

Chapter 2

Chemistry of Cereal Grains

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2.1 Introductory Remarks

Cereals are the most important staple foods for mankind worldwide and represent the main constituent of animal feed. Most recently, cereals have been additionally used for energy production, for example by fermentation yielding biogas or bioethanol. The major cereals are wheat, corn, rice, barley, sorghum, millet, oats, and rye. They are grown on nearly 60% of the cultivated land in the world. Wheat, corn, and rice take up the greatest part of the land cultivated by cereals and produce the largest quantities of cereal grains (Table 2.1) [1]. Botanically, cereals are grasses and belong to the monocot family *Poaceae*. Wheat, rye, and barley are closely related as members of the subfamily *Pooideae* and the tribus *Triticeae*. Oats are a distant relative of the *Triticeae* within the subfamily *Pooideae*, whereas rice, corn, sorghum, and millet show separate evolutionary lines. Cultivated wheat comprises five species: the hexaploid common (bread) wheat and spelt wheat (genome AABBDD), the tetraploid durum wheat and emmer (AABB), and the diploid einkorn (AA). Triticale is a man-made hybrid of durum wheat and rye (AABBRR). Within each cereal species numerous varieties exist produced by breeding in order to optimize agronomical, technological, and nutritional properties.

The farming of all cereals is, in principle, similar. They are annual plants and consequently, one planting yields one harvest. The demands on climate, however, are different. “Warm-season” cereals (corn, rice, sorghum, millet) are grown in tropical lowlands throughout the year and in temperate climates during the frost-free season. Rice is mainly grown in flooded fields, and sorghum and millet are adapted to arid conditions. “Cool-season” cereals (wheat, rye, barley, and oats) grow best in a moderate climate. Wheat, rye, and barley can be differentiated into

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Table 2.1 Cereal production in 2010 [1]

Species	Cultivated area (million ha)	Grain production (million tons)
Corn	162	844
Rice	154	672
Wheat	217	651
Barley	48	123
Sorghum + millet	76	85
Oats	9	20
Triticale	4	13
Rye	5	12

winter or spring varieties. The winter type requires vernalization by low temperatures; it is sown in autumn and matures in early summer. Spring cereals are sensitive to frost temperatures and are sown in springtime and mature in midsummer; they require more irrigation and give lower yields than winter cereals.

Cereals produce dry, one-seeded fruits, called the “kernel” or “grain”, in the form of a caryopsis, in which the fruit coat (pericarp) is strongly bound to the seed coat (testa). Grain size and weight vary widely from rather big corn grains (~350 mg) to small millet grains (~9 mg). The anatomy of cereal grains is fairly uniform: fruit and seed coats (bran) enclose the germ and the endosperm, the latter consisting of the starchy endosperm and the aleurone layer. In oats, barley, and rice the husk is fused together with the fruit coat and cannot be simply removed by threshing as can be done with common wheat and rye (*naked* cereals).

The chemical composition of cereal grains (moisture 11–14%) is characterized by the high content of carbohydrates (Table 2.2) [2, 3]. Available carbohydrates, mainly starch deposited in the endosperm, amount to 56–74% and fiber, mainly located in the bran, to 2–13%. The second important group of constituents is the proteins which fall within an average range of about 8–11%. With the exception of oats (~7%), cereal lipids belong to the minor constituents (2–4%) along with minerals (1–3%). The relatively high content of B-vitamins is, in particular, of nutritional relevance. With respect to structures and quantities of chemical constituents, notable differences exist between cereals and even between species and varieties within each cereal. These differences strongly affect the quality of products made from cereal grains. Because of the importance of the constituents, in the following we provide an insight into the detailed chemical composition of cereal grains including carbohydrates, proteins, lipids, and the minor components (minerals and vitamins).

2.2 Carbohydrates

Cereal grains contain 66–76% carbohydrates (Table 2.2), thus, this is by far the most abundant group of constituents. The major carbohydrate is starch (55–70%) followed by minor constituents such as arabinoxylans (1.5–8%), β -glucans (0.5–7%), sugars (~3%), cellulose (~2.5%), and glucofructans (~1%).

Table 2.2 Chemical composition of cereal grains (average values) [2, 3]

	Wheat	Rye	Corn	Barley	Oats	Rice	Millet
	(g/100 g)						
Moisture	12.6	13.6	11.3	12.1	13.1	13.0	12.0
Protein (N×6.25)	11.3	9.4	8.8	11.1	10.8	7.7	10.5
Lipids	1.8	1.7	3.8	2.1	7.2	2.2	3.9
Available carbohydrates	59.4	60.3	65.0	62.7	56.2	73.7	68.2
Fiber	13.2	13.1	9.8	9.7	9.8	2.2	3.8
Minerals	1.7	1.9	1.3	2.3	2.9	1.2	1.6
	(mg/kg)						
Vitamin B ₁ (thiamine)	4.6	3.7	3.6	4.3	6.7	4.1	4.3
Vitamin B ₂ (riboflavin)	0.9	1.7	2.0	1.8	1.7	0.9	1.1
Nicotinamide	51.0	18.0	15.0	48.0	24.0	52.0	18.0
Panthenic acid	12.0	15.0	6.5	6.8	7.1	17.0	14.0
Vitamin B ₆	2.7	2.3	4.0	5.6	9.6	2.8	5.2
Folic acid	0.9	1.4	0.3	0.7	0.3	0.2	0.4
Total tocopherols	41.0	40.0	66.0	22.0	18.0	19.0	40.0

2.2.1 Starch

Starch is the major storage carbohydrate of cereals and an important part of our nutrition. Because of its unique properties starch is important for the textural properties of many foods, in particular bread and other baked goods. Finally, starch is nowadays also an important feedstock for bioethanol or biogas production (for reviews see [4, 5]).

2.2.1.1 Amylose and Amylopectin

Starch occurs only in the endosperm and is present in granular form. It consists of the two water-insoluble homoglucans amylose and amylopectin. Cereal starches are typically composed of 25–28% amylose and 72–75% amylopectin [6]. Mutant genotypes may have an altered amylose/amylopectin ratio. “Waxy” cultivars have a very high amylopectin level (up to 100%), whereas “high amylose” or “amylostarch” cultivars may contain up to 70% amylose. This altered ratio of amylose/amylopectin affects the technological properties of these cultivars [7, 8]. High-amylose wheat has been suggested as a raw material for the production of enzyme-resistant starch [9].

Amylose consists of α -(1,4)-linked D-glucopyranosyl units and is almost linear. Parts of the molecules also have α -(1,6)-linkages providing slightly branched structures [10, 11]. The degree of polymerization ranges from 500 to 6,000 glucose units giving a molecular weight (MW) of 8×10^4 to 10^6 . Amylopectin is responsible for the granular nature of starch. It contains 30,000–3,000,000 glucose units and, therefore, it has a considerably higher MW (10^7 – 10^9) than amylose [12]. Amylopectin is a highly branched polysaccharide consisting of α -(1,4)-linked D-glucopyranosyl

chains, which are interconnected via α -(1,6)-glycosidic linkages, also called branch points [13]. The α -(1,4)-linked chains have variable length of 6 to more than 100 glucose units depending on the molecular site at which they are located. The unbranched A- or outer chains can be distinguished from the branched B- or inner chains, which can be subdivided into B1-, B2-, B3-, and B4-chains [14]. The molecules are “terminated” by a single C-chain containing the reducing glucose residue [15]. Amylopectin has a tree-like structure, in which clusters of chains occur at regular intervals along the axis of the molecule [16]. Short A- and B1-chains of 12–15 glucose residues form the clusters which have double-helical structures. The longer, less abundant B2-, B3-, and B4-chains interconnect 2, 3 or 4 clusters, respectively. B2-chains contain approximately 35–40, B3-chains 70–80, and B4-chains up to more than 100 glucose residues [12, 17].

2.2.1.2 Starch Granules

In the endosperm starch is present as intracellular granules of different sizes and shapes, depending on the cereal species. In contrast to most plant starches, wheat, rye, and barley starches usually have two granule populations differing in size. Small spherical B-granules with an average size of 5 μm can be distinguished from large ellipsoid A-granules with mean diameters around 20 μm [18]. In the polarization microscope native starch granules are birefringent indicating that ordered, partially crystalline structures are present in the granule. The degree of crystallinity ranges from 20 to 40% [19] and is primarily caused by the structural features of amylopectin. It is thought that the macromolecules are oriented perpendicularly to the granule surface [12, 16] with the nonreducing ends of the molecules pointing to the surface.

A model of starch granule organization from the microscopic to the nanoscopic level has been suggested [12]. At the microscopic level alternating concentric “growth rings” with periodicities of several hundreds of nanometers can be observed. They reflect alternating semicrystalline and amorphous shells [12]. The latter are less dense, enriched in amylose, and contain noncrystalline amylopectin. They further consist of alternating amorphous and crystalline lamellae of about 9–10 nm [20]. Crystalline regions contain amylopectin double helices of A- and B1-chains oriented in parallel fashion and possibly 18 nm-wide, left-handed superhelices formed from double helices. Amorphous regions represent the amylopectin branching sites, which may also contain a few amylose molecules. The lamellae are organized into larger spherical blocklets, which vary periodically in diameter between 20 and 500 nm [21]. The amylopectin double helices may be packed into different crystal types. The very densely packed A-type is found in most cereal starches, while the more hydrated tube-like B-type is found in some tuber starches, high amylose cereal starches, and retrograded starch [12, 19]. Mixtures of A- and B-types are designated C-type.

2.2.1.3 Changes in Starch Structure During Processing

In many cereal manufacturing processes flour and also starch is usually dispersed in water and finally heated. In particular heating induces a series of structural changes. This process has been termed gelatinization [22]. Depending on water content, water distribution, and intensity of heat treatment the molecular order of the starch granules can be completely transformed from the semicrystalline to an amorphous state.

The mixing of starch and excess water at room temperature leads to a starch suspension. During mixing starch absorbs water up to 50% of its dry weight (1) because of physical immobilization of water in the void space between the granules, and (2) because of water uptake due to swelling. The latter process increases with temperature. If the temperature is below the gelatinization temperature, the described changes are reversible. As the temperature increases, more water permeates into the starch granules and initiates hydration reactions. Firstly, the amorphous regions are hydrated thereby increasing molecular mobility. This also affects the crystalline regions, in which amylopectin double helices dissociate and the crystallites melt [23, 24]. These reactions are endothermic and irreversible. They are accompanied by the loss of birefringence, which can be observed under the polarization microscope. Endothermic melting of crystallites can also be followed by differential scanning calorimetry (DSC). Viscosity measurements, for example in an amylograph or a rapid visco analyzer, also allow one to monitor the gelatinization process. Characteristic points are the onset temperature (T_o ; ca. 45 °C), which reflects the initiation of the process, as well as the peak (T_p ; ca. 60 °C) and conclusion (T_c ; ca. 75 °C) temperatures. These temperatures are subject to change depending on the botanical source of the starch and the water content of the suspension. The loss of molecular order and crystallinity during gelatinization is accompanied by further granule swelling due to increased water uptake and a limited starch solubilization. Mainly amylose is dissolved in water, which strongly increases the viscosity of the starch suspension. This phenomenon has been termed “amylose leaching,” and it is caused by a phase separation between amylose and amylopectin, which are immiscible [25]. During further heating beyond the conclusion temperature of gelatinization swelling and leaching continue and a starch paste consisting of solubilized amylose and swollen, amorphous starch granules is formed. The shapes of the starch granules can still be observed unless shear force or higher temperatures are applied [23, 26].

Upon cooling with mixing the viscosity of a starch paste increases, whereas a starch gel is formed on cooling without mixing at concentrations above 6%. The second process is relevant in cereal baked goods. The changes that occur during cooling and storage of a starch paste have been summarized as “retrogradation” [22]. Generally, the amorphous system reassociates to a more ordered, crystalline state. Retrogradation processes can be divided into two subprocesses. The first is related to amylose and occurs in a time range of minutes to hours, the second is caused by amylopectin and takes place within hours or days. Therefore, amylose retrogradation is responsible for the initial hardness of a starch gel or bread, whereas amylopectin retrogradation determines the long-term gel structure, crystallinity, and hardness of a starch-containing food [27].

On cooling granule remnants that are enriched in amorphous amylopectin become incorporated into a continuous amylose matrix. Amylose molecules that are dissolved during gelatinization reassociate to local double helices interconnected by hydrated parts of the molecules, and a continuous network (gel) forms [27]. As amylose retrogradation proceeds, double helix formation increases and, finally, very stable crystalline structures are formed, which cannot be melted again by heating. Amylopectin retrogradation takes several hours or days and occurs in the granule remnants embedded in the initial amylose gel [27]. Crystallization mainly occurs within the short-chain outer A- and B1-chains of the molecules. The amylopectin crystallites melt at ca. 60 °C and, therefore, aged bread can partly be “refreshed” by heating. This so-called “staling endotherm” can be measured by DSC to evaluate amylopectin retrogradation. Amylopectin retrogradation is strongly influenced by a number of conditions and substances, including pH and the presence of low-molecular-weight (LMW) compounds such as salts, sugars, and lipids [26].

2.2.1.4 Interaction with Lipids

Amylose is able to form helical inclusion complexes in particular with polar lipids and this can occur in native (starch lipids; see below) as well as in gelatinized starch [28]. During gelatinization amylose forms a left-handed single helix and the nonpolar moiety of the polar lipid is located in the central cavity [16]. The inclusion complexes give rise to a V-type X-ray diffraction pattern. The presence of polar lipids strongly affects the retrogradation characteristics of the starch, because amylose-lipid complexes do not participate in the recrystallization process [26]. Complex formation is, however, strongly affected by the structure of the polar lipid [29]. For example, monoglycerides are more active than diglycerides and saturated fatty acids more active than unsaturated ones, because inclusion complexes are preferably formed with linear hydrocarbon chains and with compounds having one fatty acid residue. In addition, lipids, in particular lysophospholipids (lysolecithin), are minor constituents of cereal starches in amounts of 0.8–1.2% [30]. As so-called starch lipids they are associated with amylose as well as with the outer branches of amylopectin [28]. These lipid complexes lead to a delay of the onset of gelatinization and affect the properties of the starch especially in baking applications.

2.2.2 Nonstarch Polysaccharides (NSP)

Polysaccharides other than starch are primarily constituents of the cell walls and are much more abundant in the outer than in the inner layers of the grains. Therefore, a higher extraction rate is associated with a higher content of NSP. From a nutritional point of view NSP are dietary fiber, which has been associated with positive health effects. For example, cereal dietary fiber has been related to a reduced risk of chronic

life style diseases such as cardiovascular diseases, type II diabetes, and gastrointestinal cancer [31–36]. In addition, technological functionalities have been described for the arabinoxylans (AX) of wheat (reviewed by [4]) and rye.

2.2.2.1 Arabinoxylans

AX are the major fraction (85–90%) of the so-called pentosans. Different cereal species contain different amounts of AX. The highest contents are present in rye (6–8%), whereas wheat contains only 1.5–2% AX. On the basis of solubility AX can be subdivided into a water-extractable (WEAX) and a water-unextractable fraction (WUAX). The former makes up 25–30% of total AX in wheat and 15–25% in rye [37]. In particular WEAX has considerable functionality in breadmaking.

AX consist of linear β -(1,4)-D-xylopyranosyl-chains, which can be substituted at the O-2 and/or O-3-positions with α -L-arabinofuranose [38, 39]. A particular minor component of AX is ferulic acid, which is bound to arabinose as an ester at the O-5 position [40]. AX of different cereals may vary substantially in content, substitutional pattern and molecular weight [41–43]. WEAX mainly consist of two populations of alternating open and highly branched regions, which can be distinguished by their characteristic arabinose/xylose ratios, ranging between 0.3 and 1.1 depending on the specific structural region [44]. WUAX can be solubilized by mild alkaline treatment yielding structures that are comparable to those of WEAX [37, 45–48].

The unique technological properties of AX are attributable to the fact that AX are able to absorb 15–20 times more water than their own weight and, thus, form highly viscous solutions, which may increase gas holding capacity of wheat doughs via stabilization of the gas bubbles [49]. In total, WEAX bind up to 25% of the added water in wheat doughs [50]. Under oxidizing conditions, in particular under acidic pH, the so-called “oxidative gelation” [51] leads to AX gel formation by inducing di- and oligoferulic acid cross-links [52, 53]. This is thought to be one major structure-forming reaction in rye sourdoughs. Because of covalent cross-links to the cell wall structure WUAX do not dissolve in water. Although they have high water-holding capacity and assist in water binding during dough mixing they are considered to have a negative impact on wheat breadmaking as they form physical barriers against the gluten network and, thus, destabilize the gas bubbles. However, the baking performance can be affected by adding endoxylanases, which preferentially hydrolyze WUAX. This produces solubilized WUAX, which have techno-functional effects comparable to WEAX [54, 55].

Beside AX the pentosan fraction contains a small part of a water-soluble, highly branched arabinogalactan peptide [41]. It consists of β -(1,3) and β -(1,6) linked galactopyranose units with α -glycosidically bound arabinofuranose residues. The peptide is attached by 4-*trans*-hydroxyproline. Unlike AX, arabinogalactan peptides have no significant effects in cereal processing.

2.2.2.2 β -Glucans

β -Glucans are also called lichenins and are present particularly in barley (3–7%) and oats (3.5–5%), whereas less than 2% β -glucans are found in other cereals. The chemical structure of these NSP is made up of linear D-glucose chains linked via mixed β -(1,3)- and β -(1,4)-glycosidic linkages. β -Glucans show a higher water solubility than AX (38–69% in barley, 65–90% in oats) and form viscous solutions, which in the case of barley may interfere in wort filtration during the production of beer.

2.3 Proteins

The average protein content of cereal grains covers a relatively narrow range (8–11%, Table 2.2), variations, however, are quite noticeable. Wheat grains, for instance, may vary from less than 6% to more than 20%. The content depends on the genotype (cereal, species, variety) and the growing conditions (soil, climate, fertilization); amount and time of nitrogen fertilization are of particular importance. Proteins are distributed over the whole grain, their concentration within each compartment, however, is remarkably different. The germ and aleurone layer of wheat grains, for instance, contain more than 30% proteins, the starchy endosperm ~13%, and the bran ~7% [3]. Regarding the different proportions of these compartments, most proteins of grains are located in the starchy endosperm, which is the source of white flours obtained by milling the grains and sieving.

White flours are the most important grain products. Therefore, the predominant part of the literature on cereal proteins deals with white flour proteins. The amino acid compositions of flour proteins from various cereals are shown in Table 2.3. Typical of all flours is the fact that glutamic acid almost entirely occurs in its amidated form as glutamine [56]. This amino acid generally predominates (15–31%), followed by proline in the case of wheat, rye, and barley (12–14%). Further major amino acids are leucine (7–14%) and alanine (4–11%). The nutritionally essential amino acids tryptophan (0.2–1.0%), methionine (1.3–2.9%), histidine (1.8–2.2%), and lysine (1.4–3.3%) are present only at very low levels. Through breeding and genetic engineering, attempts are being made to improve the content of essential amino acids. These approaches have been successful in the case of high-lysine barley and corn.

2.3.1 Osborne Fractions

Traditionally, cereal flour proteins have been classified into four fractions (albumins, globulins, prolamins, and glutelins) according to their different solubility and based on the fractionation procedure of Osborne [57]. Albumins are soluble in water,

Table 2.3 Amino acid composition (mol-%) of the total proteins of flours from various cereals [56]

Amino acid	Wheat	Rye	Barley	Oats	Rice	Millet	Corn
Asx ^a	4.2	6.9	4.9	8.1	8.8	7.7	5.9
Thr	3.2	4.0	3.8	3.9	4.1	4.5	3.7
Ser	6.6	6.4	6.0	6.6	6.8	6.6	6.4
Glx ^a	31.1	23.6	24.8	19.5	15.4	17.1	17.7
Pro	12.6	12.2	14.3	6.2	5.2	7.5	10.8
Gly	6.1	7.0	6.0	8.2	7.8	5.7	4.9
Ala	4.3	6.0	5.1	6.7	8.1	11.2	11.2
Cys	1.8	1.6	1.5	2.6	1.6	1.2	1.6
Val	4.9	5.5	6.1	6.2	6.7	6.7	5.0
Met	1.4	1.3	1.6	1.7	2.6	2.9	1.8
Ile	3.8	3.6	3.7	4.0	4.2	3.9	3.6
Leu	6.8	6.6	6.8	7.6	8.1	9.6	14.1
Tyr	2.3	2.2	2.7	2.8	3.8	2.7	3.1
Phe	3.8	3.9	4.3	4.4	4.1	4.0	4.0
His	1.8	1.9	1.8	2.0	2.2	2.1	2.2
Lys	1.8	3.1	2.6	3.3	3.3	2.5	1.4
Arg	2.8	3.7	3.3	5.4	6.4	3.1	2.4
Trp	0.7	0.5	0.7	0.8	0.8	1.0	0.2
Amide group	31.0	24.4	26.1	19.2	15.7	22.8	19.8

^aAsx Asp+Asn, Glx Glu+Gln

while globulins are insoluble in pure water but soluble in dilute salt solutions. Prolamins are classically defined as cereal proteins soluble in aqueous alcohols, for example 60–70% ethanol. Originally, glutelins were described as proteins that were insoluble in water, salt solution, aqueous alcohols and soluble in dilute acids or bases. Later, it was ascertained that notable portions of glutelins are insoluble in dilute acids such as acetic acid, and that extraction with strong bases destroys the primary structure of proteins. Nowadays, complete solubility of glutelins is achieved by solvents containing a mixture of aqueous alcohols (e.g., 50% propanol), reducing agents (e.g., dithiothreitol), and disaggregating compounds (e.g., urea).

Regarding their functions, most of the albumins and globulins are metabolic proteins, for example enzymes or enzyme inhibitors (see Sect. 2.3.4). Oats are an exception containing considerable amounts of legume-like globulins such as 12S globulin [58]. Albumins and globulins are concentrated in the aleurone layer, bran, and germ, whereas their concentration in the starchy endosperm is relatively low. Predominantly, prolamins and glutelins are the storage proteins of cereal grains (see Sects. 2.3.2 and 2.3.3). Their only biological function is to supply the seedling with nitrogen and amino acids during germination. They are located only in the starchy endosperm; in white flours, their proportions based on total proteins amount to 70–90%. In general, none of the Osborne fractions consists of a single protein, but of a complex mixture of different proteins. A small portion of proteins does not fall into any of the four solubility fractions. Together with starch, they remain in the insoluble residue after Osborne fractionation and mainly belong to the class of lipo (membrane) proteins.

The prolamin fractions of the different cereals have been given trivial names: gliadin (wheat), secalin (rye), hordein (barley), avenin (oats), zein (corn), kafirin (millet, sorghum), and oryzin (rice). The glutelin fraction of wheat has been termed glutenin. Terms for the other glutelin fractions such as secalinin (rye), hordenin (barley), and zeanin (corn) are scarcely used today. Gliadin and glutenin fractions of wheat have been combined in the terms gluten or gluten proteins.

The content of the Osborne fractions varies considerably and depends on genotype and growing conditions. Moreover, the results of Osborne fractionation are strongly influenced by experimental conditions, and the fractions obtained are not clear-cut. Therefore, data from the literature on the qualitative and quantitative composition of Osborne fractions is differing and, in parts, contradictory. On average, the smallest proportion of total protein is present in the globulin fraction, followed by the albumin fraction. An exception is oat globulins amounting to more than 50% of total proteins. In most cereal flours, prolamins are the dominating fractions, oat prolamins, however, are minor protein components and rice flour is almost free of prolamins. Beside quantitative aspects the Osborne procedure is still useful for the preparation and characterization of flour proteins and the enrichment of different protein types.

2.3.2 Storage Proteins of Wheat Rye, Barley, and Oats

2.3.2.1 Classification and Primary Structures

Storage proteins (prolamins and glutelins) have been extensively investigated by the analysis of amino acid compositions, amino acid sequences, MW, and intra- and interchain disulfide linkages. The results indicated that, in accordance with phylogeny (see Sect. 2.1), the storage proteins of wheat, rye, and barley are closely related, whereas those of oats, in particular their glutelins, are structurally divergent. According to common structures storage proteins have been classified into three groups by two different principles. Shewry and coworkers [59] defined all storage proteins as prolamins and grouped them into the high-molecular-weight (HMW), sulfur-poor (S-poor) and sulfur-rich (S-rich) prolamins based on differences in MW and sulfur (cysteine, methionine) content. To prevent confusion, however, the term “prolamin” is not used for total storage proteins in the present paper, since classically the term prolamins comprises only the alcohol-soluble portions of storage proteins and does not include glutelins. We classified storage proteins according to related amino acid sequences and molecular masses into the following groups [60, 61]: (1) a HMW group; (2) a medium-molecular-weight (MMW) group; and (3) a LMW group. The proteins of these groups can be divided into different types on the basis of structural homologies (Table 2.4). Each type contains numerous closely related proteins; the small differences in their amino acid sequences can be traced back to substitutions, insertions, and deletions of single amino acids and short peptides.

Table 2.4 Characterization of storage protein types from wheat, rye, barley, and oats [61, 63]

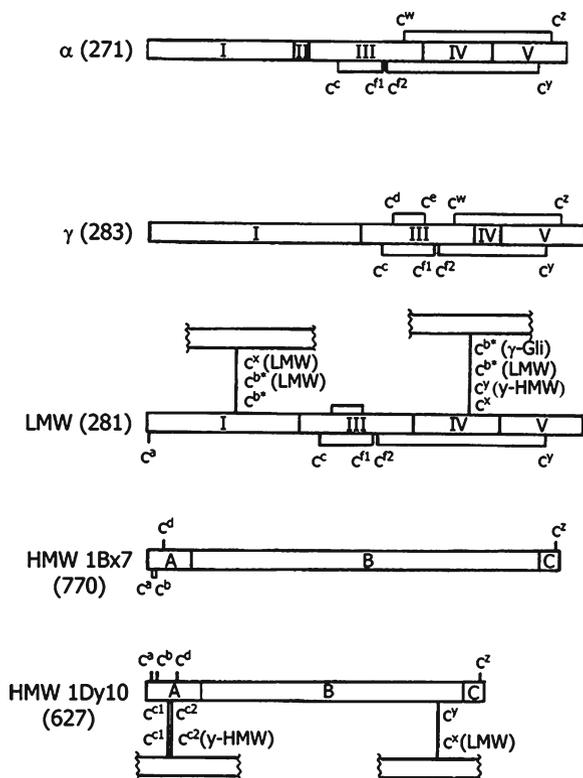
Group/type	Code	Residues	State ^a	Repetitive unit ^{b,c} (frequency)	Partial amino acid composition (mol-%) ^b						
					Q	P	F+Y	G	L	V	
HMW group											
HMW-GS x	Q6R2V1	815	a	QQPGQG (72×)	36	13	5.8	20	4.4	1.7	
HMW-GS y	Q52JL3	637	a	QQPGQG (50×)	32	11	5.5	18	3.8	2.3	
HMW-secalin x	Q94IK6	760	a	QQPGQG (66×)	34	15	6.7	20	3.7	1.5	
HMW-secalin y	Q94IL4	716	a	QQPGQG (60×)	34	12	5.0	18	3.2	1.8	
D-hordein	Q40054	686	a	QQPGQG (26×)	26	11	5.5	16	4.1	4.1	
MMW group											
ω5-gliadin	Q402I5	420	m	(Q)QQQFP (65×)	53	20	10.0	0.7	3.1	0.2	
ω1,2-gliadin	Q6DLC7	373	m	(QP)QQPFP (42×)	42	29	9.9	0.8	4.0	0.5	
ω-secalin	O04365	338	m	(Q)QPQQPFP (32×)	40	29	8.6	0.6	4.4	1.8	
C-hordein	Q40055	328	m	(Q)QPQQPFP (36×)	37	29	9.4	0.6	8.6	0.3	
LMW group											
α/β-gliadin	Q9M4M5	273	m	QPQFPFPQPYP (5×)	36	15	7.4	2.6	8.1	5.1	
γ-gliadin	Q94G91	308	m	(Q)QPQQPFP (15×)	36	18	5.2	2.9	7.2	4.6	
LMW-GS	Q52NZ4	282	a	(Q)QQPPFS (11×)	32	13	5.7	3.2	8.2	5.3	
γ-40 k-secalin ^d	–	–	m	QPQQPFP	34	18	5.5	2.4	7.4	4.7	
γ-75 k-secalin	Q9FR41	436	a	QPQQPFP (32×)	38	22	6.1	1.6	4.8	5.3	
γ-hordein	P17990	286	m	QPQQPFP (15×)	28	17	7.7	3.1	7.0	7.3	
B-hordein	P06470	274	a	QQPFPQ (13×)	30	19	7.3	2.9	8.0	6.2	
avenin	Q09072	203	m	PFVQQQQ (3×)	33	11	8.4	2.0	8.9	8.3	

^a*a* aggregative, *m* monomeric^bOne-letter-code for amino acids^cBasic unit frequently modified by substitution, insertion, and deletion of single amino acid residues^dGellrich et al. [65]

The nomenclature of types is rather confusing and inconsequential. On the one hand prolamins have been termed according to their electrophoretic mobility in acid polyacrylamide gel electrophoresis (PAGE) with band regions designated as ω (lowest mobility), γ (medium mobility), and α/β (highest mobility). On the other hand, the nomenclature is based on their apparent sizes (after reduction of disulfide bonds) as indicated by sodiumdodecyl sulfate (SDS-) PAGE; examples are HMW- and LMW-glutenin subunits (GS), HMW-secalins, D-, C-, and B-hordeins. Because of the different importance of HMW-GS for the bread-making quality of wheat, single subunits have been numbered according to their mobility on SDS-gel electrophoresis (original nos. 1–12), the genome (1A, 1B, or 1D), and the type (x or y); examples of nomenclature are HMW-GS 1Ax1, 1Bx7, and 1Dy10 [62].

The HMW group contains HMW-GS of wheat, HMW-secalins of rye, and D-hordeins of barley (Table 2.4); this type is missing in oats. HMW-GS and HMW-secalins can be subdivided into the x-type and the y-type. The proteins comprise around 600–800 amino acid residues corresponding to MW of 70,000–90,000.

Fig. 2.1 Schematic structure and disulfide bonds of α/β -gliadins, γ -gliadins, LMW-, and HMW-GS (Adapted from [64])



The amino acid compositions are characterized by high contents of glutamine, glycine, and proline. The amino acid sequences [63] can be separated into three structural domains: a nonrepetitive N-terminal domain A of ~100 residues, a repetitive central domain B of 400–700 residues, and a nonrepetitive C-terminal domain C with ~40 residues (Fig. 2.1) [64, 65]. Domain B is dominated by repetitive sequences such as QPQGQ (one-letter-code for amino acids) as a backbone with inserted sequences like YYPTSL, QQG, and QPG with remarkable differences between x- and y-types (Table 2.5). Domains A and C have a more balanced amino acid composition and more amino acid residues with charged side chains. In a native state, the proteins of the HMW group are aggregated through interchain disulfide bonds (Fig. 2.1).

The MMW group consists of the homologous ω 1,2-gliadins of wheat, ω -secalins of rye, and C-hordeins of barley including amino acid residues between 300 and 400 and MW around 40,000 (Table 2.4). Additionally, wheat contains unique ω 5-gliadins with more than 400 residues and MW around 50,000. This group, likewise, is not present in oats. The proteins of the MMW group have extremely unbalanced amino acid compositions characterized by high contents of glutamine, proline, and phenylalanine, which together account for ~80% of total residues. Most sections of the amino acid sequences are composed of repetitive units such as QPQPFP or QQQFP. This type of protein occurs as monomers and is readily soluble in aqueous alcohols and, in parts, even in water.

Table 2.5 Partial amino acid sequences of domain B of HMW-GS 1Dx2 (positions 93–338) and of HMW-GS 1Dy10 (positions 106–380) [63]

Position	Sequence ^a	Position	Sequence ^a
93	YYPSVTSPQQVS	106	YYPGVTSPRQGS
105	YYPGQASPQRPGQG	118	YYPGQASPQQPGQG
119	QQPGQG	132	QQPGKW
125	QQSGGQQG	138	QEPGQQQW
134	YYP--TSPQPGQW	147	YYP--TSLQQPGQG
146	QQPEQQPG	159	QQIGKGQQG
155	YYP--TSPQPGQL	168	YYP--TSLQQPGQGQQG
167	QQPAQG	183	YYP--TSLQHTGQR
173	QQPGGQQG	195	QQPVQG
182	QQPGGQPG	201	QQPEQG
191	YYP-TSSQLQPGQL	207	QQPGQWQQG
204	QQPAQGQQG	216	YYP--TSPQQLGQG
213	QQPGGQQG	228	QQPRQW
222	QQPGQG	234	QQSGGQQG
228	QQPGGQQG	243	HYP--TSLQQPGQGQQG
237	QQPGQG	258	HYL--ASQQPGQGQQG
243	QQPGGQQG	273	HYP--ASQQPGQGQQG
252	QQLGGQQG	288	HYP--ASQQPGQGQQG
261	YYP--TSLQQSGQGQPG	303	HYP--ASQQEPGQGQQG
276	YYP--TSLQQLGGQSG	318	QIPASQ
291	YYP--TSPQPGQG	324	QQPGGQQG
303	QQPGQL	333	HYP--ASLQQPGQGQQG
309	QQPAQG	348	HYP--TSLQQLGGQQQT
315	QQPGGQQG	363	QQPGQK
324	QQPGGQQG	369	QQPGQG
333	QQPGQG	375	QQTGGQ

^aOne-letter-code for amino acids; - deletion

The LMW group consists of monomeric proteins including α/β - and γ -gliadins of wheat, γ -40 k-secalins of rye, γ -hordeins of barley, and avenins of oats, and of aggregative proteins including LMW-GS of wheat, γ -75 k-secalins of rye, and B-hordeins of barley (Table 2.4). They have around 300 amino acid residues and MW ranging from 28,000–35,000, besides γ -75 k-secalins (~430 residues, MW ~50,000) and avenins (~200 residues, MW ~23,000). The amino acid compositions of the LMW group proteins are characterized by relatively high contents of hydrophobic amino acids besides glutamine and proline. The amino acid sequences consist of four (α/β -gliadins five) different sequence sections (Fig. 2.1). The N-terminal section I is rich in glutamine, proline, and phenylalanine forming repetitive units such as QPQPFPPQQPY (α/β -gliadins), QQPQQPFP (γ -gliadins), QQPQFS (LMW-GS), or PFVQQQQ (avenins). Section I of γ -75 k-secalins is prolonged by around 130 residues as compared to γ -40 k-secalins and that of avenins is shortened to around 40 residues. Section II is unique to α/β -gliadins and consists of a polyglutamine sequence (up to 18 Q-residues). Sections III, IV, and V possess more balanced

amino acid compositions and most of the cysteine residues that form only intrachain disulfide bonds (monomeric proteins) or both intra- and interchain disulfide bonds (aggregative proteins). The comparison of the amino acid sequences demonstrates that sections III and V contain homologous sequences, whereas section IV is, in part, unique to each type (Table 2.6). γ -Type proteins (γ -gliadins, γ -40 k-secalins [66], γ -75 k-secalins, γ -hordeins) show the highest conformity; α/β -gliadins, LMW-GS, and avenins have the lowest degree of homology within the LMW group. Most oat glutelins are globulin-like proteins and do not show any structural relationship with the HMW-, MMW-, and LMW-type proteins described above [67]. The reasons as to why they are not extractable with a salt solution are not yet clear.

As mentioned earlier, the quantitative composition of storage protein is strongly dependent on both genotype and growing conditions. Nevertheless, some constant data can be observed (Table 2.7) [68–70]: Proteins of the LMW group belong, by far, to the major components. Within this group, monomeric proteins (55–77% of total storage proteins) exceed aggregative proteins (10–25%) in the case of wheat species, whereas rye and barley are characterized by more aggregative (34–48%) than monomeric proteins (~25%). Proteins of the MMW and HMW groups belong to the minor components except ω -secalins (18%) and C-hordeins (36%) or are missing (oats). Within wheat species significant differences can be observed. Common wheat is characterized by the highest values for aggregative proteins (HMW-, LMW-GS) and a low monomeric/aggregated (m/a) ratio, and the “old” wheat species emmer and einkorn by low proportions of HMW-GS and high m/a ratios.

2.3.2.2 Disulfide Bonds

Disulfide bonds play an important role in determining the structure and properties of storage proteins. They are formed between sulfhydryl groups of cysteine residues, either within a single protein (intrachain) or between proteins (interchain). Most information on disulfide bonds is available for wheat gliadins and glutenins. With a few exceptions, ω -type gliadins are free of cysteine and, consequently occur as monomers. Most α/β - and γ -gliadins contain six and eight cysteine residues, respectively, and form three or four homologous intrachain disulfide bonds, present within or between sections III and V (Fig. 2.1) [64]. A small portion of gliadins is different from most gliadins and contains an odd number of cysteine residues. They may be linked to each other or to glutenins by an interchain disulfide bond. Homologous to γ -gliadins, γ -40 k- and γ -75 k-secalins, γ - and B-hordeins as well as avenins contain eight cysteine residues at comparable positions within sections III–V (Table 2.6). Probably they form four intrachain disulfide bonds homologous to those of γ -gliadins (Fig. 2.2). LMW-GS include eight cysteine residues, six of which form three intrachain disulfide bonds homologous to those of α/β - and γ -gliadins [64, 65]. Two cysteine residues located in sections I and IV are unique to LMW-GS; they are obviously not able to form intrachain bonds for steric reasons. They are involved in interchain bonds with residues of different proteins (LMW-GS, modified gliadins, y-type HMW-GS).

Table 2.6 Amino acid sequences of sections III, IV, and V of the LMW group [63]

Type	Positions	Sequences
(a) Section III		
α/β -gliadin	119–186	ILQQILQQQLPCMDVVLQQHNIVHGRSQVLQQSTY----QLLQELCCQHLWQIPEQSQCAIHNVVHAIIL
γ -gliadin	153–224	FIQPSLQQQLNPKCNILLQQCKPASLVSSL-WSIWVPSDCQVMRQCCQQLAQIPQQLQCAAIHSSVVHSIIM
LMW-GS	101–173	IVQPSVLOQLNPKCVFLQCCSPVAMPQRLARSQMWQQRCHVMQQCCQQLSQIPEQSRDYDAIRAITYSIIL
γ -40 k-secalin ^a	–	SIQLSLQQQLNPKCNVLLQQCSPVALVSSL-RSKIFPQSECQVMQQCCQQLAQIPPHLLQCAAIHSSVVHAIIM
γ -75 k-secalin	285–356	SIQLSLQQQLNPKCNVLLQQCSPVALVSSL-RSKIFPQSECQVMQQCCQQLAQIPQQLQCAAIHSSVVHAIIM
γ -hordein	135–206	TIQLYLQQQLNPKCKEFLQCRPVSLLSYI-WSKIVQQSSCRVMQQCCQLAQIPEQYKCTAIDSIVHAIFM
B-hordein	112–184	YVHPSILQQQLNPKCVFLQCCSPVPVQRIARSQMLQQSSCHVLLQQCCQQLQIPEQFRHEAIRAIVYSIFL
Avenin	43–114	FLQPLLLQQQLNPKCKQFLVQQCSPVAAPFL-RSQILRQAICQVTRQCCCRQLAQIPEQLRCPAHSVVQSIIL
(b) Section IV		
α/β -gliadin	187–222	HQQQKQQQPFSSQVSFQQPQQYPLGQGSFRPSQQN
γ -gliadin	225–253	QQQQQQQQQGMHIFLPLSQQQQVGGGSL
LMW-GS	174–228	QEQQGFVQAQQQPQQSGQVSSQSQSQQQQLGQCSFQQPQQQLGQQPQQQQQQQ
γ -40 k-secalin ^a	–	QQEQREGVQILLPQSHQQLVGGGAL
γ -75 k-secalin	357–381	QQEQREGVQILLPQSHQQLVGGGAL
γ -hordein	207–231	QQGQRQGVQIVQQPQQPQQVGGCVL
B-hordein	185–225	QEFPQQLVEGVSPQQQLWPQQVGGCSFQQPQQVGGQQQ
Avenin	115–155	QQQQQQQFQIQQLOQQQVFPQLQLQQQQVFPQLQQQQVFPQ
(c) Section V		
α/β -gliadin	223–273	PQAQGSVQPQQLPQF-EEIRNLALQTLPAMCNVYIPPYCTI--APFGIFGTNYR
γ -gliadin	254–308	VQQGHIQPPQPAQL-EAIRSLVQLTLPSCNVYVPPECSIMRAPFASIVAGIGGQ
LMW-GS	229–282	VLQGTFLQPHQIAHL-EAVTSIALRTLPTMCSVNVPLYSATTISVPEAVGTGV SAY
γ -40 k-secalin ^a	–	AQVQGHQPQQLSQFNVGHVQLMQLNQLPTMCNVYVPRQCPSRRHLHAMSIVCGH
γ -75 k-secalin	382–436	AQVQGHQPQQLSQL-EVVRSILVQLNQLPTMCNVYVPRQCSTIQAPFASIVTGVGH
γ -hordein	232–286	VQGGGVVQPQOLAQM-EAIRTLVQVPSMCMFNVPNCSTIKAPFVGVVTVGGGQ
B-hordein	226–274	VPQSAFLQPHQIAQL-EATTISIALRTLPMMSVNVPLYRILRGRVGPSVGV
Avenin	156–203	QLQVFNQPQMGGQI-EGMRAFALQALPAMCDVYVPPQCPVATAPLGGF

^aGellirich et al. [65]

Table 2.7 Proportions (%) of storage protein types of different wheat species, rye, and barley [68–70]

Cereal	Variety	Group				m/a ^a
		HMW	MMW	LMW		
		a	m	a	m	
Common wheat	Rektor	9.1	10.4	25.1	55.4	1.9
Spelt wheat	Schwabekorn	6.6	10.4	17.7	65.3	3.1
Durum wheat	Biodur	5.0	6.7	19.3	69.0	3.1
Emmer	Unknown	2.6	10.8	10.0	76.6	6.9
Einkorn	Unknown	3.5	12.8	19.3	64.5	3.4
Rye	Halo	9.0	17.6	48.4	25.0	0.7
Barley	Golden promise	5.0	35.8	34.1	25.1	1.6

^aa aggregative, m monomeric

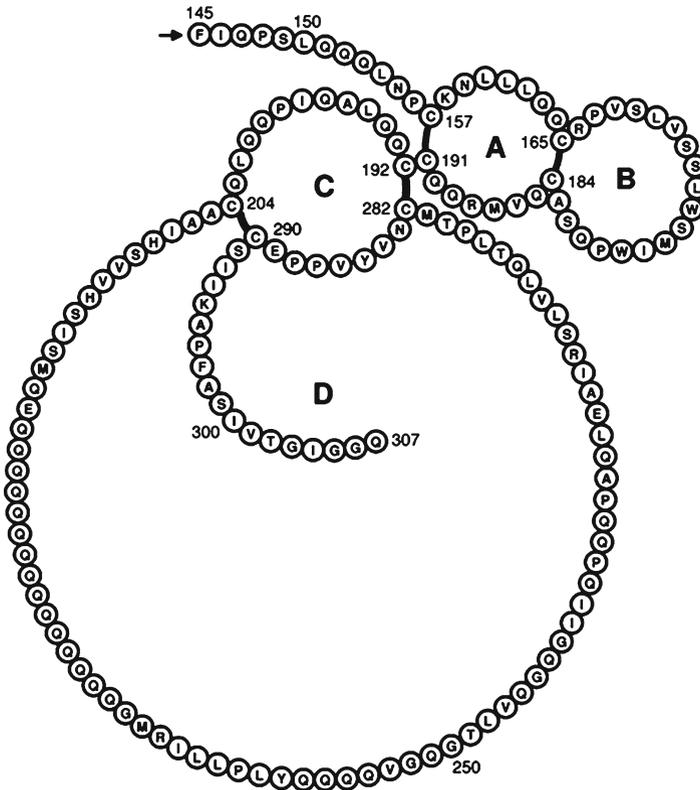


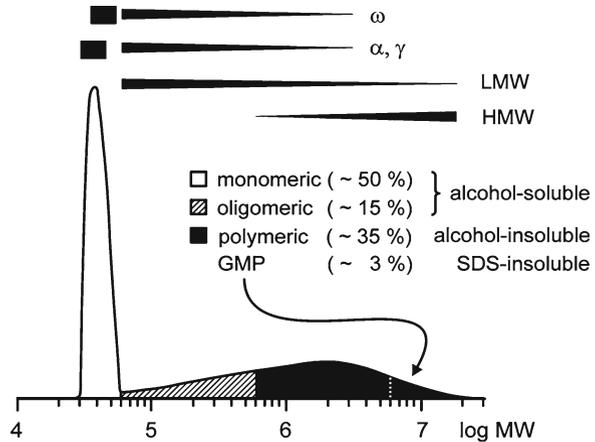
Fig. 2.2 Schematic two-dimensional structures of the C-terminal domain (sections III–V) of γ -gliadins (Taken from [94])

Because HMW-GS do not occur as monomers, it is generally assumed that they form interchain disulfide bonds. The x-type subunits, except subunit 1Dx5, have three cysteine residues in domain A and one in domain C (Fig. 2.1). Cysteines C^a and C^b were found to be linked by an intrachain bond, thus, the others (C^d, C^z) are available for interchain bonds. Subunit 1Dx5 has an additional cysteine residue at the beginning of domain B, and it has been suggested that this might form another interchain bond. Recently, a so-called head-to-tail disulfide bond between HMW-GS has been identified [71]. The y-type subunits have five cysteine residues in domain A and one in each of domains B and C. At present, interchain linkages have only been found for adjacent cysteine residues of domain A (C^{c1}, C^{c2}), which are connected in parallel with the corresponding residues of another y-type subunit, and for cysteine C^y of domain B, which is linked to C^x of section IV of LMW-GS. Thus, HMW- and LMW-GS fulfill the requirement that at least two cysteines forming interchain disulfide bonds are necessary to participate in a growing polymer; they act as “chain extenders.” The most recent glutenin model suggests a backbone formed by HMW-GS linked by end-to-end, probably head-to-tail interchain disulfide bonds [65]. LMW-GS form also linear polymers via cysteine residues of sections I and IV; they are linked to domain B of y-type HMW-GS. y-Type HMW-secalins of rye have a second cysteine in domain C, which opens the possibility that an intrachain disulfide bond within domain C is formed inhibiting an interchain bond for polymerization [72]. As far as is known, D-hordeins possess ten (!) cysteine residues [63]; the formation of a regular polymer backbone appears to be impossible.

2.3.2.3 Molecular Weight Distribution

Most information on the quantitative MW distribution (MWD) of native storage (gluten) proteins is available for wheat, because MWD of gluten proteins has been recognized as one of the main determinants of the rheological properties of wheat dough. Native gluten proteins consist of monomeric α/β - and γ -gliadins with MW around 30,000 and monomeric $\omega 5$ - and $\omega 1,2$ -gliadins with MW between 40,000 and 55,000. They are alcohol-soluble and amount to ~50% of gluten proteins (Fig. 2.3). Besides monomers the alcohol-soluble fraction contains oligomers with MW roughly ranging between 60,000 and 600,000. They are formed by modified gliadins with an odd number of cysteine residues and LMW-GS via interchain disulfide bonds and account for ~15% of gluten proteins. Composition and quantity of the oligomeric fraction are strongly determined by the conditions of alcohol extraction, for example by temperature and duration. The remaining proteins (~35%) are alcohol-insoluble and mainly composed of LMW-GS and HMW-GS linked by disulfide bonds. Their MW ranges approximately from 600,000 to more than 10 million. The largest polymers termed “glutenin macropolymers” (GMP) are insoluble in SDS solutions and have MW well into the multimillions indicating that they may belong to the largest proteins in nature [73, 74]. Their amounts in flour (20–40 mg/g) are strongly correlated with dough strength and bread volume. GMP is characterized by higher ratios of HMW-GS to LMW-GS and x-type to y-type HMW-GS in

Fig. 2.3 Molecular weight distribution of native wheat storage (gluten) proteins (Modified from [73])



comparison with total glutenins; the HMW-GS combination 1Dx5+1Dy10 appears to produce higher GMP concentrations than 1Dx2+1Dy12 [73].

Rye storage proteins have a strongly different MWD as compared to wheat. Although rye shows higher proportions of aggregative to monomeric storage proteins than wheat (Table 2.7), the proportions of polymers is much lower (~23%) and the amount of GMP (~5 mg/g flour) strongly reduced [75, 76]. The deficiency of polymeric proteins is balanced by the higher proportion of oligomers (~30%), whereas the proportion of rye monomers (~47%) is similar to that of wheat. Obviously rye storage proteins consist of many more chain terminations (e.g., γ -75 k-secalins, γ -type HMW-secalins) and less chain extenders than wheat, which apparently prevents gluten formation during dough mixing. Information about the MWD of native barley and oat proteins is not yet available.

2.3.2.4 Influence of External Parameters

Many studies have substantiated that both structures and quantities of storage proteins are exposed to a continuous change from the growing period of plants to the processing of end products. Because of the importance of wheat as a unique “bread cereal,” most investigations have been focused on gluten proteins. In principle, however, the effect of external parameters is similar for all cereal proteins.

Fertilization

The supply with minerals during growing is essential for optimal plant development. Nitrogen (N) fertilization is, in particular, important for common wheat, because a high N supply provides a high flour protein content and thus, increased bread volume. Fertilization with different N amounts demonstrated that the quantities

of albumins/globulins are scarcely influenced, whereas those of gluten proteins increase with higher N supply [77]. The effects on gliadins are more pronounced than on glutenins resulting in an elevated gliadin/glutenin ratio. Particularly, the proportions of ω -type gliadins are strongly enhanced by high N supply.

In the past, sulfur (S)-containing fertilizers were not widely used for cereal crops, because air pollution from industry and traffic provided sufficient amounts of S in the soil. The massive decrease in the input of S from atmospheric deposition over the last decades reduced S availability in soils dramatically, and has led to a severe S deficiency in cereals, which exerts a large influence on protein composition and technological properties. In the case of wheat, S deficiency provokes a drastic increase of S-free ω -type gliadins and a decrease of S-rich γ -gliadins and LMW-GS [78]. Moreover, S deficiency has been reported to impair dough and bread properties [79].

Infections

The infection of cereal plants with *Fusarium* strains induces a premature fading of individual spikelets and then, a fading of the whole ears. An outbreak of this infection is often accompanied by mycotoxin contamination of grains and flours, for example by the trichothecene deoxinivalenol. Studies on wheat demonstrated that infection with *Fusarium* caused a distinct reduction in the content of both total glutenins and HMW-GS and impaired dough and bread quality [80]. Glutenins of common wheat have been shown to be more strongly affected than those of emmer and storage proteins of naked barley [81, 82].

Germination

Proteins as well as other constituents are stable in dry grains. Water supply, however, induces germination of grains accompanied by the activation of enzymes, in particular, amylases and peptidases. The latter have been shown, for instance, to cause a fast degradation of prolamins of wheat, rye, barley, and oats during germination [83]. Studies on the Osborne fractions of wheat demonstrated that both monomeric gliadins and polymeric glutenins were strongly degraded during germination for 168 h at 15–30 °C, whereas albumins/globulins were scarcely affected [84]. The degradation of gluten proteins has a drastic negative effect on the bread-making quality of wheat.

Oxidation

Grains contain a considerable amount of LMW thiols such as glutathione, which are known to affect the structures and functional properties of polymeric storage proteins by thiol/disulfide interchange reactions during dough preparation [64]. To prevent this deleterious effect on the bread-making quality of wheat, week-long storage of

flour under air (direct oxidation of LMW thiols) and treatment with L-ascorbic acid (indirect oxidation catalyzed by glutathione dehydrogenase) are recommended.

Enzymes

Breads prepared from rye and wheat sourdoughs are of increasing consumer interest due to the improvement of sensorial and nutritional quality, the prolongation of shelf life, and the delay in staling. Wheat storage proteins, however, which are responsible for the viscoelastic and gas-holding properties of dough and for the texture of the bread crumb, are profoundly degraded [85]. Protein degradation during fermentation is primarily due to acidic peptidases present in flour and activated by the lowered pH caused by *Lactobacillus* strains. The strongest decrease was found for the glutenin macropolymer and total glutenins. The extent of the decrease of monomeric gliadins was lower and more pronounced for the γ -type than for the α/β - and ω -types.

Heat

The baking process involves a drastic heat-treatment of proteins, with temperatures of more than 200 °C on the outer layer (crust) and near 100 °C in the interior (crumb) of bread. HPLC analysis of crust proteins from wheat bread indicated serious structural damage of both gliadins and glutenins [86]. With respect to crumb, the extractability of total gliadins with 60% ethanol is strongly reduced compared with those from flours. The single gliadin types are affected differently, ω -type gliadins less and α/β - and γ -types much more. Most gliadins can be recovered in the glutenin fraction after reduction of disulfide bonds suggesting that major heat-induced cross-links of gliadins to glutenins are disulfide bonds.

High Pressure

The effect of hydrostatic pressure is similar to that of heat. Treatment of gluten with pressure in the range of 300–600 MPa at 60 °C for 10 min provokes a strong reduction of gliadin extractability [87]. Within gliadin types, cysteine containing α/β - and γ -type gliadins, but not cysteine-free ω -type gliadins, are sensitive to pressure and are transferred to the ethanol-insoluble glutenin fraction. Cleavage and rearrangement of disulfide bonds have been proposed as being responsible for pressure-induced aggregation.

2.3.2.5 Wheat Gluten

Wheat is unique among cereals in its ability to form a cohesive, viscoelastic dough, when flour is mixed with water. Wheat dough retains the gas produced during

fermentation and this results in a leavened loaf of bread after baking. It is commonly accepted that gluten proteins (gliadins and glutenins) decisively account for the physical properties of wheat dough. Both protein fractions are important contributors to these properties, but their functions are divergent. Hydrated monomeric and oligomeric proteins of the gliadin fraction have little elasticity and are less cohesive than glutenins; they contribute mainly to the viscosity and extensibility of dough. In contrast, hydrated polymeric glutenins are both cohesive and elastic, and are responsible for dough strength and elasticity. Thus, gluten is a “two-component glue,” in which gliadins can be understood as a “plasticizer” or “solvent” for glutenins [65]. A proper mixture (~2:1) of the two is essential to give desirable dough and bread properties.

Native gluten proteins are amongst the most complex protein networks in nature due to the presence of several hundred different protein components. Even small differences in the qualitative and quantitative protein composition decide on the end-use quality of wheat varieties. Numerous studies demonstrated that the total amounts of gluten proteins (highly correlated with the protein content of flour), the ratio of gliadins to glutenins, the ratio of HMW-GS to LMW-GS, the amount of GMP, and the presence of specific HMG-GS determine dough and bread quality.

Amongst chemical bonds disulfide linkages (Fig. 2.1) play a key role in determining the structure and properties of gluten proteins. Intrachain bonds stabilize the steric structure of both monomeric and aggregative proteins; interchain bonds provoke the formation of large glutenin polymers. The disulfide structure is not in a stable state, but undergoes a continuous change from the maturing grain to the end product (e.g., bread), and is chiefly influenced by redox reactions. These include (1) the oxidation of free SH groups to S-S linkages, which supports the formation of large aggregates, (2) the presence of chain terminators (e.g., glutathione and gliadins with an odd number of cysteine), which stop polymerization, and (3) SH-SS interchange reactions, which affect the degree of polymerization of glutenins. Consequently, oxygen is known to be essential for optimal dough development and oxidizing agents, for example potassium bromide, azodicarbonimide, and dehydroascorbic acid (the oxidation product of ascorbic acid) have been found to be useful as bread improvers [88].

Conversely, reducing agents such as cysteine and sodium metabisulfite are used to soften strong doughs, accompanied by decreased dough development and resistance and increased extensibility. They are specifically in use as dough softeners for biscuits. The overall effect is to reduce the average MW of glutenin aggregates by SH/SS interchange.

Beside disulfide bonds, dityrosine and isopeptide bonds have been described as further covalent cross-links between gluten proteins. Compared with the concentration of disulfide bonds (~10 μmol per g flour) tyrosine-tyrosine cross-links (~0.7 nmol per g flour) appear to be only of marginal importance [89]. Interchain cross-links between lysine and glutamine residues (isopeptide bonds) are catalyzed by the enzyme transglutaminase (TG). Addition of TG to flour results in a decrease in the quantity of extractable gliadins and an increase of the glutenin fraction and the nonextractable fraction [90]. Thereby, dough properties and bread-making quality can be positively influenced, similar to the actions of chemical oxidants.

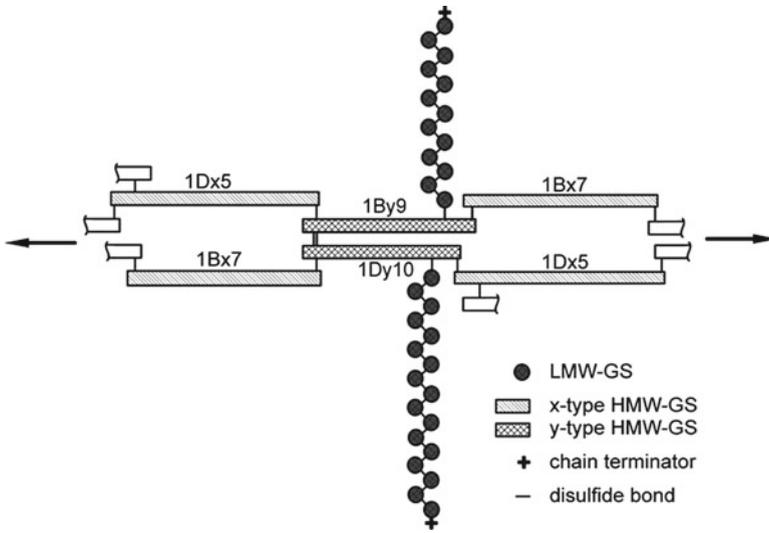


Fig. 2.4 A model double unit for the interchain disulfide structure of LMW-GS and HMW-GS of glutenin polymers (Adapted from [65])

The covalent structure of gluten proteins is complemented by noncovalent bonds (hydrogen bonds, ionic bonds, hydrophobic bonds). Glutamine, predestinated for hydrogen bonds, is the most abundant amino acid in gluten proteins (Table 2.4) and chiefly responsible for the water-binding capacity of gluten. In fact, dry gluten absorbs about twice its own weight of water. Moreover, glutamine residues are involved in frequent protein-protein hydrogen bonds. Though the number of ionizable side chains is relatively low, ionic bonds are of importance for the interactions between gluten proteins. For example, salts such as NaCl are known to strengthen dough, obviously via ionic bonds with glutenins [91]. Hydrophobic bonds can also contribute to the properties of gluten. Because the energy of hydrophobic bonds increase with increased temperature, this type of noncovalent bonds is particularly important for protein interactions during the oven phase.

Both covalent and noncovalent bonds determine the native steric structures (conformation) of gliadins and glutenins. Studies on the secondary structure have indicated that the repetitive sequences of gliadins and LMW-GS are characterized by β -turn conformation, whereas the nonrepetitive sections contain considerable proportions of α -helix and β -sheet structures [92]. The nonrepetitive sections of α/β -, γ -gliadins, and LMW-GS include intrachain disulfide bonds, which are concentrated in a relatively small area and form compact structures including two or three small rings and a big ring (Fig. 2.2) [93]. The nonrepetitive sections A and C of HMW-GS are dominated by α -helix and β -sheet structures, whereas the repetitive section B is characterized by regularly repeated β -turns [94]. They form a loose β -spiral similar to that of mammalian connective tissue elastin; β -spirals have been proposed to transfer elasticity to gluten.

A range of models has been developed to explain the structure and functionality of glutenins. Most recently, the experimental findings on disulfide bonds were transformed into a two-dimensional model [65] (Fig. 2.4). HMW-GS and LMW-GS polymerize separately, both forming linear backbone polymers. Both polymers are cross-linked by a disulfide bond between section IV of LMW-GS and section B of γ -type HMW-GS. The backbone of HMW-GS is established by end-to-end, probably head-to-tail linkages. LMW-GS polymers are linked between two sections I and between sections I and IV. The polymerization of HMW-GS and LMW-GS is terminated by chain terminators, either by modified gliadins or LMW thiol compounds.

2.3.3 Storage Proteins of Corn, Millet, Sorghum, and Rice

Overall, the storage proteins of corn, sorghum, millet, and rice are, in part, related and differ significantly from those of wheat, rye, barley, and oats. According to the amino acid composition they contain less glutamine and proline and more hydrophobic amino acids such as leucine [56]. Corn storage proteins, called zeins, can be subgrouped into alcohol-soluble monomeric zeins and cross-linked zeins alcohol-soluble only on heating or after reduction of disulfide bonds. With respect to different structures zeins have been divided into four different subclasses [95]. α -Zeins are the major subclass (71–85% of total zeins), followed by γ - (10–20%), β - (1–5%) and δ -zeins (1–5%), respectively [96]. α -Zeins are monomeric proteins with apparent MW of 19,000 and 22,000 determined by SDS-PAGE. Their amino acid sequences contain up to ten tandem repeats [97]. Proteins of the other subclasses are cross-linked by disulfide bonds and their subunits have apparent MW of 18,000 and 27,000 (γ -zein), 18,000 (β -zein), and 10,000 (δ -zein).

In many ways the storage proteins of sorghum and millet called kafirins are similar to zeins. Sorghum kafirins have also been subdivided into α -, β -, γ - and δ -subclasses based on solubility, MW, and structure [98]. α -Kafirins are monomeric proteins and represent the major subclass accounting for around 65–85% of total kafirins. Proteins of the other subclasses are highly cross-linked and alcohol-soluble only after reduction of disulfide bonds. On average, each of them accounts for less than 10% of total kafirins [99, 100]. Within the numerous millet species and varieties the proteins of foxtail millet were studied in detail [101]. SDS-PAGE of unreduced kafirins revealed bands with apparent MW ranging from 11,000 to 150,000. After the reduction of disulfide bonds two major bands with MW of 11,000 (subunit A) and 16,000 (subunit B) were obtained. Unreduced proteins with higher MW were formed by cross-links of A and/or B subunits. The storage proteins of rice are characterized by the highly unbalanced ratio of prolamins to glutelins (~1:30) [102]. Both fractions show the lowest proline content (~5 mol-%) amongst cereal storage proteins [56]. SDS-PAGE patterns of rice prolamins (oryzins) showed a major band with MW 17,000 and a minor band with MW 23,000 [103]. The apparent MW of glutelin subunits was in a range from 20,000 to 38,000.

2.3.4 *Metabolic Proteins*

Most proteins of the albumin and globulin fractions are metabolic proteins, mainly enzymes and enzyme inhibitors. The corresponding extensive studies have been summarized by Kruger and Reed [104] and recently by Delcour and Hoseney [105]. Many of these proteins are located in the embryo and aleurone layer; others are distributed throughout the endosperm. They have nutritionally better amino acid compositions than storage proteins, particularly because of their higher lysine contents. Those enzymes that hydrolyze carbohydrates and proteins and, thereby, provide the embryo with nutrients and energy during germination, are of most significant importance.

2.3.4.1 **Hydrolyzing Enzymes**

Carbohydrate-Degrading Enzymes

The many carbohydrate-degrading enzymes include α -amylases, β -amylases, debranching enzymes, cellulases, β -glucanases, and glucosidases. Amylases are enzymes that hydrolyze the polysaccharides in starch granules. They can be classified as endohydrolases, which attack glucosidic bonds within the polysaccharide molecules and exohydrolases, which attack glucosidic bonds at or near the end of chains. The most important enzyme of the endohydrolase type is α -amylase. The enzyme hydrolyzes α -1,4-glucosidic bonds of amylose and amylopectin and produces a mixture of dextrans together with smaller amounts of maltose and oligosaccharides; the pH-optimum is about 5. The other major amylase type is β -amylase, an exohydrolase, which hydrolyzes α -1,4-glucosidic bonds near the nonreducing ends of amylose and amylopectin to produce maltose. Its pH-optimum is similar to that of α -amylase. Both amylase types exist in multiple forms or isoenzymes with different chemical and physical properties. Neither α - nor β -amylase can break α -1,6-glucosidic bonds present in amylopectin. For this kind of hydrolysis debranching enzymes are present in cereal grains. Along with α -glucosidases they assist α - and β -amylases in a more complete conversion of starch to simple sugars and small dextrans. A number of other carbohydrate degrading enzymes exist, their amounts, however, are very low compared to amylases. Examples are α - and β -glucosidases, cellulases, and arabinoxylanases.

Proteolytic Enzymes

Enzymes that hydrolyze proteins are called proteinases, proteases, or peptidases. They attack the peptide bond between amino acid residues and include both endo- and exopeptidases. The latter are divided into carboxypeptidases, when acting from the carboxy terminal and aminopeptidases, when acting from the amino terminal.

The most important proteolytic enzymes are acidic peptidases. They exist in multiple forms having pH-optima between 4.2 and 5.5 and include both endo- and exotypes. On the basis of their catalytic mechanism they can be classified as serine, metallo-, aspartic, and serine peptidases. According to their biological function to provide the embryo with amino acids, their activity is highest during the germination of grains.

Other Hydrolyzing Enzymes

Lipases are the most important enzymes that hydrolyze ester bonds. They attack triacylglycerols yielding mono- and diacylglycerols and free fatty acids. Lipase activity is important, because free fatty acids are more susceptible to oxidative rancidity than fatty acids bound in triacylglycerols. The activity varies widely among cereals with oats and millet having the highest activity. Exogenous lipases are in use to improve the baking performance of wheat flour.

Phytase is an esterase that hydrolyzes phytic acid to inositol and free phosphoric acid. Even partial hydrolysis of phytic acid by phytase is desirable from a nutritional point of view, because the strong complexation of cations such as zinc, calcium, and magnesium ions by phytic acid is significantly reduced.

2.3.4.2 Oxidizing Enzymes

Lipoxygenase is present in high levels in the germ. It catalyzes the peroxidation of certain polyunsaturated fatty acids by molecular oxygen. Its typical substrate is linoleic acid containing a methylene-interrupted, doubly unsaturated carbon chain with double bonds in the *cis*-configuration.

Polyphenoloxidases preferably occur in the outer layers of the grains. They catalyze the oxidation of phenols, such as catechol, pyrogallol, and gallic acid, to quinons by molecular oxygen. Peroxidase and catalase may be classified as hydroperoxidases catalyzing the oxidation of a number of aromatic amines and phenols, for example ferulic acid in arabinoxylans, by hydrogen peroxide. Other oxidizing enzymes are ascorbic acid oxidase and glutathione dehydrogenase.

2.3.4.3 Enzyme Inhibitors

Many investigators have isolated and characterized enzyme inhibitors from germ and endosperm. Most important inhibitors are targeted on hydrolyzing enzymes to prevent the extensive degradation of starch and storage proteins during grain development and to defend plant tissues from animal (insect) or microbial enzymes. Predominant classes are amylase and protease inhibitors concentrated in the albumin/globulin fractions. Amylase inhibitors can be directed towards both cereal and noncereal amylases and protease inhibitors towards proteases from both cereals and

animals. Some inhibitors appear to be bifunctional inhibiting amylases as well as proteases.

2.4 Lipids

2.4.1 *Lipid Composition*

Cereal lipids originate from membranes, organelles, and spherosomes and consist of different chemical structures. Depending on cereal species average lipid contents of 1.7–7% in the grains are present (Table 2.2). Lipids are mainly stored in the germ, to a smaller extent in the aleurone layer and to the lesser extent in the endosperm. In particular oats are rich in lipids (6–8%) in contrast to wheat and rye (1.7%). Cereal lipids have similar fatty acid compositions, in which linoleic acid reaches contents of 39–69%, while oleic acid and palmitic acid make up 11–36% and 18–28%, respectively [106, 107]. Although wheat lipids are only a minor constituent of the flour, they greatly impact the baking performance and have, therefore, been extensively studied.

While triglycerides are the dominating lipid class in the germ and the aleurone layer, phospho- and glycolipids are present in the endosperm (Fig. 2.5). Depending on the extraction rate wheat flour contains 0.5–3% lipids [108]. Extraction with a polar solvent at ambient temperature, i.e., water-saturated butanol, dissolves the nonstarch lipids that make up approximately 75% of the total flour lipids [109]. The residual 25% are the so-called starch lipids. The composition of the nonstarch lipids is given in Table 2.8. They contain about 60% nonpolar lipids, 24% glycolipids, and 15% phospholipids. By extraction with solvents of different polarities they can be further subdivided into a free and a bound fraction. The nonpolar lipids are mainly present in the free lipid fraction, whereas glyco- and phospholipids are part of the bound fraction, in which they can be associated, for example with proteins [106, 107]. The major glycolipid class is the digalactosyldiglycerides. Starch lipids are primarily composed of lysophospholipids, which form inclusion complexes with amylose helices already in native starch [28].

2.4.2 *Effects of Lipids on the Baking Performance of Wheat Flour*

Only nonstarch lipids affect the rheological properties of wheat doughs. Interactions between starch lipids and starch are sufficiently strong so that this lipid fraction is not available before the starch gelatinizes. Studies with nonstarch lipids have shown that only the polar lipids have a positive effect on baking performance, whereas the

Fig. 2.5 Polar lipids that affect the baking performance of wheat

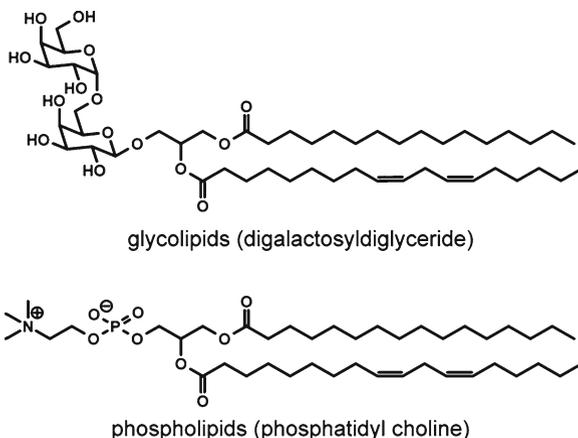


Table 2.8 Composition of nonstarch lipids of wheat flour [107]. Content (g/100 g) based on total lipid

Nonstarch lipids: 1.70–1.95 g/100 g flour			
Polar	36–42	Nonpolar	58–64
<i>Phospholipids</i>	14–16	Sterol esters	1.9–4.2
Acylphosphatidyl ethanolamine	4.2–4.9	Triglycerides	39.5–49.4
Acyllysophosphatidyl ethanolamine	1.6–2.3	Diglycerides	3.3–5.4
Phosphatidyl ethanolamine/phosphatidyl glycerol	0.7–1.1	Esterified monogalactosyldiglycerides/monoglycerides	2.7–3.9
Phosphatidyl choline	3.8–4.9	Esterified sterolglycerides	0.8–4.2
Phosphatidyl serine/phosphatidyl inositol	0.4–0.7		
Lysophosphatidyl ethanolamin	0.3–0.5		
Lysophosphatidyl glycerol	0.2–0.3		
Lysophosphatidyl choline	1.4–2.1		
<i>Glycolipids</i>	22–26		
Monogalactosyldiglycerides	5.0–5.9		
Monogalactosylmonoglycerides	0.9–0.4		
Digalactosyldiglycerides	12.6–16.5		
Digalactosylmonoglycerides	0.6–3.4		

nonpolar lipids have the opposite effect [110]. In particular glycolipids have been shown to contribute to the high baking performance of wheat flour [29, 111–113], whereas the functionality of the phospholipids has been found to be less important. If the term “specific baking activity” would be defined, polar lipids would be found to affect the baking performance of wheat flour to a considerably greater extent than proteins. The addition of only 0.13% polar lipids would yield the same increase of loaf volume as a protein content that would be increased by 1%. Polar lipids affect dough properties in many ways, i.e., the dough handling properties are improved

and the gas-holding capacity during proofing is increased enabling a prolonged oven spring, increased loaf volume, better crumb resilience, and, in some cases, retardation of bread staling.

2.4.3 Modes of Action of Polar Lipids in Baking

The high baking activities of polar lipids, in particular of the glycolipids, might be explained by modes of action based on the formation of liquid films at the dough liquor/gas cell interface. Possible modes of action are the direct influence of the surfactants on the liquid film lamellae and gas cell interfaces through direct adsorption resulting in an increase of surface activity as suggested by Gan et al. [49, 114] and Sroan et al. [115] as the secondary stabilizing mechanism in the so-called dual film theory. It suggests the presence of liquid lamellae, providing an independent mechanism of gas cell stabilization. As shown recently, the effects of different surface active components may be explained by the type of monolayer that they form [116].

However, in particular the positive effect of some polar lipids such as acylated sterol glucosides and sterol glucosides cannot be explained with this mode of directly stabilizing the liquid film lamellae. Here another mode of action could be the answer, for example the indirect stabilization of the dough liquor/gas cell interface through this type of surfactant [116, 117]. These polar lipid classes have a positive influence on the phase behavior of the endogenous lipids present in the dough liquor in that they lead to an increase in surface activity of the endogenous lipids and hence a better availability and accumulation at the liquid film lamellae/gas cell interface, thus increasing gas cell stabilization, and consequently the bread volume.

Inclusion complexes between amylose helices and polar lipids with one fatty acid residue are responsible for two effects. Complexes present in native starch (starch lipids) increase the temperature of gelatinization and, thus, prolong the oven spring. Inclusion complexes between amylose helices and polar lipids with one fatty acid residue may also form during and after the gelatinization process and are responsible for the anti-staling effect of some polar lipids, for example monoglycerides.

2.5 Minor Constituents

2.5.1 Minerals

The mineral content of cereals ranges from ca. 1.0 to 2.5% (Table 2.2). Compared to other foods this is an intermediate concentration with milk, meat, and vegetables having somewhat lower mineral contents and pulses, which are extraordinarily rich in minerals (mean mineral content ~3.5%). As cereals are among the most important staple foods, and are consumed in high quantities, they are important sources of

minerals in the human diet. The major portion of the minerals (>90%) is located in the outer layers of the grains, namely in the bran, the aleurone layer, and the germ. Consequently, products made from whole grains should increasingly be introduced into human nutrition to benefit from the mineral content of cereals.

2.5.2 *Vitamins*

Cereals contain vitamins in concentrations ranging from below 1 to ca. 50 mg/kg, depending on the compound (Table 2.2). Thus, cereals are a good source of vitamins from the B-group, and, in industrial countries, they cover about 50–60% of the daily requirement of B-vitamins. The most important fat-soluble vitamins are the tocopherols, which are present in concentrations exceeding 20 mg/kg. Like the minerals, vitamins are concentrated in the outer layers of the grains, in particular in the aleurone layer as well as in the germ. Therefore, milling of cereals into white flour will remove most of the vitamins. Consequently, the use of whole-grain products or products enriched in vitamin-containing tissues will be of nutritional benefit for the consumer.

Abbreviations

AX	Arabinoxylans
DSC	Differential scanning calorimetry
GMP	Glutenin macropolymer
GS	Glutenin subunits
HMW	High-molecular-weight
HPLC	High-performance liquid chromatography
LMW	Low-molecular-weight
m/a	Monomeric/aggregated
MMW	Medium-molecular-weight
MW	Molecular weight
MWD	Molecular weight distribution
NSP	Nonstarch polysaccharides
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecyl sulfate
TG	Transglutaminase
WEAX	Water-extractable arabinoxylans
WUAX	Water-unextractable arabinoxylans

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