under the control of an endosperm-specific glutelin promoter. To localize the product in plastids, CrtI was designed as a fusion with the ribulose-

1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit. An alternative construct was made by co-transformation with constructs carrying the PSY/CrtI gene as described above and the LCY gene under the control of a glutelin promoter. By the latter approach, the carotenoid content of edible rice endosperm was 1.6 μg/g dry weight (Ye et al., 2000). However, in 2005, Golden Rice2 was developed and the carotenoid content was increased up to 23- fold (37 μg/g of dry weight) compared to the original Golden Rice. This content is close to a realistic level for palliating VAD in children (Paine et al., 2005). Expression of carotenoid biosynthetic genes in other cereals, such as wheat, requires further scientific investigation.

Minerals

Micronutrient malnutrition ("hidden hunger") now afflicts over 40% of the world's population and is increasing especially in many developing nations. Today, deficiencies of iron and iodine are of most concern to the nutrition community and healthcare officials although other nutrient deficiencies, including zinc, selenium, calcium and magnesium may be prevalent in some global regions. The consequences of malnutrition create immense economic and societal costs to nations. Micronutrient malnutrition greatly increases mortality and morbidity rates, diminishes cognitive abilities of children and lowers their educational attainment, reduces labor productivity, stagnates national development efforts, contributes to continued high population growth rates and reduces the livelihood and quality of life for all those affected (Welch and Graham 1999).

Past programs to combat micronutrient malnutrition have relied primarily on interventions directed at food fortification or nutrient supplementation programs (Yip 1997). Unfortunately, these approaches have not proven to be sustainable for various reasons and do not reach all the people at highest risk of developing micronutrient malnutrition. Remarkably, the nutrition community has never embraced agriculture as an important 'tool' to use in fighting 'hidden hunger'. Breeding for micronutrient-enriched staple plant foods is a possibility that should be pursued (Graham et al., 1999). Success in such a breeding effort would target those groups of people most at risk of developing micronutrient malnutrition because these sectors of societies are dependent on these foods for their sustenance. Furthermore, a plant breeding approach would be sustainable; once micronutrient-dense lines of staple plant foods are developed; there is little additional cost to continue their lineage in ongoing breeding programs for the foreseeable future (Welch and Graham 2002).

The breeding steps include at least (1) identification of a useful genetic variation and the most promising parents, (2) long-term crossing and back-crossing activities, (3) stability of the target traits (e.g., high grain Zn concentrations) across the different environments that feature huge variation in soil and climatic conditions, and finally (4) adaptation of the newly developed biofortified genotypes over a range of crop and soil management practices applied in the target regions or countries. The acceptance of biofortified crops by producers is a further issue that needs a special attention (Cakmak 2008).

Iron (Fe) and Zinc (Zn)

Increasing the Zn and Fe concentration of food crop plants, resulting in better crop production and improved human health is an important global challenge. Among micronutrients, Zn deficiency is occurring in both crops and humans (Welch and Graham 2004). Zinc deficiency is currently listed as a major risk factor for human health and cause of death globally. According to a WHO report on the risk factors responsible for development of illnesses and diseases, Zn deficiency ranks 11th among the 20 most important factors in the world and 5th among the 10 most important factors in developing countries. Hotz and Brown (2004) reported that Zn deficiency affects, on average, one-third of world's population, ranging from 4 to 73% in different countries. Zinc deficiency is responsible for many severe health complications, including impairments of physical growth, immune system and learning ability, combined with increased risk of infections, DNA damage and cancer development (Hotz and Brown 2004; Gibson 2006; Prasad 2007).

A high risk of tissue hypoxia and heart failure, which can lead to death in young children and pregnant women, is associated with Fe deficiency (Viteri 1998). Maternal anaemia, aggravated by blood loss during child birth, is reported to be responsible for most of the maternal mortality during birth in the world (20% of all maternal deaths are attributed to Fe deficiency anaemia (Maberly et al., 1994). Babies born to mothers that are iron-deficient are commonly stunted and unhealthy. Children suffering from Fe deficiency have poor attention spans, impaired fine motor skills and less capacity for memory (Walter et al., 1997). Iron deficiency in pregnant women may cause irreversible damage to fetal brain development leading to irreversible damage to intellectual development in their babies (Gordon 1997). Iron deficiency in pregnant women is correlated to infant prematurity and low birth weight; this can result in long-term frailties such as immune system dysfunctions and growth failure

(McGuire 1993). Iron deficiency reduces both physical performance and work productivity (Maberly et al., 1994).

A wide range of wheat germplasm is being studied at CIMMYT with respect to the concentration of Fe and Zn in the whole grain and environmental interactions on their concentrations (Welch and Graham 2002). Based on a range of reports and survey studies, the average concentration of Zn in whole grain of wheat in various countries is between 20 to 35 $mg.kg^{-1}$ (Cakmak et al., 2004). Most of the seed-Zn is located in the embryo and aleurone layer, whereas the endosperm is very low in Zn concentration (Ozturk et al., 2006). Zn concentrations were found to be around 150 $mg.kg^{-1}$ in the embryo and aleurone layer and only 15 $mg.kg^{-1}$ in the endosperm. Fe concentrations (dry weight basis) in wheat grain from plants grown in El Batan (Mexico) in 1994 were 28.8–56.5 $mg.kg^{-1}$ (mean = 37.2 $mg.kg^{-1}$). Clearly, enough genetic variation exits within the wheat germplasm to substantially increase Fe and Zn concentrations in wheat grain. There was a high correlation between grain-Fe and grain-Zn concentrations in the wheat lines studied. While there was significant genotype × environmental interactions obtained for Fe and Zn grain concentrations, there was still a strong genetic component to Fe and Zn accumulation in the grain (Welch and Graham 2002). Very recently, new wild emmer wheat accessions have been identified showing simultaneously very high concentrations of Zn (up to 139 $mg.kg^{-1}$), Fe (up to 88 $mg.kg^{-1}$) and protein (up to 380 $g.kg^{-1}$) in seeds and high tolerance to drought stress and Zn deficiency in soil (Peleg et al., 2008). This finding indicates that it should be possible to improve Fe and Zn levels in wheat grain through plant breeding. Additional research has also shown that there is no negative linkage between grain yield and Fe and Zn density in the grain (Welch and Graham 2002).

Selenium (Se)

Selenium (Se) is an integral component required for normal cell metabolism in humans and animals. Selenium (Se) is an essential micronutrient for humans and animals, with antioxidant, anti-cancer and anti-viral effects (Arthur 1999). Soils are frequently low in available Se, and hence the food systems of many countries are deficient in Se (Lyons et al., 2003; Rayman 2002). Wheat is an important dietary source of Se. For example, in Australia it is estimated to supply nearly half the Se intake of most people (Lyons et al.,, 2003). However, Se concentration in wheat grain is highly variable. Published values range from 0.02–0.60 $mg.kg^{-1}$ for most of the world's wheat (Alfthan and Neve 1996). Se availability in soils

depends upon soil pH, redox potential, calcium carbonate level, cation exchange capacity, and organic carbon, iron (Fe) and aluminium (Al) levels. In alkaline soils, most Se is present as selenates, which are highly soluble and easily taken up by plants. In acidic, poorly aerated soils, Se occurs mainly as insoluble selenides and elemental Se. In lateritic soils high in Fe, Se binds strongly to Fe to form poorly soluble ferric hydroxide-selenite complexes (Lyons et al., 2005). Fertilisation of food crops with selenate to increase human Se intake (an example of *agronomic* biofortification) has nevertheless been successful, especially in Finland (Aro et al., 1995). A strategy of breeding staple crops with enhanced ability to load more micronutrients into the edible portion of the plant (e.g. grain) (*genetic* biofortification) offers a sustainable, cost-effective alternative to conventional fortification, which is more likely to reach those most in need. Exploiting the genetic variation in crop plants for micronutrient density is likely to be an effective method to improve the nutrition of entire populations. It could be argued that plants may be expected to show more genotypic variability for plant-essential micronutrients than for a non-essential element like Se. However, studies have demonstrated significant genetic variability in the edible parts of some crops (Yang et al., 2003; Zhang et al., 2003). Findings from wheat studies have been variable. Some have found no evidence for genetic variability among wheat cultivars for Se density in grain (Grela 1996; Tveitnes et al., 1996), while another found higher concentrations of Se, Zn, lithium (Li), magnesium (Mg) and phosphorus (P) in hulled wheat (*Triticum spelta* L. and *Triticum dicoccum* Schrank) grown together with modern bread wheats (*Triticum aestivum* L.) (Piergiovanni et al., 1997), and a Russian study suggested that commercial wheat cultivars may vary in their ability to accumulate Se (Seregina et al., 2001). Lyons et al., (2005) have detected no significant genetic variability among commercial bread or durum wheat varieties, triticale or barley. More research is needed to determine whether sufficient genetic variation in grain Se density in wheat exists to enable the selection of this trait for plant breeding purposes

References

Alfthan G, Neve J (1996) In: J Kumpulainen and J Salonen (Eds) Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention, pp 161–167. Royal Society of Chemistry, Cambridge

Alvarez I, Isabel MG, Pimentel E, Ludevid D, Torrent M (1998) Planta 205: 420–427

Anai T, Koga M, Tanaka H, Kinoshita T, Rahman SM, Takagi Y (2003) Plant Cell Rep. 21: 988-92

Aro A, Alfthan G, Varo P (1995) Analyst 120: 841–843

Arthur JR (1999) Proc. Nutr. Soc. 58: 507–512

Baldo BA, Wrigley CW (1984) Adv. Cereal Sci. Technol. 6: 289-356

Behall KM, Scholfield DJ, Hallfrisch JG, Liljeberg-Elmståhl HGM (2006) Diabetes Care 29: 976–981

Belderok B, Mesdag H, Donner DA (2000) Bread-Making Quality of Wheat. Springer, New York

Bermink MR (1994) In: Nielson SS (ed) Introduction to the Chemical Analysis of Foods, pp 169–180, Jones and Bartlett Publishers, Boston

Bicar EH, Woodman-Clikeman W, Sangtong V, Peterson JM, Yang SS, Lee M, Scott MP (2008) Transgenic Res. 17: 59–71

Bilgiçli N, Elgün A, Herken EN, Türker S, Ertaş N, İbanoğlu S (2006) J. Food Eng. 77: 680–686

Bilgiçli N, İbanoğlu S (2007) J. Food Eng. 78: 681-686

Bilgiçli N, İbanoğlu S, Herken EN (2007) J. Food Eng. 78: 86-89

Brennan ChS, Cleary LJ (2005) J. Cereal Sci. 42: 1-13

Bright SWJ, Shewry PR (1983) Crit. Rev. Plant Sci. 1: 49–93

Cakmak I (2004) IFS Proceedings No. 552, pp 1–28, International Fertiliser Society, York

Cakmak I (2008) Plant Soil 302: 1–17

Cornell H (2003) In: Cauvain SP (ed) Bread Making: Improving Quality. Woodhead Publishing, Cambridge

Dalmo, RA, Bøgwald, J (2008) Fish Shellfish Immunol. 25: 384-396

Ehrenbergerova J, Belcrediova N, Pryma J, Vaculova K, Newman CW (2006) Plant Foods Hum. Nutr. 61: 145–150

Ehrenbergerová J, Březinová Belcredi N, Psota V, Hrstková P, Cerkal R, Newman CW (2008) Plant Foods Hum. Nutr. 63:141-145

Englyst HN, Kingman SM, Hudson GJ, Cummings JH (1996) Br. J. Nutr. 75: 749–755

Fassler C, Arrigoni E, Venema K, Hafner V, Brouns F, Amado R (2006) Eur. J. Nutr. 45: 445–453

Ficco DBM, Riefolo C, Nicastro G, De Simone V, Di Gesù AM, Beleggia R, Platani C, Cattivelli L, De Vita P (2009) Field Crops Res. 111: 235-242

Gibson RS (2006) Proc. Nutr. Soc. 65: 51–60

Gordon N (1997) Brain Dev. 19: 165–170

Graham RD, Senadhira D, Beebe S, Iglesias C, Monasterio I (1999) Field Crops Res. 60: 57–80

Grela ER (1996) J. Sci. Food Agr.71: 399– 404

Guenoune D, Landau S, Amir R, Badani H, Devash L, Wolf S, Galili D (2002) J. Agric. Food Chem. 50: 2256–2260

Hesser JM (1994) Int. Food Ing. 172: 50–52

Hidalgo A, Brandolini A, Pompei C, Piscozzi R (2006) J. Cereal Sci. 44: 182-193

Hidalgoa A, Brandolini A (2008) Food Chem. 1: 444-448

Hotz C, Brown KH (2004) Food Nutr. Bull. 25: 94–204

Cheng MK (1998) M. Phil. dissertation, The Chinese University of Hong Kong

Katsube T, Kurisaka N, Ogawa M, Maruyama N, Ohtsuka R, Utsumi S, Takaiwa F (1999) Plant Physiol. 120: 1063–1073

Keeler SJ, Maloney CL, Webber PY, Patterson C, Hirata LT. Falco SC., Rice JA (1997) Plant Mol. Biol. 34: 15–29

Konik-Rose Ch, Thistleton J, Chanvrier H, Tan I, Halley P, Gidley M, Kosar-Hashemi B, Wang H, Larroque O, Ikea J, McMaugh S, Regina A, Rahman S, Morell M, Li Z (2007) Theor. Appl. Genet. 115: 1053–1065

Li Z, Chu X, Mouille G, Yan L, Kosar-Hashemi B, Hey S, Napier J, Shewry P, Clarke B, Appels R, Morell MK, Rahman S (1999) Plant Physiol. 120: 1147–1156

Li Z, Sun F, Xu S, Chu X, Mukai Y, Yamamoto M, Ali S, Rampling L, Kosar-Hashemi B, Rahman S, Morell MK (2003) Funct. Integr. Genomics 3: 76–85

Lindsay DG (2002) Phytochem. Rev. 1: 101–111

Liu QQ (2002) Ph.D. dissertation, Yangzhou University and The Chinese University of Hong Kong

Lopez HW, Krespine V, Guy C, Messager A, Demigne C, Remesy C (2001) J. Agric. Food Chem. 49: 2657-2662

Lopez HW, Ouvry A, Bervas E, Guy C, Messager A, Demigne C, Remesy C (2000) J. Agric. Food Chem. 48: 2281-2285

Lyons GH, Ortiz-Monasterio I, Stangoulis J, Graham R (2005) Plant Soil 269: 369–380

Lyons GH, Stangoulis JCR, Graham RD (2003) Nutr. Res. Rev. 16: 45– 60

Maberly GF, Trowbridge FL, Yip R, Sullivan KM, West CE (1994) Ann. Rev. Public Health 15: 277–301

Maruta Y, Ueki J, Saito H, Nitta N, Imaseki H (2001) Mol. Breed. 8: 273–284

McGuire J (1993) SCN News 9: 1–10

Molberg O, Uhlen AK, Jensen T, Flæte NS, Fleckenstein B, Arentz–Hansen E, Raki M, Lundin KEA, Ludvig M (2005) Gastroenterol. 128: 393-401

Morell MK, Li Z, Regina A, Rahman S, d'Hulst C, Ball SG (2006) In: Plaxton WC, McManus MT (eds) Control of primary metabolism in plants. Annual plant reviews, pp 258–289. Blackwell, Oxford

Murphy DJ (2006) In Vitro Cell. Dev. Biol.: Plant 42: 89–99

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Peña RJ (2007) J. Cereal Sci. 46: 293-307

Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A, Ozkan H, Braun H-J, Sayers Z, Cakmak I (2006) Physiol. Plant. 128: 144–152

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Nat. Biotechnol. 23: 482–487

Paredes-López O, Osuna–Castro JA (2006) In: Shetty K, Paliyath G, Pometto AL, Levin RE (eds) Functional foods and biotechnology. 650 p, Marcel Dekker Inc., New York

Peleg Z, Saranga Y, Yazici A, Fahima T, Ozturk L, Cakmak I (2008) Plant Soil 306: 57-67

Piergiovanni AR, Rizzi R, Pannacciulli E, Della Gatta C (1997) Int. J. Food Sci. Nutr. 48: 381–386

Prasad AS (2007) J. Nutr. 137: 1345–1349

Rave K, Roggen K, Dellweg S, Heise T, Dieck TH (2007) Br. J. Nutr. 98: 929-936

Rayman MP (2002) Proc. Nutr. Soc. 61: 203–215

Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T, Kosar- Hashemi B, Li Z, Rahman S, Morell MK (2006) Proc. Natl. Acad. Sci. 103: 3546–3551

Seregina II, Nilovskaya NT, Ostapenko NO (2001) Agrokhimiya 1: 44–50

Shimizu C, Kihara M, Aoe S, Araki S, Ito K, Hayashi K, Watari J, Sakata Y, Ikegami S (2008) Plant Foods Hum. Nutr. 63: 21-25

Singh J, Sharp PJ, Skerritt JH (2000) J. Sci. Food Agric. 81: 216–226

Smith AM (2001) Biomacromol. 2: 335–341

Spaenij-Dekking L, Kooy-Winkelaar Y, van Veelen P, Wouter Drijfhout J, Jonker H, van Soest L, Smulders MJM, Bosch D, Gilissen LJWJ, Koning F (2005) Gastroenterol. 129: 797-806

Sun SSM, Liu QQ (2004) In Vitro Cell. Dev. Biol. Plant 40: 155–162

Šramková Z, Havrlentová M, Gregová E, Šturdík E, Hauptvogel P (2008) Chem. Listy 102: 1154

Tang G, Galili G, Zhuang X (2007) Metabolomics 3: 357–369

Theriault A, Chao JT, Wang Q, Gapor A, Adeli K (1999) Clinical Biochem. 32: 309–319

Torrent M, Alvarez I, Geli MI, Dalcol I, Ludevid D (1997) Plant Mol. Biol. 34: 139–149

Tozawa Y, Hasegawa H, Terakawa T, Wakasa K (2001) Plant Physiol. 126: 1493–1506

Tveitnes S, Singh BR, Ruud L (1996) Fert. Res. 45: 163–167

Viteri FEV (1998) In: Howson CP, Kennedy ET, Horwitz A (eds) Prevention of Micronutrient Deficiencies. pp. 45–102. National Academy Press, Washington DC

Walter T, Peirano P, Roncagliolo M (1997) In: Fischer PWF, L'Abbe MR, Cockell KA, Gibson RS (eds) Trace Elements in Man and Animals – 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals, pp. 217–219, National Research Council of Canada, Ottawa

Wandelt CI, Khan MRI, Craig S, Achroeder HE, Spencer D, Higgins TJV (1992) Plant J. 2: 181–192

Weickert MO, Pfeiffer AF (2008) J. Nutr. 138: 439-42

Welch RM (2002) J. Nutr. 132: 495S-499S

Welch RM (2005) Food Nutr. Bull. 26: 419-21

Welch RM, Graham RD (1999) Field Crops Res. 60: 1–10

Welch RM, Graham RD (2002) Plant Soil 245: 205–214

Welch RM, Graham RD (2004) J. Exp. Bot. 55: 353–364

Wood P. (1997) In: Campbel GM, Webb C, McKee S (eds) Proceedings of an international conference on Cereals: Novel Uses and Processes, held June 4-6, 1996. Springer, Manchester

Yamamori M, Fujita S, Hayakawa K, Matsuki J, Yasui T (2000) Theor. Appl. Genet. 101: 21–29

Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Science 287: 303–305

Yip R (1997) Euro. J. Clin. Nutr. 51: S16–S24

Yonekura-Sakakibara K, Saito K (2006) Biotechnol. Lett. 28: 1983–1991

Zhu XH, Galili G (2003) Plant Cell 15: 845–853