Chapter 1

Cereals and Legumes

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Chapter Outline

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PART I: CEREALS

I. INTRODUCTION

Cereals are members of the large monocotyledonous grass family, the Gramineae, which mainly consists of wheat, maize, barley, oats, rice, and sorghum (Anderson *et al.*, 2000). Cereal-based foods have been a staple dietary source for the world's population for centuries. Cereal grains contain the macronutrients (protein, fat, and carbohydrate) required by humans for growth and maintenance, contributing approximately 70% and 50% of the total calories and protein, respectively (Topping, 2007). Cereal grains also supply important minerals, vitamins, and other micronutrients essential for optimal health. They still provide 20% of magnesium and zinc, 30–40% of carbohydrate and iron, 20–30% of riboflavin and niacin, and over 40% of thiamine in the diet (Marston and Welsh, 1980). Global cereal production per capita fluctuated around 280 kg per year during the first half of the twentieth century, as seen in Figure 1.1 (Gilland, 2002). The world production of cereal is projected to be 3555 Mt, with per capita production of 378 kg (Gilland, 1998). Cereal-based food, especially whole grains, have shown the potential for health promotion, linking to reduced risk of several chronic diseases such as coronary heart disease, type 2 diabetes, and certain types of cancer (Truswell, 2002; Montonen *et al.*, 2003; Slavin, 2000; Slavin *et al.*, 1999). These beneficial effects are attributed to numerous phytochemicals contained in the grains. Therefore, this chapter will discuss those biochemical changes taking place during development, germination, and storage of cereal grains, with particular attention to wheat.

II. CEREAL GRAIN STRUCTURE

The different tissues constituting the cereal seed are generally described in terms of their embryogenic origin and structure (Evers *et al.*, 1999). The cereal seed is composed of three main tissues: the embryo, the endosperm, and the aleurone layer surrounding the storage endosperm. This is illustrated for wheat in Figure 1.2, in which the endosperm comprises over 80% of the grain weight while the aleurone cells and the germ tissue containing the embryo embedded in the surrounding scutellum account for 15% and 3%, respectively. The peripheral tissues of the grain overlying the starchy endosperm are made up successively from the outer to the inner surface: the outer pericarp, the inner pericarp, seed coat, hyaline layer, and the aleurone layer (Barrona *et al.*, 2007). The germ comprises the embryonic axis and the scutellum. The anatomical structure of all cereal grains is essentially similar with some minor

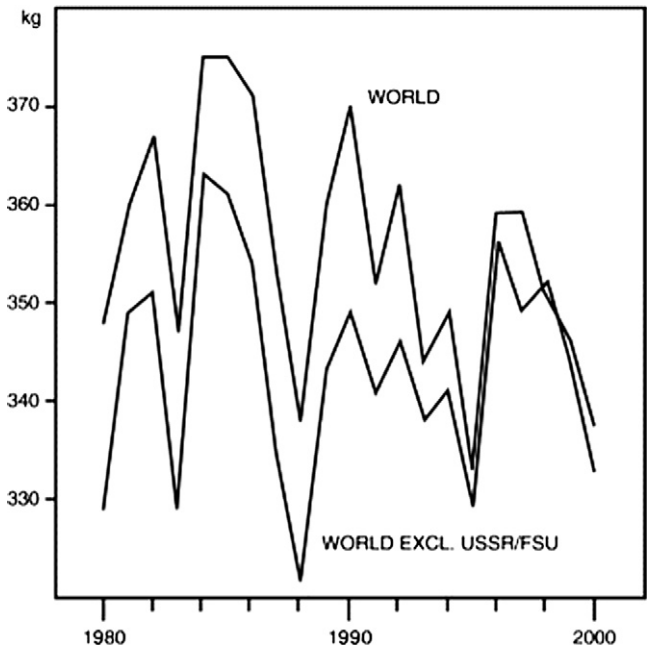


FIGURE 1.1 Cereal production per capita, 1980–2000. *(Sources: FAOSTAT; US Bureau of the Census; adapted from Gilland, 2002.)*

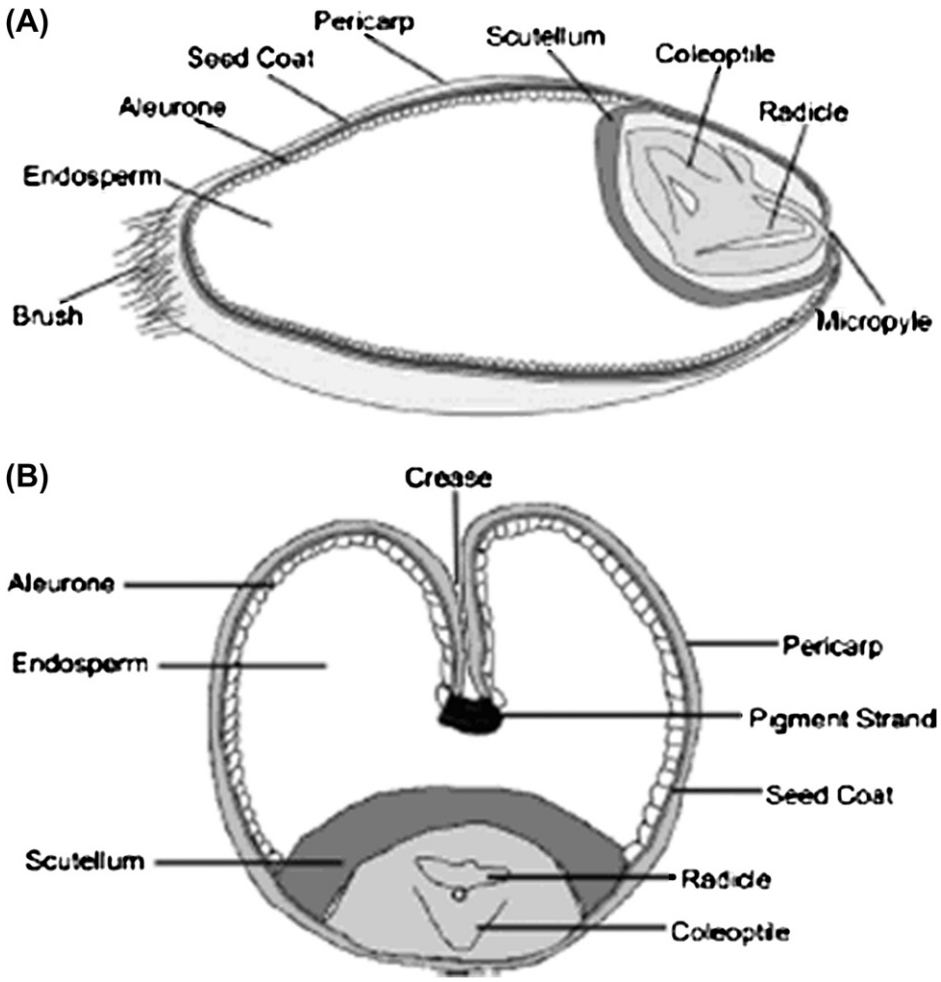


FIGURE 1.2 Diagram of wheat grain showing major structures in (A) longitudinal and (B) transverse sections *(Rathjen et al., 2009 with permission).*

differences. For instance, wheat and maize are surrounded by a fruit coat or pericarp and seed coat or testa, together referred to as a naked caryopsis. In the case of barley, oats, and rice, however, an additional husk is found surrounding the caryopsis or kernel of the grain.

III. CEREAL GRAIN COMPOSITION

Cereal grains are highly nutritious, and the major components of the grain are proteins (approximately 10−15%) and starch (approximately 60−70% of grain), with non-starch polysaccharides derived from the cell walls accounting for about 3−8% of the total (Saulniera *et al.*, 2007). The composition of cereals varies and highly depends on grain variety, growing conditions, husbandry, and infection (Tester, 1995). The starchy endosperm constitutes the major portion of the cereal seed and provides the nutrients necessary for embryo development during germination. The nutrients are made available by the release of enzymes from the aleurone layer and embryo, which hydrolyze the endosperm reserves. These reserves are contained in discrete storage bodies identified as starch granules and protein bodies. These components have major effects on the use of cereal grain due to their physiochemical properties during milling and food processing. It is worth noting that the non-digestible carbohydrates in cereal grains have received increased attention as a significant source of dietary fiber, and are purported to have an impact on the nutritional quality and health-enhancing effects of cereal foods (Salmeron *et al.*, 1997). In addition, polysaccharides in cereals are associated with many other substances, mostly with proteins, polyphenols, and phytate, which can modify mineral binding by dietary fiber (Vitali *et al.*, 2008).

A. Amyloplasts

Amyloplasts are plastids or organelles responsible for the storage of starch granules. The rate of starch synthesis in cereal grains is one of the factors affecting both grain size and yield (Kumar and Singh, 1980). In the mature endosperm of wheat, barley, and rye, starch is found as two distinct fractions based on the size of the granules. The primary or A-type starch granules range in size from 20 to 45 μm, while the secondary or B-type granules rarely exceed 10 μm in diameter (Evers, 1973). Examination of the particle size distribution in wheat endosperm starch by Evers and Lindley (1977) showed that those starch granules less than 10 μm in diameter accounted for approximately one-third of the total weight of starch. The presence of these two starch granule types in wheat kernels was confirmed in studies by Baruch *et al.* (1979). They found that the size of the starch was affected by seasonal changes in much the same way as grain yield and protein content. The starch granule occupies only a very small part of the total plastid during initial kernel development but accounts for close to 93% at maturity (Briarty *et al.*, 1979). In the mature endosperm, A-type starch granules account for only 3% of the total number of granules although they represent 50−70% of the total weight, owing to their larger size (Evers and Lindley, 1977). The smaller B-type granules, however, make up 97% of the total number of starch granules but account for only 25−50% of the overall weight.

Isolated starch granules also contain protein, most of which can be removed by washing repeatedly with water. A small part of the protein, however, remains strongly associated with the granule itself. Lowy *et al.* (1981) found that this protein fraction is readily extracted with salt solution and suggested that it is associated with the starch granule surface. This extractable fraction accounts for 8% of the total protein in the starch granule. The major protein fraction has a molecular weight of around 30,000 and is associated with both A- and B-type starch granules. Based on amino acid analysis, this protein is quite different from wheat gluten. An additional protein fraction was extracted from A-type starch granules but only following gelatinization in the presence of sodium dodecyl sulfate. This fraction was quite different, and based on electrophoresis, was thought to be part of the internal granule components.

B. The Starch Granule

Starch granule shape can be characteristic of a genus and species (Ellis, 1998). The shape and size of the starch granule vary with the different cereals (Table 1.1). The size distribution of the starch granules in the amyloplasts and the composition of starch granules and their properties change during granule development. The large A-type starch granules of wheat, barley, and rye are lenticular, while the smaller B-type starch granules are spherical or polyhedral. The starch granules of rice, oats, and maize are irregular and polyhedral in shape, those of rice being comparable in size to the B-type starch granules of wheat and barley, while those of maize are larger (Ellis, 1998). Starch is composed of amylose and amylopectin, with the level of amylose ranging from 20% to 30% for most cereal starches (Katz *et al.*, 1993). In the case of certain varieties of maize, barley, and rice, the starch is composed almost exclusively

TABLE 1.1 Structure and Amylose Content of Some Whole Granular Cereal Starches

Source	Granule Shape	Granule Size (nm)	Amylose Content (%)
Wheat	Lenticular or round	20−25	22
Maize	Round or polyhedral	15	28
Waxy maize	Round	15 (5−15)	1
High-amylose	Round or irregular sausage-shaped	25	52
Barley	Round or elliptical	20−25	22
Rice	Polygonal	3−8	17−19[a] 21−23[b]
Oats	Polyhedral	3−10	23−24

[a] *Japonica*
[b] *Indica*
Adapted from Lineback (1984).

of amylopectin; these are referred to as 'waxy'. High-amylose starches are also found, for example, in the case of amylomaize.

A portion of amylose in the starch granule is complexed with lysophospholipids as a function of the stage of development of the endosperm, at which the amylose is formed (Morrison, 1993). B-type granules are initiated later in grain development than A-type granules. The proportions of amylose and of lysophospholipids rose in both A-type and B-type starch granules during grain development of wheat and barley (Morrison and Gadan, 1987; McDonald, 1991).

C. Biosynthesis of Starch

Sucrose is believed to be the main source of carbon for starch synthesis in the cereal endosperms, and is converted to starch via a series of enzyme-catalyzed reactions (Duffus, 1993). Starch synthesis is achieved through the action of starch synthase, which can utilize either adenosine diphosphoglucose (ADPG) or uridine diphosphoglucose (UDPG) as a substrate (Recondo and Leloir, 1961). ADPG appears to be the more active glucosyl donor and is formed by the action of ADPG-pyrophosphorylase (Preiss and Levi, 1979).

$$\text{ATP} + \alpha\text{-Glucose-1-P} \rightarrow \text{ADPG} + \text{PP}_\text{i}$$

The amount of inorganic pyrophosphate (PP_i) in developing grains is controlled by the enzyme alkaline inorganic pyrophosphatase (EC 3.6.1.1). This enzyme limits the accumulation of PP_i and was thought to be the controlling factor in starch synthesis, as PP_i inhibited ADPG-pyrophosphorylase in sweet corn (Amir and Cherry, 1972). The activity of both ADPG-pyrophosphorylase and alkaline pyrophosphatase was studied by Kumar and Singh (1983) during the development of wheat grain. Their results, shown in Figure 1.3, indicate that both enzymes increased steadily, reaching a maximum 28 days after anthesis, but then declined with maturity. The rapid increase in alkaline pyrophosphatase activity 14 days after anthesis corresponded with the period of rapid starch synthesis. The inability of the intermediate metabolites of sucrose−starch conversion to inhibit the activity of alkaline pyrophosphatase eliminated any possible regulatory role for this enzyme in starch biosynthesis.

D. Sucrose Starch Conversion in Developing Grains

The amount of free sugars formed during the development of wheat was examined by Kumar and Singh (1981) in relation to grain size and starch content. Their results, summarized in Figure 1.4, indicate that the non-reducing sucrose reached a maximum level 14 days after anthesis, then declined and leveled off after 28 days. Starch synthesis

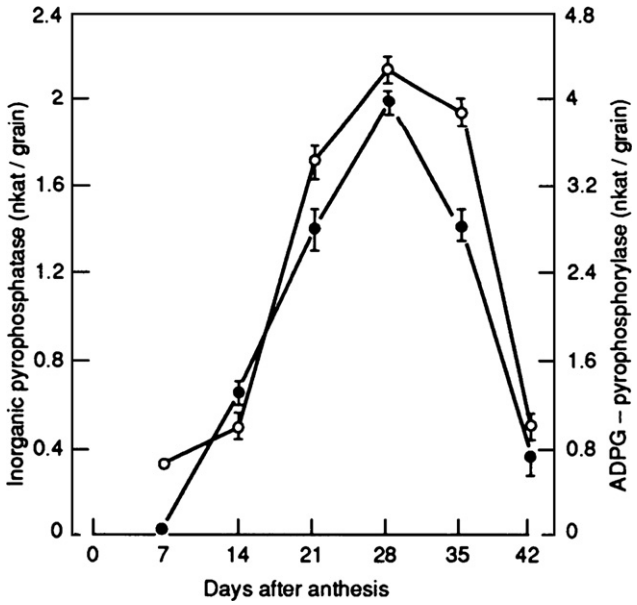


FIGURE 1.3 **Activity of alkaline inorganic pyrophosphatase (○) and ADPG-pyrophosphorylase (●) during wheat grain development** *(Kumar and Singh, 1983).*

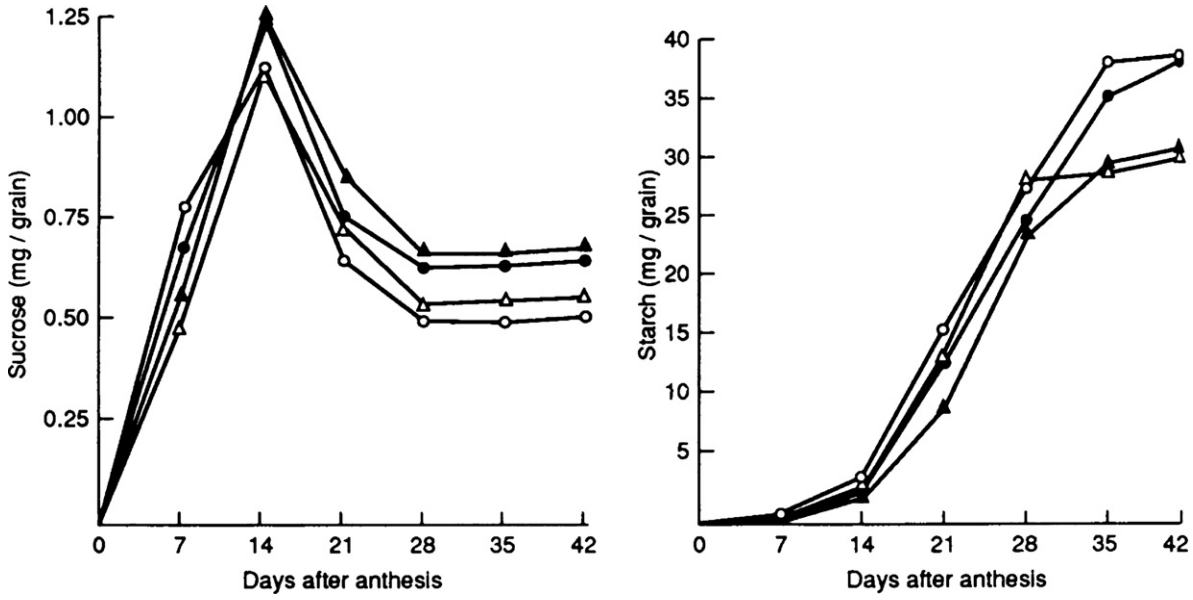


FIGURE 1.4 **Changes in sucrose and starch (mg per grain) during the development of four wheat grains** *(Kumar and Singh, 1981).*

was negligible after 7 days but increased markedly after 14 days, then continued until 35 days after anthesis. The rapid decline in sucrose and reducing sugars once starch synthesis commenced suggested the involvement of hydrolytic enzymes, including invertase. The activity of this enzyme was found by Kumar and Singh (1980) to decrease to negligible levels after 21 days compared to the rather rapid rise in sucrose−UDP glucosyl transferase activity (Figure 1.5). The latter enzyme, also referred to as sucrose synthetase, catalyzes the first step in the formation of starch from sucrose, as discussed later in this section. The parallel activities of sucrose−UDP glucosyl transferase and starch synthesis suggested that this enzyme played a major role in the hydrolysis of sucrose. Kumar and Singh (1984) suggested that the initial role of invertase was to provide substrates for energy-liberating respiratory enzymes needed for sustaining active cell division.

Chevalier and Lingle (1983) reported that insoluble invertase was located mainly in the outer pericarp, with only slight activity in the endosperm (Figure 1.6). These researchers monitored sucrose synthetase activity, which was

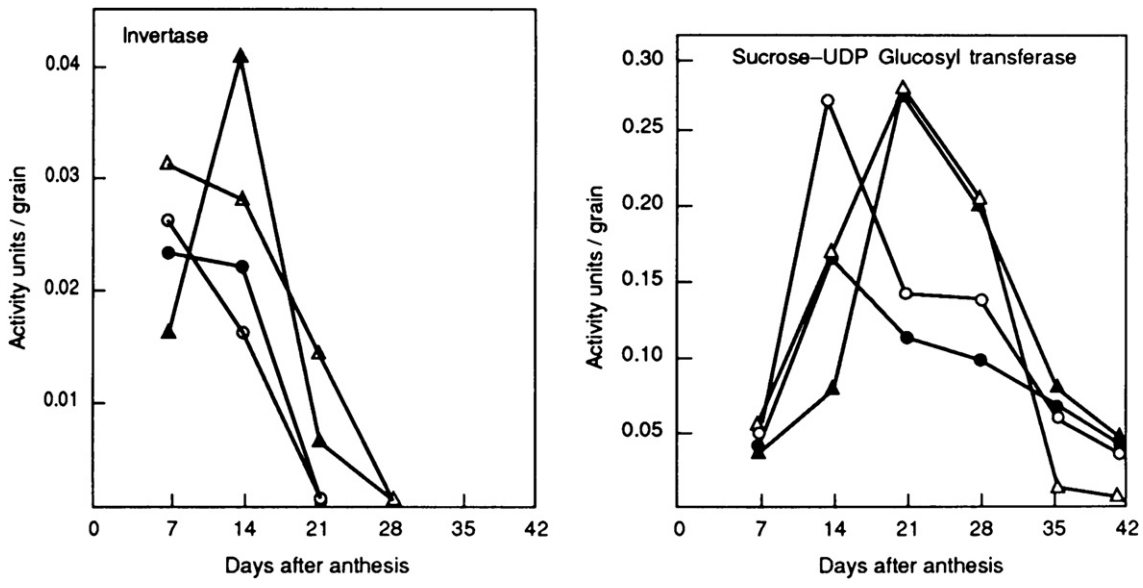


FIGURE 1.5 **Changes in invertase and sucrose-UDP glucosyl transferase activities during the development of wheat grains** *(Kumar and Singh, 1980).*

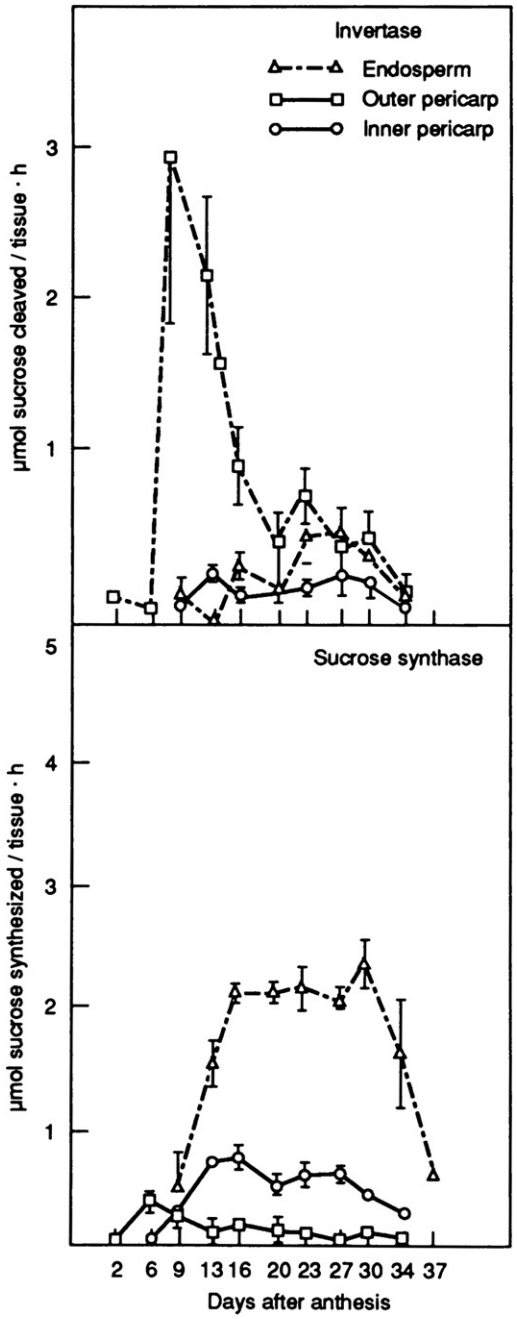


FIGURE 1.6 Distribution of invertase and sucrose synthetase activities in the endosperm and pericarp of developing wheat grains. *(Reproduced from Chevalier and Lingle, 1983.)*

found predominantly in the endosperm. Using whole wheat kernels, Kumar and Singh (1980) monitored invertase activity during the early stages of grain development and that of sucrose synthetase as the grain matured. Chevalier and Lingle (1983) found an increase in free sucrose in mature wheat and barley kernels, which was consistent with earlier research with wheat (Cerning and Guilbot, 1973), barley (Laberge *et al.*, 1973), and rice (Singh and Juliano, 1977). The marked rise in sucrose observed by Lingle and Chevalier (1980) in the endosperm fraction was accompanied by decreasing sucrose synthetase activity. This decline in synthetase activity was considered to be an important factor in controlling grain filling. It appeared to be responsible for accumulation of sucrose in extracellular spaces such as the endosperm cavity since the endosperm was now unable to utilize any incoming sucrose. The overall effect was to prevent any more sucrose from entering the kernel.

Kumar and Singh (1984) confirmed the accumulation of sucrose up to 14 days after anthesis, which represented rapid translocation from photosynthetic parts to the wheat endosperm followed by active starch synthesis. Previous work by Chevalier and Lingle (1983) demonstrated the movement of sucrose from the phloem to the endosperm in developing wheat and barley kernels. Using wheat endosperm slices, Rijven and Gifford (1983) also found sucrose to be the preferred substrate for starch synthesis as it was not hydrolyzed before its uptake by the endosperm.

E. Starch Synthesis

The *in vivo* synthesis of starch involves phosphorylase or synthetase leading to the formation of the linear polymer amylose. Once sucrose enters the endosperm it becomes the starting point for amylose synthesis. The first step involves its conversion to UDPG by sucrose synthetase (sucrose−UDP glucosyl transferase):

$$\text{Sucrose} + \text{UDP} \xrightarrow{\text{Sucrose−UDPG glucosyl transferase}} \text{UDPG} + \text{Fructose-1-P}$$

Following this, fructose-1-P is converted to glucose-1-P by phosphoglucoisomerase, hexokinase, and phosphoglucomutase. Glucose-1-P is metabolized to ADPG by ADPG-pyrophosphorylase:

$$\text{Glucose-1-P} + \text{ATP} \xrightarrow{\text{ADPG-phosphorylase}} \text{ADPG} + \text{PP}_\text{i}$$

The absence of any detectable PP_i suggested that it is rapidly hydrolyzed by pyrophosphatase since, as discussed earlier, it is a potent inhibitor of ADPG-phosphorylase (Amir and Cherry, 1971). Amylose synthesis, as discussed earlier, can be mediated directly by starch synthetase involving UDPG or indirectly by ADPG-starch synthetase to ADPG via glucose-1-P:

$$\text{ADPG} + \text{Primer (Gn)} \xrightarrow{\text{ADPG-starch synthetase}} \text{Glycosyl primer} \rightarrow (\text{Gn} + 1) + \text{ADP}$$

It appeared, however, that the ADPG reaction was the preferred one for starch biosynthesis in developing wheat grains (Kumar and Singh, 1984). In this reaction glucose is repeatedly transferred from ADPG to a small glucan primer until the elongated starch chain is formed. The extremely small amount of glucose-1-P in the developing grain suggested that it was rapidly utilized, pointing to a possible regulatory role for phosphoglucomutase, the enzyme responsible for its formation, in starch biosynthesis. Kumar and Singh (1984) proved conclusively that the termination of starch accumulation in mature wheat grains was due to the loss in synthetic capacity of the endosperm and not due to the unavailability of sucrose.

Joshi *et al.* (1980) attempted to explain the regulation of starch biosynthesis in normal and Opaque-2 maize during development of the endosperm. Opaque-2 maize was nutritionally superior although it had decreased grain yield and a lower protein and starch content. These researchers monitored the activities of sucrose−UDP glucosyl transferase, glucose-6-phosphate ketoisomerase, and soluble and bound ADPG-starch glucosyl transferase in the developing endosperm for 30 days following pollination. Except for sucrose-UDP glucosyl transferase, all the other enzymes were much lower in Opaque-2 maize, compared to the normal maize during the latter stages of endosperm development. The lower activity of these enzymes was responsible for the reduced amount of starch in Opaque-2 maize, which had 15% less starch content per endosperm. This was accompanied by a decreased protein synthesis in the Opaque-2 endosperm, which explained the reduced enzyme synthesis during the later stages of endosperm development.

F. Starch Synthesis: Amylopectin

Biosynthesis of the branched chain amylopectin requires the formation of the amylose via phosphorylase or synthase as described in the previous section. The branch points (α-(1,6)-D-glucosidic linkage) required for amylopectin are introduced by the branching enzyme Q-enzyme (EC 2.4.1.18). Borovsky *et al.* (1979) concluded that the introduction of 1,6-branch points is a random process in which the Q-enzyme interacts with two 1,4-glucan chains held together in a possible double-helix arrangement.

Amylose and amylopectin are synthesized concurrently in the ratio of 1:4 for ordinary starches (Robyt, 1984). Several hypotheses have been developed to explain the side-by-side occurrence of amylose and amylopectin in the starch granule, although our understanding of starch biosynthesis remains incomplete (Erlander, 1958; Geddes and Greenwood, 1969; Marshal and Whelan, 1970). One such hypothesis suggested that some mechanism was operating which protected the linear polymer from the branching enzyme (Whelan, 1958, 1963). The participation of

phospholipids in the regulation of amylopectin was proposed by Vieweg and De Fekete (1976), since phospholipids inhibit the action of the branching enzyme. Thus, only amylose without attached phospholipids could theoretically be converted, although this remains to be verified. Another hypothesis, discussed earlier, is the possible specificity of the branching enzyme for a double-helix arrangement involving the shorter amylopectin chains (Borovsky *et al.*, 1979; Robyt, 1984).

G. Protein Bodies

Protein bodies are membrane-bound cellular organelles containing storage proteins located in the starchy endosperm of cereals (Pernollet, 1978, 1982). They are also found in the aleurone layer, although these differ in composition, structure, and function. While the protein bodies in the endosperm have only a storage function, those in the aleurone layer possess both synthetic and secretory functions (Simmonds and O'Brien, 1981). Protein bodies in the aleurone layer are 2−4 μm in diameter with globoid and crystalline inclusions, while those in the starchy endosperm have a homogeneous granular structure devoid of inclusions. These differences have been confirmed in wheat, barley, maize, and rice by examination of their ultrastructural differences, as indicated in Table 1.2.

In members of the *Triticum* species, these protein bodies vanish as the grain matures, as observed for wheat seeds (Simmonds, 1972; Pernollet and Mossé, 1983) and rye seeds (Parker, 1981). This results in the conversion of the spherical protein granules into irregularly shaped protein masses which eventually become the matrix protein, which is no longer bound by a membrane between the starch granules.

H. Origin of Protein Bodies

The origin of protein bodies in the endosperm is still unclear. Most researchers support their synthesis on the rough endoplasmic reticulum (RER) (Campbell *et al.*, 1981; Miflin *et al.*, 1981; Miflin and Burgess, 1982; Parker and Hawes, 1982), although Bechtel *et al.* (1982a, b) favored secretion of the wheat storage proteins. Irrespective of the mechanism proposed, initiation and formation of the protein bodies involve the active participation of the Golgi apparatus. Pernollet and Camilleri (1983) examined protein body formation and development in wheat endosperm and found the polypeptides stored in all protein bodies to be similar. Earlier work by Tanaka *et al.* (1980) suggested that only one kind of protein was stored in wheat endosperm. The presence of all cell storage proteins in the protein bodies, however, pointed to a common synthetic pathway operating in wheat seeds. The polypeptides in the protein bodies were similar to those in the endoplasmic reticulum. This suggested that the storage proteins were secretory proteins discharged into the endoplasmic reticulum before being translocated to the protein bodies. This model conflicted with the soluble mode of gliadin synthesis proposed by Bechtel *et al.* (1982a, b), but was in agreement with studies carried out by Greene (1981) and Donovan *et al.* (1982). These researchers reported that messenger RNAs encoding gliadin molecules were translated on polysomes bound to the endoplasmic reticulum.

TABLE 1.2 Ultrastructural Differences Between Aleurone Layer and Starchy Endosperm Protein Bodies

	Aleurone Layers		Endosperm	
Species	Diameter (μm)	Structure	Diameter (μm)	Structure
Wheat	2−3	Two kinds of inclusion	0.1−8	No inclusion; granular structure
	4−5	One globoid and one crystalloid	1−2	No inclusion; lamellar structure
Barley	2−3	Two kinds of inclusion	2	No inclusion; lamellar structure
	4−5	One globoid and one crystalloid	1−2	No inclusion; lamellar structure
Rice	1.5−4	Globoid	2−5	No inclusion; homogeneous
	1−3	Globoid	2−5	No inclusion; homogeneous
Maize			1−2	No inclusion; homogeneous

Adapted from Pernollet (1978). Reprinted with permission. Copyright © Pergamon Press.

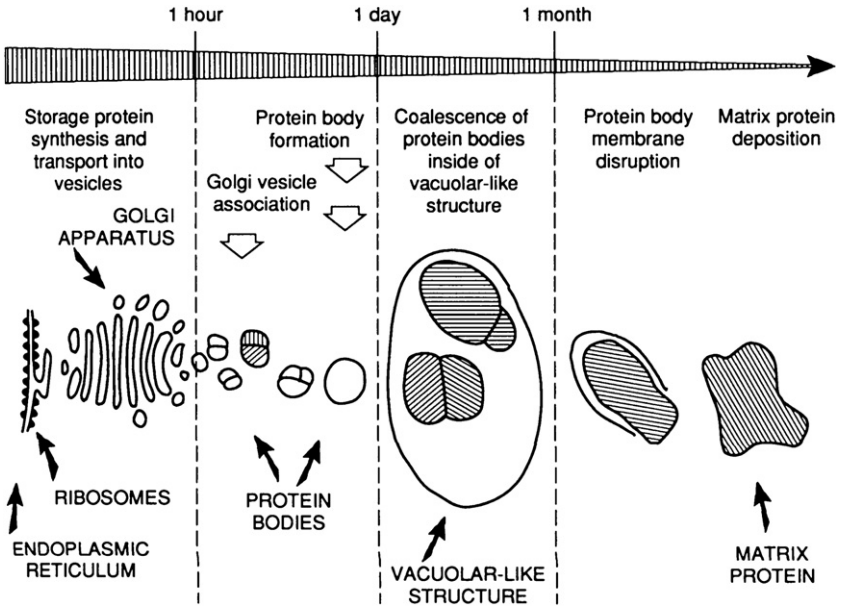


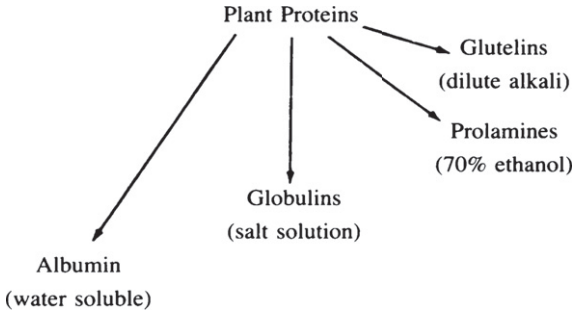
FIGURE 1.7 Schematic diagram of wheat endosperm protein body formation and evolution *(Pernollet and Camilleri, 1983).*

Three distinct stages were noted by Pernollet and Camilleri (1983) during the development of wheat protein bodies. The initial stage involved protein synthesis of storage proteins and their association as small vesicles into bodies of 5−10 μm in the first month after anthesis. During the next stage, the formation of the small protein bodies slowed down and instead they coalesced into much larger bodies (50−100 μm). The instability of the membrane of these large protein bodies and the mechanical pressure of the developing starch granules resulted in disruption of the membrane with the release of the protein bodies into the matrix protein. This loss of the protein bodies and the matrix protein formation is characteristic of the final stage of development of the mature wheat endosperm. The model proposed by Pernollet and Camilleri (1983) in Figure 1.7 summarizes the sequence of events leading to the formation of protein bodies in wheat and their eventual disruption.

The protein bodies of barley are similar to those of wheat, but differ quiet markedly from those of maize. In maize the membrane is derived from the endoplasmic reticulum, which completely encloses the protein bodies. This differs from wheat and barley, where the endoplasmic reticulum is disrupted by wheat and barley protein body aggregates which are not completely surrounded by this membrane. Oparka and Harris (1982) reported that rice protein bodies were surrounded by a membrane derived from the endoplasmic reticulum.

I. Classification of Plant Proteins

Plant proteins were first classified by Osborne (1895) as albumin, globulin, prolamins, and glutelins on the basis of solubility in different solvents as summarized in Scheme 1.1.



SCHEME 1.1

Several modifications have since been introduced to improve extraction of these fractions. Current practice extracts a combined albumin−globulin fraction as salt-soluble protein while the prolamines are extracted with aqueous propan-1-ol or propan-2-ol plus a reducing agent (Shewry *et al.*, 1980). This method is appropriate for the study of the basic genetic products but quite inappropriate from a technological point of view, as reducing agents result in the re-establishment of new disulfide bonds that change the solubility of the fractions. To prevent denaturation of the glutelin fraction by alkali extraction, alternative extractants such as buffers containing the detergent sodium dodecyl sulfate (SDS) at pH 10 are used (Moreaux and Landry, 1968). The relative proportion of the Osborne protein fractions in the seeds of wheat, barley, maize, and rye are summarized in Table 1.3.

J. Prolamins

The major storage proteins present in the starchy endosperm of wheat, barley, and maize are the alcohol-soluble proteins, the prolamins. These account for 30−60% of the total grain nitrogen depending on species, nutritional status, and genotype of the plant (Bright and Shewry, 1983; Shewry *et al.*, 1981). The prolamin fractions identified for different cereal species are listed in Table 1.4.

Prolamins derive their name from their unusually high content of proline and amide nitrogen (glutamine). This protein fraction is deficient in the essential amino acid lysine. Oats and rice differ substantially from other cereals in containing very little prolamin (5−10%), with the major storage proteins being globulin and a glutelin-like compound, respectively. Thus, these cereals have much more lysine, making them nutritionally superior. Electrophoretic separation of the different prolamin fractions on the basis of molecular size is accomplished by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS−PAGE). This permits identification of different polypeptide patterns in prolamins, which vary considerably among different cultivars of the same species. PAGE is a widely used technique for varietal identification of single seeds of wheat and barley. When there are only minor differences, two-dimensional isoelectric focusing (IEF) and PAGE can be effectively applied. Using

TABLE 1.3 Relative Proportions (%) of the Osborne Protein Fractions in Cereal Seeds

Cereal	Non-protein N	Albumins	Globulins	Prolamins	Glutelins	Residues
Barley[a]	11.6		15.6	45.2	18.0	5.0
Wheat[b]		33.1		60.7		6.2
Maize[a]	4.4	0.9	1.5	55.4	22.9	–
Rice[c]		15.7		6.7	61.5	15.4
Oats[d]	11		56	9	23	–

[a] *% Total seed N (%)*
[b] *% Recovered seed N (%)*
[c] *% Total protein (%)*
[d] *% Recovered protein (%)*
From Bright and Shewry (1983) with permission.

TABLE 1.4 Prolamin Fractions of Cereal Grain

Species	Trivial name
Wheat	Gliadin
Maize	Zein
Barley	Hordein
Oats	Avenin

TABLE 1.5 Prolamin Fractions of Wheat, Barley, and Maize

Wheat	MW	Barley	MW	Maize	MW
α-Gliadin	32,000	B-hordein	35,000−46,000	20K	20,000−21,000
β-Gliadin	40,000	C-hordein	45,000−72,000	22K	22,000−23,000
ω-Gliadin	40,000−72,000	D-hordein	100,000	9K	9,000−10,000
HMW subunits	95,000−136,000			14K	13,000−14,000

MW: molecular weight; HMW: high molecular weight.

these procedures the polypeptides identified for prolamin fractions in wheat, barley, and maize are summarized in Table 1.5.

The wheat gliadins are classified into two groups based on their electrophoretic mobility at low pH. The first group includes the fastest fraction, α-gliadin, followed by β-, γ-, and ω-gliadins, while the second group, with a much higher apparent molecular weight (95,136,000), is referred to as high-molecular-weight units (HMU). All the gliadin fractions are deficient in lysine and threonine. Three groups of hordein protein were separated from barley by SDS−PAGE and referred to as B, C, and D. They differed from each other in apparent molecular weights and amino acid composition (Miflin and Shewry, 1977). The C fraction had only trace amounts of sulfur amino acids while the D fraction was rich in glycine (13%). Lysine was particularly low in all the hordein protein fractions (< 1%) while B and C hordeins were also deficient in threonine.

The zein component of maize protein, although not well defined, was composed of two major and two minor fractions. The two major fractions had apparent molecular weights of 20,000−21,000 and 22,000−23,000, while minor fractions were 9,000−10,000 and 13,000−14,000, referred to as 22K, 9K, and 14K zein, respectively. All of these fractions were deficient in lysine. Unlike the other cereal grains, the major storage proteins of oats were 12S and 7S globulins as prolamins accounted for less than 15% of the total grain nitrogen (Peterson and Smith, 1976). Burgess and Miflin (1985) showed that 7S globulin was located mainly in the embryo while 12S globulin, the larger fraction, was predominant in the endosperm. Based on SDS−PAGE it appeared that the globulin and prolamin fractions were localized in different protein bodies.

K. Protein Synthesis

The development of cereal seed protein is associated with at least three stages. The first stage is characterized by rapid cell division in which protein synthesis remains quite low. When cell division ceases this is followed by an increase in the RER and accumulation of soluble nucleotides (Briarty *et al.*, 1979; Jenner, 1968). This results in a rapid synthesis of storage proteins which is related to initiation and synthesis of messenger RNA (mRNA) as well as the efficiency of mRNA translation. The accumulation of mRNA in developing wheat seeds was correlated with protein synthesis by Greene (1983). Using labeled [5-^{3}H] uridine and L-[^{3}H] leucine, he studied the synthesis, functioning, and stability of storage protein RNAs. Three developmental stages were apparent:

1. A change from seed protein synthesis of non-storage to storage protein.
2. An increase in the rate of accumulation of poly(A)+RNA.
3. An increase in the level of transcription mRNA.

A direct relationship between mRNA levels and the rate of protein synthesis is shown in Figure 1.8. Synthesis of the gliadin peptide was predominant from 15 to 25 days following flowering and paralleled the increase in poly(A)+ RNA. Thus, the storage protein gene expression in wheat endosperm is an mRNA-limiting process based on the amount of storage protein that the mRNA synthesized near the end of endosperm cell division. Okita and Greene (1982) previously identified mRNAs as the major messenger species in Cheyenne responsible for gliadin synthesis 20−25 days after anthesis. For a more detailed review of cereal proteins the article by Laszity (1984) is recommended.

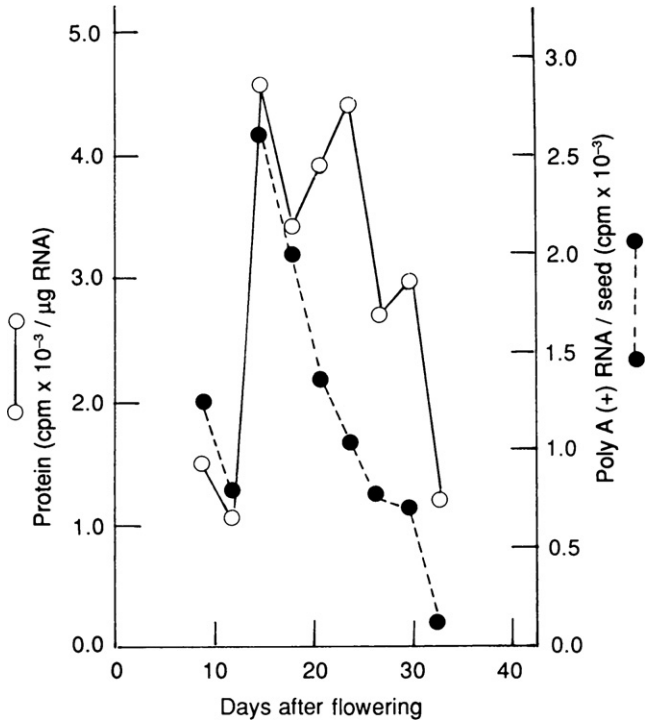


FIGURE 1.8 **Development profiles of poly(A) + RNA accumulation and *in vitro* protein synthesis capacity in wheat** *(Greene, 1983).*

TABLE 1.6 Lipid Content of Whole-grain Cereals

Cereal	Crude Fat (%)
Wheat	1.8
Maize kernel	0.4–1.7
Barley	3.3–4.6
Oats	5.4
Rice	1.9–3.1

L. Lipids

Lipids are distributed throughout the cereal grain as part of the intracellular membranes and spherosomes. They are stored as triglyceride-rich droplets in the spherosomes of the aleurone layer which are found clustered around the aleurone grains or with the plasmalemma (Buttrose, 1971; Chamura, 1975; Morrison *et al.*, 1975; Morrison, 1978). Spherosomes are also present in the embryo, scutellum, and coleoptile (Buttrose and Soeffly, 1973; Jelseman *et al.*, 1974). Lipids are also found in starch, primarily as monoacyl lysophosphatidyl ethanolamine, and lysophosphatidylcholine, and as inclusion complexes with amylose inside the starch granule. There appears to be a correlation between the amylose and lipid content of cereals, for example, waxy maize has little lipid while high-amylose or amylomaize starch has a higher lipid content than normal maize starch (Acker and Becker, 1971). The distribution of lipids in mature cereal kernels is shown in Table 1.6.

The major fatty acids present in grain lipids are linoleic, oleic, palmitic, and linolenic acids, in order of decreasing amounts (Price and Parsons, 1975). The cereal lipids can be separated into polar and non-polar lipids by solvent fraction. For example, in the case of a hard red spring wheat, Waldron, the polar and non-polar lipids accounted for

TABLE 1.7 Distribution of Wheat Lipids within Wheat Tissues[a,b]

Total Lipids							
				Endosperm (44.8%)			
Germ (30.4%)		Aleurone Layer (24.8%)		*Non-starch (29.2%)*		*Starch (15.6%)*	
Non-polar lipids (24.1%)	Polar lipids (6.3%)	Non-polar lipids (17.9%)	Polar lipids (6.9%)	Non-polar lipids (9.7%)	Polar lipids (19.5%)	Non-polar lipids (0.7%)	Polar lipids (14%)

[a] *Adapted from Hargin and Morrison (1980)*
[b] *calculated and adapted from data by Hargin and Morrison (1980)*
Data are expressed as a percentage of total lipids.

49.6% and 50.4% of the total lipids, respectively (Hargin and Morrison, 1980). The distribution of these lipid fractions within the wheat tissues is shown in Table 1.7.

The germ contains one-third of the total wheat lipids, of which 80% are neutral triglycerides. Aleurone lipids account for one-quarter of the total lipids, with 80% being non-polar in nature. The endosperm, however, accounts for almost half of the whole kernel lipids. The endosperm starch is associated with 15.6% of the total lipids, of which 96% are phospholipids. The predominant phospholipid in starch endosperm is lysophosphatidyl-choline (Hargin and Morrison, 1980).

The biosynthesis of lipids begins with the formation of fatty acids by a multistep process involving a multienzyme complex, the acyl protein carrier (ACP) fatty acid synthetase. Once formed, they are esterified with glycerol to triglycerides, which serve as an important source of energy during germination of cereals. They are responsible for maintaining the embryo and aleurone layer during the initial stages of germination until sugars are provided from the starchy endosperm.

IV. GERMINATION OF CEREALS

Germination of cereals is important in the malting industry, which depends on a certain degree of starch degradation. In the production of baked products, however, it is important that most of the starch granules remain intact. Thus germination or sprouting of cereal grains affects the grading of wheat and cereal grains as a result of the damage it causes. According to the *Grain Primer* (US Department of Agriculture, 1957), sprouting of wheat is defined as 'kernels which have the germ end broken open from germination, and kernels from which sprouts have broken off'. This is prevalent during wet weather when the moisture content is increased. The preharvest germination of the wheat reduces grain yield, flour yield, and flour quality. This has an adverse effect on the breadmaking properties of the flour because of the enhanced hydrolysis of the dough starch by α-amylase (Buchanan and Nicholas, 1980). If the activity of α-amylase is excessive it produces a bread product with a wet, sticky crumb.

A. Mobilization of Cereal Starches by α-Amylase

The native starch granule in wheat is attacked by certain α-amylase isoenzymes. Two groups were separated by Sargeant (1979) during germination of wheat, one of which hydrolyzed the starch granules. Halmer (1985) pointed out that since starch hydrolysis is normally carried out with soluble and not native granular starch granules, it is difficult to relate total amylolytic activity, as measured in the laboratory, to the granule-degrading activity of the cereal grain *in vivo*. The hydrolysis of starch by α-amylases is characterized by the endocleavage of amylose and amylopectin (Abbott and Matheson, 1972).

B. Biosynthesis of α-Amylase during Germination

The importance of α-amylase activity in baking and brewing has focused considerable attention on its secretion during germination. A major controversy has centered on whether the site of α-amylase biosynthesis is in the scutellum or aleurone layer (Akazawa and Hara-Nishimura, 1985). In the case of barley grains, α-amylase formation *de novo* has been reported in both the scutellum and aleurone layer (Briggs, 1963, 1964; Chrispeels and Varner,

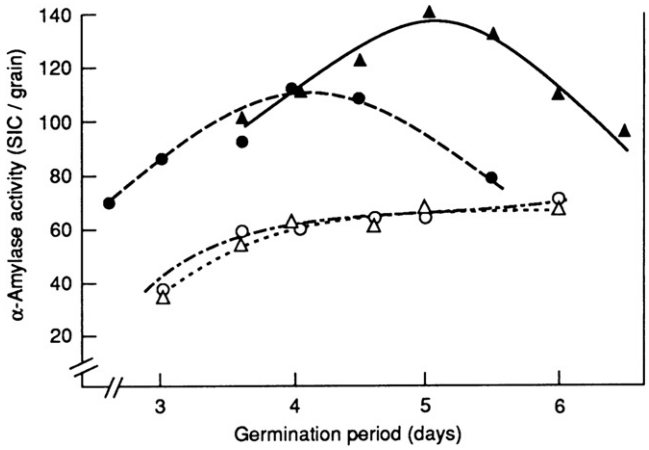


FIGURE 1.9 α-Amylase activity in decorticated barley germinated in the K_2SO_4 and GA_3. No additives (○); K_2SO_4 (50 mM) (Δ); GA_3 (50 μg/ml) (●); GA_3 (50 μg/ml) and K_2SO_4 (50 mM) (▲) *(Raynes and Briggs, 1985).*

1967). The biosynthesis and secretion of this enzyme appear to involve the plant hormone gibberellin GA_3. This hormone is produced by the embryo, and triggers the production of α-amylase as well as other hydrolytic enzymes in the aleurone layer (Briggs *et al.*, 1981). The increase in enzyme activity was attributed to an increase in the level of α-amylase mRNA (Bernal-Lugo *et al.*, 1981; Higgins *et al.*, 1976). In the case of wheat, the aleurone layer also becomes the target of hormone-induced enzymes, including increased synthesis of α-amylase (Filmer and Varner, 1967; Melcher and Varner, 1971). Varty *et al.* (1982) found that the plant hormone abscisic acid inhibited both transcription and translation of α-amylase mRNA in isolated wheat aleurone tissue. This explained the ability of abscisic acid to inhibit the induction of α-amylase by GA_3 (Chrispeels and Varner, 1967). Studies by Raynes and Briggs (1985) showed increased α-amylase production in decorticated barley grains germinated with or without gibberellic acid. Their results, shown in Figure 1.9, indicate that the onset and amount of enzyme activity were affected by GA_3 and K_2SO_4. The presence of K_2SO_4 appeared to delay the destruction of α-amylase (Briggs, 1968). Based on studies with rice scutellum, calcium also appears to play a role in the biosynthesis and secretion of α-amylase with the possible involvement of calmodulin (Mitsui *et al.*, 1984).

A number of researchers reported that the major isoenzyme form of α-amylase in germinated mature grain or aleurone tissue incubated with GA_3 was α-AMY1 (MacGregor, 1983; Marchylo *et al.*, 1981; Sargeant, 1979, 1980). This differed from α-amylase production in pre-mature excised embryo/scutellar tissue, where α-AMY2 was the predominant isoenzyme formed even in the presence of GA_3. While this tissue normally produces little α-amylase activity in pre-mature wheat grain, once removed from the caryopsis it starts synthesizing α-amylase, resulting in the characteristic cytological changes associated with germination. Cornford *et al.* (1987) further examined the production of α-amylase in embryo/scutellar tissue from pre-mature wheat and found that it was influenced by embryo age. While both α-AMY1 and α-AMY2 forms were detected by rocket-line immuno-electrophoresis in the presence of GA_3, it was the production of α-AMY2 that was stimulated by the addition of this growth substance. Abscisic acid inhibited the production of α-AMY1 and several α-AMY2 bands, although four active α-AMY2 bands were still detected. This switching of developmental to germinative mode by the excised embryos, in terms of α-amylase production, may be due to the loss of abscisic acid from the embryo (Triplett and Quatrano, 1982).

MacGregor and Matsuo (1982) conducted a detailed study on initial starch degradation during germination in endosperms of barley and wheat kernels. The kernels examined were all carefully split longitudinally through the crease without distorting any of the structural features (Figure 1.10). Using scanning electron microscopy, similar physical changes were evident in both barley and wheat kernels during the initial stages. Starch degradation appeared to commence at the endosperm—embryo junction, then moved along the junction to the dorsal edge of the kernel. This effect was only observed once extensive degradation of the cell wall material and protein matrix in the endosperm had occurred. These results were consistent with earlier work showing that α-amylase synthesis during germination commenced in the embryo (Gibbons, 1979, 1980; Okamoto *et al.*, 1980). Irrespective of where α-amylase is synthesized, it is ultimately discharged into the endosperm, where starch hydrolysis takes place.

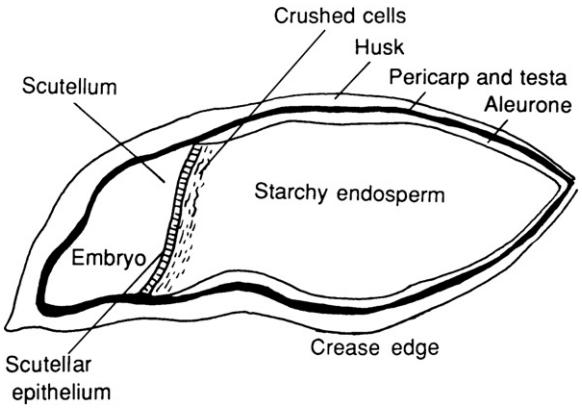


FIGURE 1.10 Longitudinal section of barley kernel cracked open through the crease edge *(MacGregor and Matsuo, 1982).*

C. α-Amylase Activity in Germinated Cereals

During the course of germination, starch is degraded by α-amylases and simple sugars are released (Kruger, 1972a,b). Lineback and Ponpipom (1977) monitored the degradation of starch during the germination of cereals, including wheat and oats. They found that an increase in α-amylase activity was accompanied by a rise in free sugars in all cereals examined. The amount produced reflected the degree of damaged starch in the flour milled from the germinated seed. Although the highest α-amylase activity was associated with germinated wheat, the degradation of starch was less than in the other cereals. Starch degradation in wheat was evident by erosion of the granule surface and the equatorial groove. The starch granule from oats was much more resistant to enzyme attack, however, with little damaged starch in the milled flour.

Germination studies conducted on five wheat cultivators by Reddy *et al.* (1984a) showed α-amylase development to be temperature dependent. Wheat kernels germinated in growth chambers at 15.5°C developed the highest enzyme activity compared to 20°C for the field-grown kernels. The activity did not increase significantly until the third day of germination and then rose markedly after 6 days.

D. Effect of Germination on Flour Quality

Lukow and Bushuk (1984) examined the effect of germination on wheat flour quality. Using flours from two cultivars of Canadian hard red spring wheat they found that α-amylase activity was quite low but increased 1600- and 3000-fold during germination. This marked increase in enzyme activity was accompanied by a rise in reducing sugars, which explained the inferior baking characteristics of the germinated flours. The major effect of α-amylase activity was to reduce the water-binding properties of the flour by degradation of the gelatinized starch. The overall result was the production of bread in which the crumbs were damp and sticky (Jongh, 1967; Thomas and Lukow, 1969).

Kruger and Matsuo (1982) studied the effect of preharvest sprouting on the pasta-making quality of durum wheat. α-Amylase activity increased 155- and 320-fold when germinated for 72 and 120 hours, respectively. While cooling during semolina and spaghetti production decreased α-amylase activity, it did not destroy the enzyme immediately. These researchers noted that α-amylase was still active during the first 6 minutes of cooking spaghetti and accounted for the production of reducing sugars, the substantial loss of solids, and the detrimental effect on spaghetti quality.

E. Treatment of Sprouted Grain: Reduction of α-Amylase

Germination of wheat grains commences at harvest time with an adverse effect on quality (Meredith and Pomeranz, 1985). The major culprit is α-amylase activity, which increases during germination, while β-amylase activity remains unchanged. Various methods have been examined to improve the properties of the sprouted grain. Since the starch fraction of sprouted wheat was of good quality, efforts focused on inhibiting α-amylase activity using heat or chemical agents (Bean *et al.*, 1974; Cawley and Mitchell, 1968; McDermott and Elton, 1971; Westermarck-Rosendahl *et al.*, 1979). Early research by Schultz and Stephan (1960), for example, reported an improvement in structure when wheat was treated with acids. Fuller *et al.* (1970) used hydrochloric acid followed by neutralization with ammonia to reduce α-amylase activity, but their method proved impractical. Several α-amylase inhibitors were

TABLE 1.8 Effect of Sodium Polyphosphate on the Falling Number of Sprout-damaged Wheat

Chemical Agent	Concentration[a] (%)	Falling Number
Sodium polyphosphate	0.1	147[b]
	0.5	175[c]
	1.0	250[c]

[a]*Based on meal weight (moisture content 15.0%)*
[b]*Difference significant at 5%*
[c]*Difference significant at 1%*
Adapted from Westermarck-Rosendahl *et al.* (1979).

examined by Westermarck-Rosendahl *et al.* (1979) to improve the baking qualities of sprouted wheat. The most promising agents were trisodium phosphate, disodium phosphate, sodium polyphosphate, SDS, calcium steoryl lactylate, and citric acid. Evaluations were based on the falling number test values for grain samples in which the optimum for baking flour was around 200 seconds (Greenaway, 1969). These α-amylase inhibitors caused an increase in falling number values well above 200 seconds, as shown in Table 1.8 for sodium polyphosphate.

The falling number test measures the time it takes for a plunger to fall freely through a suspension of flour in water and the effect of starch amylolytic degradation on the viscosity of the flour/water paste. The faster the decrease in viscosity of the flour paste, the lower the falling number value. Further research by Westermarck-Rosendahl *et al.* (1980) showed that the most promising of the 23 enzyme inhibitors examined were trisodium phosphate and disodium hydrogen phosphate. These were particularly effective in reducing the stickiness problem associated with flours from sprouted wheat as well as improving crumb characteristics. Alternative solutions discussed by Meredith and Pomeranz (1985) included the elimination of sprout-susceptible lines through breeding and selection programs.

F. Mobilization of Proteins during Germination

Essential amino acids increase during germination or sprouting of cereal grains (Dalby and Tsai, 1976; Tsai *et al.*, 1975). For example, both lysine and tryptophan increased during the germination of wheat, barley, oats, and rice. The extent of the increase was directly related to the decrease in prolamin content of the grain. A substantial increase in lysine of 50% was noted for wheat, compared to only a slight increase in oats (Figure 1.11). The level of prolamin in oats, however, was much lower than that in wheat. Jones and Tsai (1977) reported an increase in the lysine and tryptophan content of the embryo of normal maize and a corresponding decrease in the endosperm. The higher level of lysine is required for embryo growth and development, as observed previously by Singh and Axtell (1973) in studies on barley embryo and endosperm proteins. The precursors for lysine biosynthesis in maize may be provided by mobilization of zein reserves in the endosperm.

The release of amino acids during germination of wheat was investigated by Tkachuk (1979). After 122 hours of germination at 16.5°C the proline and glutamine content increased 100- and 80-fold, respectively, while lysine increased only 12-fold (Table 1.9). These results represent the changes taking place in the whole wheat kernels and may not reflect changes occurring in the embryo or aleurone layer. Nevertheless, they do illustrate that considerable proteolysis occurs during germination, which could be a method for assessing the extent of germination.

Kruger (1984), using high-performance liquid chromatography in the gel permeation mode, monitored the molecular weight profiles of buffer-soluble (0.5 M sodium phosphate buffer, pH 7.0, containing 0.5 M sodium chloride) proteins in both sound and germinated wheat kernels. Of the molecular weight protein group examined, the low-molecular-weight peptides and amino acids exhibited the largest change. This was further evidence for the increased solubilized amino nitrogen, particularly amino acids, during germination. Little change occurred during the first 2 days of germination compared to after 6 days. Further studies by Kruger and Marchylo (1985) examined mobilization of protein during the germination of five wheat cultivars. Six major protein components were eluted, of which only the low-molecular-weight species underwent major changes during germination. These results confirmed earlier work by Kruger (1984) and Lukow and Bushuk (1984) which showed that a very rapid hydrolysis of wheat endosperm proteins occurs following limited endopeptidase activity during the initial period of germination.

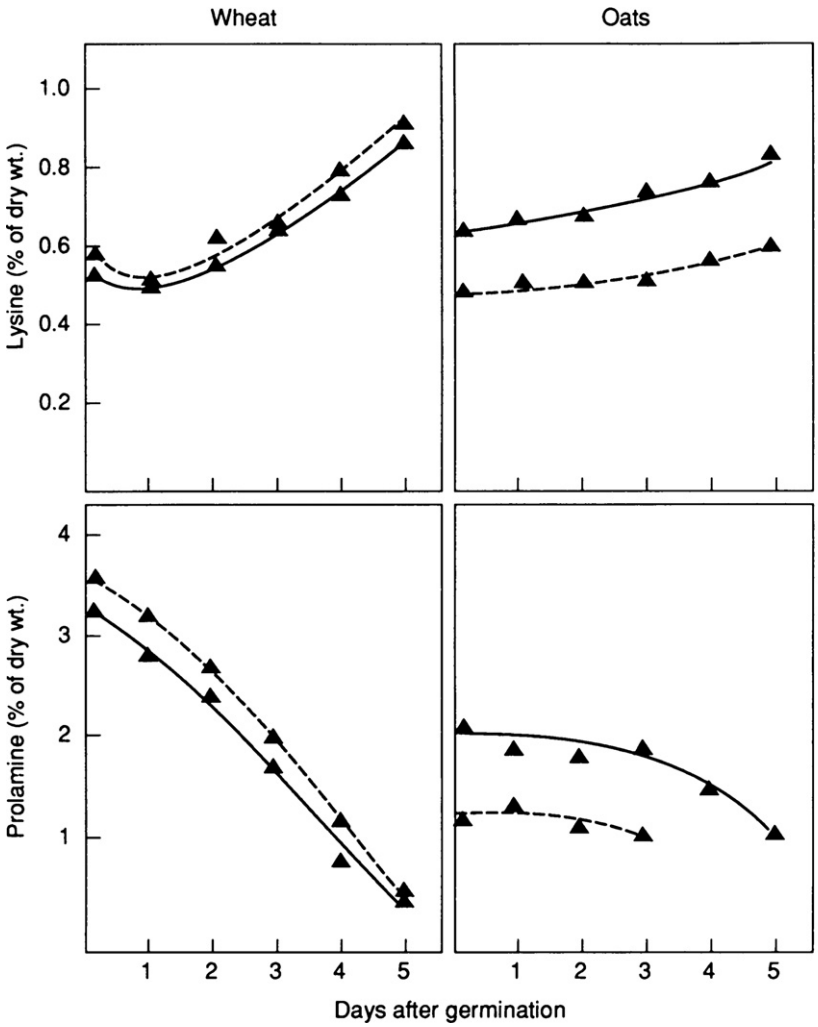


FIGURE 1.11 Changes in protein and prolamin content during germination of wheat and oats. *(Adapted from Dalby and Tsai, 1976.)*

Increased release of free amino acids during germination suggests extensive mobilization of the storage proteins during this period. The mechanism controlling this process remains poorly understood. A number of proteases has been found in wheat grain, including endopeptidases, carboxypeptidases, and aminopeptidases (Grant and Wang, 1972; Kruger, 1973; Preston and Kruger, 1976a, b, 1977; Kruger and Preston, 1978). Of these, carboxypeptidase is prominent in the endosperm, where it represents one-quarter of the total endopeptidase activity (Preston and Kruger, 1976a). These enzymes have a negligible effect on the endosperm reserves during the first 2 days of germination, possibly because of their compartmentalization, the presence of protease inhibitors, or insolubilization of the substrate. During the course of germination there is limited endopeptidase activity resulting in the formation of intermediate products which are then degraded by carboxypeptidase to amino acids (Kruger and Marchylo, 1985). Only a fraction of the storage proteins is affected at any time, which explains the similarity in protein patterns for sprouted and mature seeds.

G. Lipid Mobilization during Germination

Germination and sprouting of cereal grains are accompanied by an increase in total lipid content (Lorenz, 1980; Rahnotra *et al.*, 1977). The presence of lipase in ungerminated wheat and barley seeds is extremely low but develops as soon as germination commences (Huang and Moreau, 1978; Taverner and Laidman, 1972). In sharp contrast to these cereals, oats are rich in lipase activity (Matlashewski *et al.*, 1982). Lipase (triacylglycerol lipase, EC 3.1.1.30) hydrolyzes triacyiglycerols, diacyiglycerols, and possibly monoacylglycerols, producing fatty acids. The major

TABLE 1.9 Effect of Germination at 16.5°C on the Production of Selected Free Amino acids in Wheat cv. 'Neepawa'

	Germination Period (Hours)	
Amino Acid (μmole/g N)	0	122
Tryptophan	47	50
Lysine	5.7	63
Histidine	2.2	72
Glutamic acid	64	95
Methionine	2.4	27
Isoleucine	5.1	140
Leucine	6.0	170
Tyrosine	4.5	72
Phenylalanine	4.2	150
Proline	7.8	790
Glutamine	12	920

Adapted from Tkachuk (1979).

difficulty involved in measuring lipase activity is due to the insolubility of the substrate in aqueous solution. This difficulty has been partially overcome using water-soluble substrates such as *p*-nitrophenyl (Pnp) acetate, or butyrate, or by forming a stable emulsion with olive oil. A specific method for assaying lipase was developed by Matlashewski *et al.* (1982) using radioactive triacylglycerols in which the fatty acid moiety was labeled. Using this method, Baxter (1984) examined lipase activity in both germinated and ungerminated barley. The results obtained in Figure 1.12 show that lipase activity increased slowly during the initial 2 days of germination but then rose sharply after 3 days. Two distinct lipase fractions were separated with similar molecular weights (400,000 range) but different ionic properties. The major fraction (I) was associated with the embryo, while the smaller lipase fraction (II) was located in the endosperm. Taverner and Laidman (1972) identified lipase in wheat embryo and endosperm, each induced by different factors. Urquardt *et al.* (1984) separated oat embryos from the rest of the kernel and monitored changes in lipase activity during germination. The initial increase in lipase activity appeared to be primarily in the bran layer,

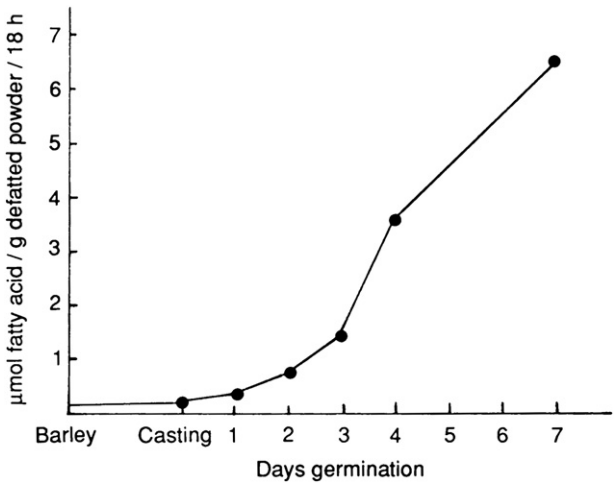


FIGURE 1.12 **Lipase activity in aqueous extracts of barley (variety Sonja) during germination.** *(Baxter, 1984.)*

with little or no activity in the endosperm (Urquardt *et al.*, 1983). While the primary role of lipase is hydrolysis of the storage triacylglycerols, its physiological role remains obscure.

V. STORAGE OF GRAINS

Following harvest, cereal grains such as wheat are stored in either sacks or bulk silos. These grains are traditionally recognized for their keeping quality, which is affected by moisture, temperature, and invasion by rodents, insects, bacteria, and fungi. Postharvest grain losses worldwide appear to be around 3−10%, sometimes up to 15%, depending on local conditions and resources (Harris, 1984). This section will focus on the effects of moisture and temperature on the quality of grains.

A. Respiration

When cereal grains are dry very little respiration occurs. If the moisture content of the seeds rises above 14%, respiration increases until a critical moisture level is attained. At this point respiration accelerates rapidly with the subsequent heating of the grain. This marked rise in respiration is attributed in part to germination and growth of molds such as *Aspergillus* and *Penicillium.* Grain respiration is affected by moisture, temperature, and oxygen tension, although the moisture content is of paramount importance in the commercial storage of cereal grains.

1. Effect of Moisture Content

Exposure of grain will result in the uptake of moisture until equilibrium is reached with the water vapor in the atmosphere. Thus, the moisture content of grain is controlled by the relative humidity in the environment, which in terms of grain storage is the nature of the interstitial atmosphere. When exposed to an atmosphere of uniform relative humidity at a constant temperature, the relative humidity of the stored grain reaches an equilibrium referred to as the equilibrium relative humidity (ERH). The relationship between relative humidity and moisture content is defined by the sorption isotherm, the shape of which is sigmoid. This is due to the larger equilibrium moisture content during desorption compared to adsorption at a given ERH. Figure 1.13 shows the moisture isotherm obtained at 30°C for maize with the characteristic sigmoid curve resulting from the greater water content of the desorption isotherm (Denloye and Ade-John, 1985).

The equilibrium moisture content is quite low in grains and only after the isotherm reaches 80% relative humidity does the moisture content rise exponentially with relative humidity (Oxley, 1948). The moisture content that is regarded as safe for grain is that in equilibrium with 70% relative humidity (Pixton and Warburton, 1971). Microbial growth will only occur above 75% relative humidity, resulting in extensive deterioration of the grain.

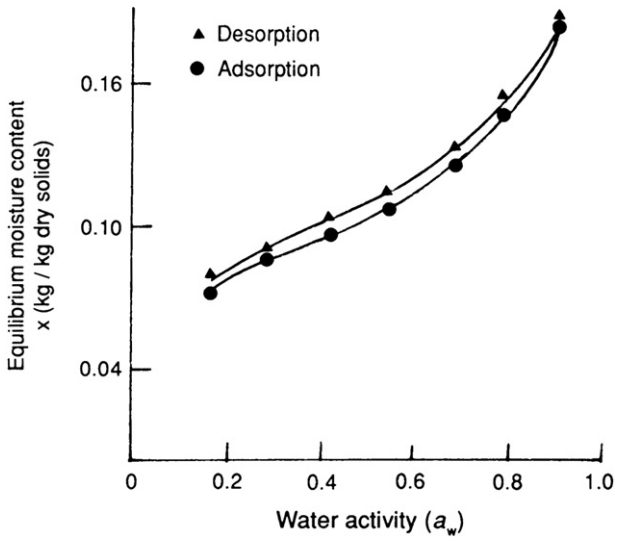


FIGURE 1.13 Moisture sorption isotherms for maize (30°C). *(Reprinted with permission from Denloye and Ade-John, 1985. © Pergamon Press.)*

Under extremely wet conditions the grain may be harvested at a moisture content that is too high for safe storage. This necessitates the use of drying to reduce the moisture content of the grain, which can then be stored with minimal loss in seed viability, nutritive value, and breadmaking properties (Bushuk, 1978). Spillane and Pelhate (1982) attempted to bypass the drying step by storing barley harvested with a high moisture content (> 30%) under ventilated conditions. Unless the rise in grain temperature, due to respiration, could be controlled, an explosive growth of yeasts and bacteria would take place. This was prevented by continuously ventilating the silo for a month, which removed a good portion of the heat generated by respiration, and so reduced the final grain temperature to below the critical point of 16°C. The moisture content of the grain was reduced to 16% and the relative humidity of the grain environment to around 80% at the end of the storage period. Under these conditions the growth of yeasts and bacteria was suppressed while quality factors remained intact.

2. Effect of Temperature

The ERH is affected only slightly by changes in temperature. Ayerst (1965) reported that a rise or fall of 10°C resulted in a 3% change in ERH over a relative humidity range of 40–90%. At higher relative humidities the change never exceeded 1% (Pixton and Warburton, 1975). Using Manitoba wheat, Pixton (1968) showed that at 10% moisture content the ERH increased by 6% when heated at 70°C compared to only 2% when the moisture content was 14%. Prolonging the heating for more than an hour produced further change. Denloye and Ade-John (1985) noted a decrease in the equilibrium moisture content for maize kept at a constant relative humidity as temperature was changed from 30°C to 50°C (Figure 1.14).

Since grain is stored in bulk, the movement of heat and moisture in the stored grain is extremely important. Anderson *et al.* (1943) first showed that movement of moisture occurred over a temperature gradient from high to low. This process was extremely slow and involved diffusion with some convection currents. The main effect of heating appears to be related to the translocation of moisture brought about by the temperature gradients in the grain.

B. Prolonged Storage of Grains and Flour

Pixton *et al.* (1975) monitored the changes in quality of wheat stored for 16 years under conditions of low temperature (4.5–0.5°C) and low oxygen concentrations (< 2% by volume). Two different pest-free dry wheats, Manitoba and Cappelle, with respective moisture contents of 11.9% and 12.6%, were placed in bins in 1-ton lots. The moisture content did not change significantly over this period. The crude protein and salt-soluble protein content remained unchanged for both wheats irrespective of the storage conditions. A slight increase in the total fat of 0.5% was observed for both wheat varieties, which was attributed to carbohydrate metabolism during the long storage period. This was based on the slight reduction in total sugars observed in these wheat samples by Pixton and Hill

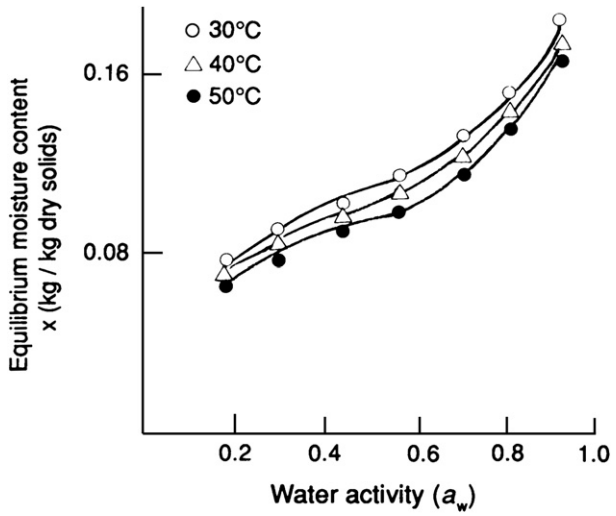


FIGURE 1.14 Desorption moisture isotherm for maize at different temperatures. *(Reprinted with permission from Denloye and Ade-John, 1985. © Pergamon Press.)*

(1967) after 8 years, although maltose and sucrose changed very little during the subsequent storage period. These researchers also monitored vitamin B, which remained unchanged throughout the storage period.

A high viability was observed for wheats stored at 4.5°C (96%) compared to only one-third viability when held at ambient temperature for the same period. As long as the wheat was protected from atmospheric moisture, rapid temperature changes, and insects, the baking quality remained intact, although some supplementation with fungal α-amylase was required.

PART II: LEGUMES

I. INTRODUCTION

The term legume encompasses more than 13,000 different species, all of the family Leguminosae. Legumes play a dominant role in the diets of humans across the globe. Of the thousands of species, however, only relatively few are widely grown commercially: soybeans, peanuts, dry beans, peas, broadbeans, chickpeas, and lentils. Of these seven, soybean is by far the most widely produced. Many other species of legumes play an important role in local food production in various corners of the world, but they are too numerous to be discussed in this chapter. Legumes are perhaps best known for their high plant protein content, due to nitrogen fixation allowed by the symbiotic relationship with bacteria. Table 1.10 shows the Food and Agriculture Organization (FAO) estimations for the world 2007 production of the major food legumes. This portion of the chapter will discuss the composition of legume seeds and the biochemical changes that occur during seed development, germination, storage, and fermentation.

II. LEGUME SEED STRUCTURE

Despite great variation in the macronutrient composition of legumes, their basic seed structure is the same. Mature seeds contain three major components: the seed coat (testa), the embryo, and the endosperm. Most legume seeds, however, have very little endosperm at maturity, as the cotyledons of the embryo make up a majority of the seed weight and contain the necessary stores for growth. Thus, the cotyledons provide the great majority of the nutritional components of interest to food value, with the exception of fiber and calcium, of which a significant portion is found in the seed coat (Kadam *et al.*, 1989). The structure of a typical soybean seed is shown in Figures 1.15 and 1.16. Size, shape, color, and thickness of the seed coat vary among the different legumes, although the basic structure prevails.

III. LEGUME SEED COMPOSITION

A. Proximate Composition

Food legumes vary greatly in their nutrient composition, depending on the type and variety of seed, soil conditions, and environmental factors. The proximate compositions of some major food legumes grown in the USA are presented

TABLE 1.10 World Production of Legumes in 2007

Legume	Million Tonnes
Beans, dry	19.3
Beans, green	6.4
Broad beans, dry	4.9
Peanuts, with shells	34.9
Lentils	3.9
Peas, dry	10.1
Peas, green	8.3
Soybeans	216.1

Data from the Food and Agriculture Organization (2008).

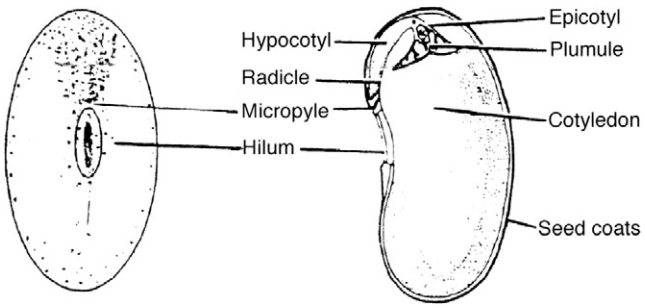


FIGURE 1.15 Structure of a soybean seed. *(From Liu, 1997, p. 4. With kind permission from Springer Science + Business Media BV.)*

in Table 1.11. The protein content of the selected legumes ranges from 19.30% to 26.12% of the edible portion, although crude protein content has been reported to range between 15% and 45% (Kadam *et al.*, 1989), with some soybean varieties containing as much as 50% protein (Vaidehi and Kadam, 1989). Carbohydrate content ranges from 24% to 68% (Reddy *et al.*, 1984b), and appears to be inversely related to the lipid content. Legume seeds high in carbohydrates have low lipid content, and vice versa. A classical example is peanut, which has a very high lipid content (49.24%) and relatively low carbohydrate content (16.13%) (Table 1.11). Potassium is by far the most abundant mineral in most food legumes (Iqbal *et al.*, 2006; USDA 2008), with soybeans containing as much as 1.80 g/100 g edible portion (Table 1.11). Phosphorus, copper, iron, calcium, and magnesium are some of the important minerals found in significant amounts in legumes. Niacin and pantothenic acid account for the most quantitatively important vitamins in legumes, and most are also a good source of folate.

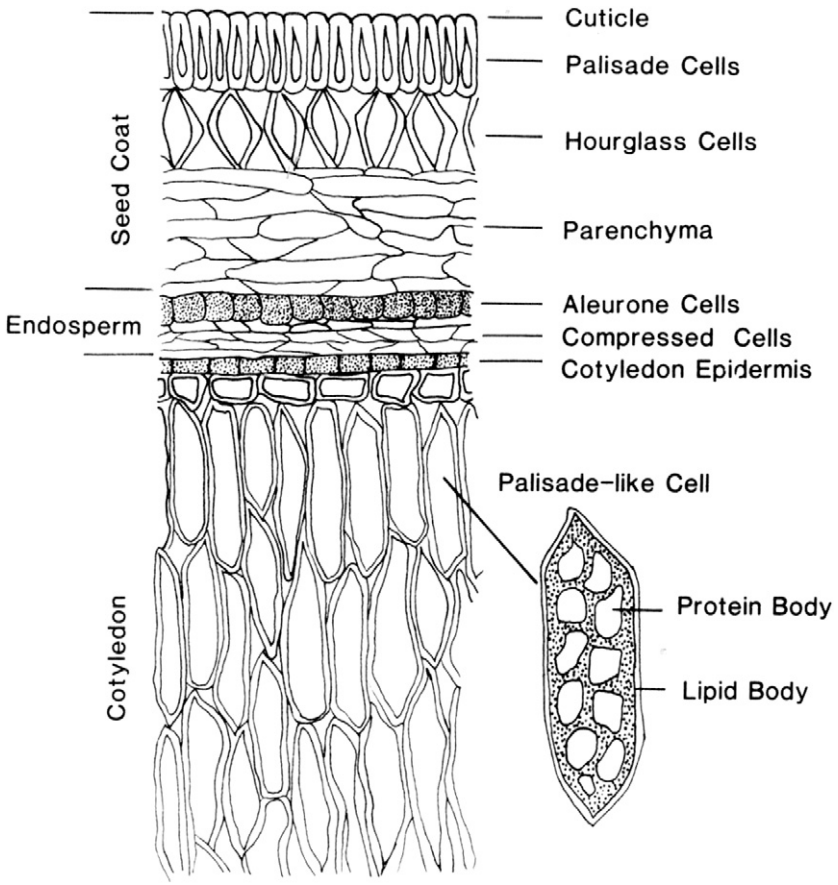


FIGURE 1.16 Cross-section of soybean seed coat. *(From Bair, 1979. © Craig Bair.)*

TABLE 1.11 Proximate Composition of Some Important Food Legumes

Nutrient	*Glycine max* L.	*Cicer arietinum*	*Arachis hypogaea*	*Pisum sativum*	*Vicia faba*	*Lens culinaris*	*Phaseolus vulgaris*
Water (g)	8.54	11.53	6.50	11.27	10.98	10.40	11.02
Protein (g)	36.49	19.30	25.80	24.55	26.12	25.80	21.60
Total lipid (g)	19.94	6.04	49.54	1.16	1.53	1.06	1.42
Ash (g)	4.87	2.48	2.33	2.65	3.08	2.67	3.60
Carbohydrate, by difference (g)	30.16	60.65	16.13	60.37	58.29	60.08	62.36
Fiber, total dietary (g)	9.30	17.4	8.50	25.5	25	30.50	15.2
Calcium (mg)	277	105	92	55	103	56	123
Iron (mg)	15.70	6.24	4.58	4.43	6.70	7.54	5.02
Magnesium (mg)	280	115	168	115	192	122	171
Phosphorus (mg)	704	366	376	366	421	451	352
Potassium (mg)	1797	875	705	981	1062	955	1483
Sodium (mg)	2	24	18	15	13	6	5
Zinc (mg)	4.89	3.43	3.27	3.01	3.14	4.78	3.65
Copper (mg)	1.658	0.847	1.144	0.866	0.824	0.519	0.841
Manganese (mg)	2.517	2.204	1.934	1.391	1.626	1.33	1.06
Selenium (mg)	17.8	8.2	7.2	1.60	8.2	8.30	3.2

Nutrient values are per 100 g edible portion.
Data from USDA (2008).

B. Protein

1. Nitrogen Fixation

Biological nitrogen fixation (BNF) is achieved by diazotrophs, microorganisms that possess the enzyme nitrogenase, which converts atmospheric dinitrogen (N_2) into organic nitrogen (usually ammonia). Legumes benefit from BNF by forming symbiotic associations with some nitrogen-fixing bacteria. The soil bacteria *Azorhizobium*, *Bradyrhizobium*, and *Rhizobium*, in association with legumes, are responsible for most of the nitrogen fixed biologically (Freiberg *et al.*, 1997). The bacteria inhabit specialized organs called nodules on the roots of the legumes, which is the site where nitrogen fixation takes place. The process of legume nodulation is a complex one, and is controlled by several genetic and environmental factors (Hirsch, 1992; Schubert, 1995).

Nodulation may be seen to proceed through three stages: preinfection, nodule initiation, and differentiation, with the flavonoids in the seed coat thought to serve as chemoattractants that induce *Rhizobium* nod genes (see review by Hirsch, 1992). Nitrogenase is sensitive to oxygen concentration, requiring very low partial pressure of oxygen in order to fix atmospheric nitrogen. Legume root nodules, as part of the symbiotic relationship with the bacteria, synthesize leghemoglobin in response to being infected or inoculated by the bacteria. Leghemoglobin is an oxygen-binding protein that maintains a low enough oxygen tension to protect the oxygen-labile nitrogenase enzyme from inactivation but high enough to make bacterial respiration possible. It has been reported that the apoprotein portion of leghemoglobin is synthesized by the plant, while the bacteria contribute the heme (iron complexed with a porphyrin ring) (O'Brian *et al.*, 1987). Another study, however, suggests that both apoprotein and heme components of leghemoglobin are synthesized by the plant (Santana *et al.*, 1998). The entire process of atmospheric nitrogen fixation can be reduced to the following chemical equation:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16\ P_i$$

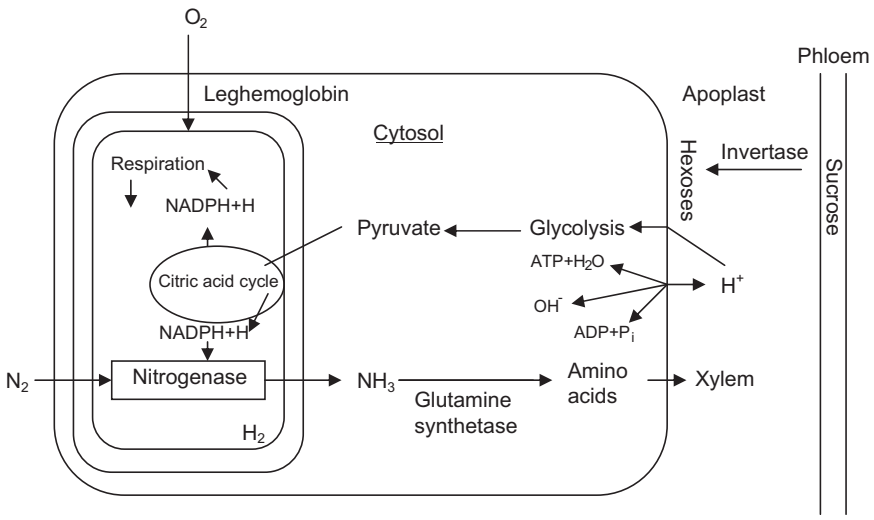


FIGURE 1.17 Model metabolic and transport pathways in infected cells of legume nodules. *(Redrawn from Schubert, 1995, p. 102. With kind permission from Springer Science + Business Media BV.)*

The energy (ATP) required to drive this process, as well as other metabolites necessary for the survival of the symbiotic bacteria, is supplied by the host plant in the form of sucrose through the vascular bundles in the inner cortex of the nodule (Serraj *et al.*, 1999). Soil acidity, drought, and soil mineral nitrogen content are a few of the environmental factors that influence the rate of nitrogen fixation (Schubert, 1995). The ammonia formed, present in its ammonium ion form, is released into the cytosol across a diffusion gradient (Schubert, 1995). Figure 1.17 presents a good schematic summary of the metabolic and transport pathways in the nodule.

2. Classification

Most of the protein in legumes is located in the cotyledons and embryonic axis, with the seed coat containing very little protein (Singh *et al.*, 1968). Legume seed proteins can be classified based on their functionality into structural and storage proteins. Structural proteins, sometimes referred to as enzymatic or catalytic proteins, are made up of protease inhibitors, lectins, lipoxygenases, and amylase inhibitors. Together they make up a small percentage of the total protein in the seeds, are found in the cotyledon, and are responsible for cell metabolism (Duranti and Gius, 1997). These structural proteins are albumins, soluble in water, and influence the postharvest taste and digestibility of food legumes. Specific examples and their effects will be examined in a later section. The storage proteins, which make up the bulk of legume seed proteins, are insoluble in water but soluble in salt solution and belong to the globulin class of proteins. Found primarily in the parenchyma cells of the cotyledons, storage proteins are contained in small membrane-bound organelles called protein bodies (Tombs, 1967; Duranti and Gius, 1997; Herman and Larkins, 1999), and range in size from 2 to 20 μm in diameter (Vaidehi and Kadam, 1989). Storage proteins provide the carbon and nitrogen building blocks necessary for seed growth during germination. They are further classified based on their sedimentation coefficients into four main fractions: 2S, 7S, 11S, and 15S. Fractions with higher sedimentation coefficients (up to 18S) have been reported in some soybean strains (Duranti and Gius, 1997). None of the fractions are, however, homogeneous. The 2S and 15S fractions are made up mainly of enzyme inhibitors and allergenic factors (Vaidehi and Kadam, 1989). The 7S globulins make up the bulk of the 7S fraction, while 11S globulin is the only protein in the 11S fraction. The 7S and 11S globulins together account for 50% of proteins in some soybeans (Vaidehi and Kadam, 1989), but usually contribute more than 70% of total proteins in most soybean and legume seeds (Kimura *et al.*, 2008; Natarajan *et al.*, 2006). Some legumes, e.g. French bean and cowpea, have a predominance of 7S globulins as the storage protein (Kimura *et al.*, 2008).

3. Protein Structure and Properties

The 7S and 11S globulins, because they make up the bulk of legume seed proteins, have been extensively studied. These globulins may be structurally similar, but differences exist from one legume to another in their subunit profiles and amino acid sequences, that give rise to differences in overall protein functionality.

The 7S globulins in different species of legumes are referred to by different names. The most abundant of the 7S globulins in soybeans is β-conglycinin. It is a trimeric glycoprotein made up of three types of subunit, α, α′, and β (Natarajan *et al.*, 2006; Rickert *et al.*, 2004). Different combinations of the subunits give rise to heterogeneous fractions with different functional properties among varieties of soybean (Rickert *et al.*, 2004). The α and α′ subunits are each composed of a core region with 418 amino acid residues, and extension regions with 125 and 141 residues, respectively (Maruyama *et al.*, 1999, 2002). The β subunit has only a core region made up of 416 amino acid residues (Maruyama *et al.*, 1999). In the study by Maruyama *et al.* (1999), the relationship between structure and physicochemical properties of these β-conglycinin subunits was studied. Their results indicated that the subunits differed in their thermal stabilities, solubilities, emulsifying abilities, surface hydrophobicities, and heat-induced associations. They also found that these properties varied with changes in conditions such as pH and ionic strength. They concluded that the core regions of the subunits were responsible for determining surface hydrophobicity and thermal stability, while solubility, heat-induced association, and emulsifying ability depended on the extension regions, the carbohydrate moieties, and the core regions.

The predominant 7S protein in common beans (*Phaseolus vulgaris* L.) is known as phaseolin. It has 420 amino acid residues at synthesis, but loses 21 residues during maturation (Slightom *et al.*, 1983). Like the soybean 7S protein, phaseolin is a trimeric protein, with α, β, and γ subunits (Blagrove *et al.*, 1983; Slightom *et al.*, 1983). In general, legume 7S proteins show pH and ionic strength-dependent association and dissociation equilibria (Duranti and Gius, 1997).

Glycinin, the 11S globulin in soybean seeds, is a hexamer consisting of five types of subunit, G1, G2, G3, G4, and G5, with G1 and G2 being allergens (Natarajan *et al.*, 2006). Each subunit is composed of an acidic and a basic polypeptide linked by a single disulfide bridge (Staswic *et al.*, 1981). The amino acid sequences of the polypeptides differ between and within species, giving rise to heterogeneous fractions with different functional properties. For example, differences in gel strength of different glycinin fractions have been found to be dependent on the amino acid sequence of the acidic

TABLE 1.12 Amino Acid Content of Some Important Food Legumes

Amino Acid	*Glycine max* L.	*Cicer arietinum*	*Arachis hypogaea*	*Pisum sativum*	*Vicia faba*	*Lens culinaris*	*Phaseolus vulgaris*
Tryptophan (g)	0.591	0.185	0.250	0.275	0.247	0.223	0.256
Threonine (g)	1.766	0.716	0.883	0.872	0.928	0.895	0.909
Isoleucine (g)	1.971	0.828	0.907	1.014	1.053	1.078	0.954
Leucine (g)	3.309	1.374	1.672	1.760	1.964	1.809	1.725
Lysine (g)	2.706	1.291	0.926	1.772	1.671	1.740	1.483
Methionine (g)	0.547	0.253	0.317	0.251	0.213	0.212	0.325
Cystine (g)	0.655	0.259	0.331	0.373	0.334	0.327	0.235
Phenylalanine (g)	2.122	1.034	1.337	1.132	1.103	1.230	1.168
Tyrosine (g)	1.539	0.479	1.049	0.711	0.827	0.667	0.608
Valine (g)	2.029	0.809	1.082	1.159	1.161	1.238	1.130
Arginine (g)	3.153	1.819	3.085	2.188	2.411	1.928	1.337
Histidine (g)	1.097	0.531	0.652	0.597	0.664	0.702	0.601
Alanine (g)	1.915	0.828	1.025	1.080	1.070	1.042	0.905
Aspartic acid (g)	5.112	2.270	3.146	2.896	2.916	2.758	2.613
Glutamic acid (g)	7.874	3.375	5.390	4.196	4.437	3.868	3.294
Glycine (g)	1.880	0.803	1.554	1.092	1.095	1.014	0.843
Praline (g)	2.379	0.797	1.138	1.014	1.099	1.042	0.916
Serine (g)	2.357	0.973	1.271	1.080	1.195	1.150	1.175

Amino acid values are per 100 g edible portion.
Data from USDA (2008).

polypeptide chain (Nakamura *et al.*, 1984). Several studies have been conducted into the structure–function relationship of glycinin (Mori *et al.*, 1981; Nakamura *et al.*, 1984; Riblett *et al.*, 2001; Khatib *et al.*, 2002).

4. Protein Quality

Protein quality is generally defined by its amino acid composition, digestibility, and bioavailability. It is well known that legume proteins are low in the essential sulfur-containing amino acid methionine, while being especially rich in lysine. The second limiting amino acid in legume protein is tryptophan, but in a few legumes (cowpeas, lentils, and greenpeas) it was the most limiting amino acid (Iqbal *et al.*, 2006). The effect of these deficiencies is observed more markedly on growth than on protein requirements for maintenance (Patwardhan, 1962). The amino acid profiles of some important food legumes are presented in Table 1.12. The nutritional value of the legume proteins can be assessed by a variety of methods. The most commonly used include the essential amino score with reference to the FAO/World Health Organization (WHO) standard amino acid profile and protein efficiency ratio (based on growth response in experimental animals, usually rats). The digestibility coefficient of legume proteins varies greatly, from 51% to 92% (Patwardhan, 1962), and is influenced by the presence of anti-nutritional factors (Duranti and Gius, 1997).

C. Carbohydrates

1. Overview

The total carbohydrate of dry pulses varies greatly, ranging from 24% in winged bean seeds to 68% found in cowpea seeds (Table 1.13). In general, the total carbohydrate may consist of soluble and insoluble fractions. Soluble

TABLE 1.13 Total Carbohydrate and Starch Contents of Food Legumes

Legume	Total Carbohydrate	Starch	Amylose	Gelatinization Temperature (°C)
Winged bean seed	24.0–42.2	–	–	–
Smooth pea	56.6	36.9–48.6	5.3–8.7	65–69
Wrinkled pea	–	24.0–36.6	10.2–15.1	>99
Great bean	61.2–61.5	44.0	9.9	–
California small white bean	–	57.8	7.7	–
Red kidney bean	56.3–60.5	31.9–47.0	17.5–37.2	64–68
Navy bean	58.4	27.0–52.7	22.1–36.0	68–74
Pinto bean	–	51.0–56.5	25.8	–
Pink bean	–	42.3	14.9–35.3	–
Black eye bean	–	41.2	15.8–38.3	–
Black gram	56.5–63.7	32.2–47.9	43.9	71.5–74
Bengal gram	60.1–61.2	37.2–50.0	31.8–45.8	–
Mung bean	53.3–61.2	37.0–53.6	13.8–35.0	63–69
Red gram	57.3–58.7	40.4–48.2	38.6	–
Soybean	25.2–33.5	0.2–0.9	15.0–20.0	73–81
Broad bean	57.3	41.2–52.7	22.0–35.0	
Lentil	59.7	34.7–52.8	20.7–45.5	58–61
Cowpea	56.0–68.0	31.5–48.0	–	–
Lupine seed	–	0.3–3.5	–	–

Values are reported in g/100 g on a dry weight basis.
Data from Reddy *et al.* (1984b).

carbohydrate may include monosaccharides and oligosaccharides, whereas the insoluble fraction may include starch and dietary fiber and other polysaccharides. These carbohydrate components differ in their functionality and impact on human health. Individual pulse seeds may differ in their total carbohydrate content and carbohydrate composition, which may lead to their different nutritional values and food utilizations.

2. Insoluble Carbohydrate

a. Starch

Starch is the primary component of legume carbohydrates. Legume seeds vary greatly in their starch content and composition. As shown in Table 1.13, California small white beans contain 57.8% of starch, while soybeans contain as little as 0.2% of starch. In general, soybean, lupine, and winged beans have a lower starch level. The legume seed starches may have a high amylose concentration and differ significantly in their amylose and amylopectin ratios. The amylose content of legumes varies from 5.3% in smooth peas to 43.9% in black gram, as shown in Table 1.13 (Reddy *et al.*, 1984b).

The gelatinization temperature of various legume starches generally ranges from 60°C to 90°C (Table 1.13), which is comparable to the gelatinization temperature of corn starch and higher than that of waxy maize starch. Gelatinization temperature is determined by the structure and composition of the starch: a substantial amount of amylopectin promotes the gelatinization process while the degree of amylopectin branching in the starch varies the gelatinization temperature. Other factors that may alter the gelatinization temperature include the presence of bound lipids, protein and phosphate, and starch granule size.

It has recently been realized that starch may not be completely hydrolyzed and absorbed after digestion, although legume starch may contribute significantly to total energy intake. The starch components that are not hydrolyzed in the human gastrointestinal tract are classified as resistant starch (RS). Resistant starch may have an improved glycemic index (GI), an indicator of the effect of carbohydrates on blood glucose level. Many factors may alter starch digestibility and the formation of RS. These may include the inherent properties of starch such as granular structure and ratio of amylose and amylopectin, presence or treatment of heat, moisture content, interaction of starch with other chemicals, and storage and processing conditions (Sajilata *et al.*, 2006; Siddhuraju and Becker, 2005; Bravo *et al.*, 1998; Tovar and Melito, 1996). For instance, thermal treatment decreased RS concentration from 2.4% to 1.9% in the field pea, from 3.3% to 2.5% in lentil beans, and from 3.4% to 2.3% in chickpea (Rochfort and Panozzo, 2007). In contrast, steam-heating increased RS concentration from 1.9% to 6.0% in black beans, and from 0.8% to 4.0% in lima beans, whereas dry pressure cooking increased RS from 0.8% to 2.1% in lima beans, but had no effect on that in black beans, suggesting the effect of inherent properties and treatment of heat and moisture on the formation of RS (Tovar and Melito, 1996).

The effect of gelatinization on digestibility was reported by Sandhu and Lim (2007), who compared the pasting temperature of several legumes: mung bean, chickpea, field pea, lentil, black gram, and pigeon pea. Mung bean yielded the lowest pasting temperature at 50.2°C, compared to 51.4°C for chickpea, suggesting higher digestibility. Sandhu and Lim confirmed mung bean's high digestibility, as the level of RS was low at 50.3%. In comparison, pigeon pea starch, with 78.9% RS, has a low digestibility and GI. As Sandhu and Lim concluded, mung bean with high digestibility is suitable for malnourished patients, while pigeon pea is more preferable for diabetic patients.

Soaking and autoclaving also altered the digestibility of starch in mucuna beans, which has 28% starch composition (Siddhuraju and Becker, 2005). After soaking in water, the percentage of digestible starch increased from 67.4% to 87.2%. This was accompanied by a significant decrease in the amount of RS from 88.3 g/kg in raw seeds to 48.1 g/kg after soaking. Earlier work by Chau and Cheung (1997) found that soaking two Chinese legume seeds increased starch digestibility by 36.4−98.2%, while heat treatments increased starch digestibility by 6−7-fold, and germination increased starch digestibility by 1−2-fold. The content of RS in raw and cooked legume seeds is listed in Table 1.14.

b. Dietary Fiber

Legume seeds are excellent sources of dietary fibers. Their total fiber concentration ranges from about 1.2% (w/w) in black gram, Bengal gram, red gram, and mung beans, to 25.6% (w/w) in Bengal gram beans (Table 1.15). Total fiber concentration and composition may vary greatly in the same type of legume bean. For instance, total fiber concentration varied from 1.2% to 25.6% and cellulose content ranged from 1.1% to 13.7% in Bengal gram beans

TABLE 1.14 Resistant Starch (RS) Content in Raw and Cooked Legume Seeds

Legume	Raw (% RS)	Cooked (% RS)
Field pea[a]	2.4	1.9
Lentil[a,b]	3.3–6.53	2.5
Chickpea[a,b]	3.4–5.7	2.3
Black bean[c]	1.9	6.0
Red bean[c]	0.8	NA
Lima bean[c]	2.0	4.0
Kidney bean[b]	4.6–6.6	NA
Soybean[b]	0.2–0.3	NA
Soybean faba bean[b]	0.3–5.6	NA
Pea – smooth[b]	5.6–7.1	NA
Pea – wrinkled[b]	9.6–10.3	NA
Wheat bran	0.4	NA

NA: not available
[a]*Data from Rochfort and Panozzo (2007)*
[b]*Mikulikova* et al. *(2008)*
[c]*Tovar and Melito (1996)*

TABLE 1.15 Fiber Compositions of Food Legumes

Legume	Total Fiber	Cellulose	Lignin	Hemicellulose
Winged bean seed	3.4–12.5	–	0.7–1.0	1.36
Smooth pea	4.6–7.0	0.9–4.9	0.5–0.9	1.0–5.1
Wrinkled pea	7.6	1.2–4.2	0.3–1.0	0.9–6.6
Great bean	4.5–6.7	–	–	–
Red kidney bean	3.7	2.5–5.9	2.7–3.1	0.3
Navy bean	3.4–6.6	3.2	0.1	0.5–4.9
Pinto bean	4.3–7.2	9.0	1.8–3.0	4.0
Pink bean	–	6.0	0.2	–
Black eye bean	3.1	4.9	0.1	–
Black gram	1.2–19.5	5.0	3.8	10.7
Bengal gram	1.2–25.6	1.1–13.7	2.9–7.1	0.6–9.1
Mung bean	1.2–12.8	2.5–4.6	2.2–7.2	0.3–9.1
Red gram	1.2–20.3	7.3	2.9	10.1
Soybean	2.4–5.5	–	–	7.6
Broad bean	8.0	1.0	0.7–1.1	4.0–4.6
Lentil	2.6	4.1	2.6	6.0
Cowpea	1.7–4.0	–	0.6–1.8	–
Lupine seed	3.0	–	0.7–0.8	9.3–9.9

Values are reported in g/100 g on a dry weight basis.
Data from Reddy *et al.* (1984b).

(Salunkhe *et al.*, 1985). Legume bean fiber may contain significant levels of cellulose, hemicellulose, and lignins, along with minor amounts of pectic substance, arabinogalactan, and xyloglucan (Sathe *et al.*, 1984).

Growing evidence indicates the potential health beneficial effects of legume bean fiber and other dietary fibers. These beneficial effects may include, but are not limited to, an increase in fecal bulk and fecal moisture, reduction of plasma cholesterol level, improved GI, and reduced risk of colon cancer (Nwokolo, 1996). Dietary fiber molecules cannot be digested by the human small intestine but are fermented by the microorganisms in the colon to short-chain fatty acids. These reduce local pH, enhance intestinal content passage, and lead to enhanced elimination of bile acids. The reduced risk of colon cancer may be attributed to the production of short-chain fatty acids. Dietary fiber may absorb and trap bile acids and enhance their elimination in feces. This effect may stimulate the conversion of cholesterol to bile acids in liver and reduce the plasma cholesterol level.

3. Soluble Carbohydrate

Legume seeds contain significant levels of water soluble carbohydrates, including trace amounts of monosaccharides, such as glucose and arabinose in soybeans, and measurable concentrations of disaccharides and oligosaccharides, often including sucrose, raffinose, stachyose, verbascose, and ajugose (Sathe *et al.*, 1984; Nwokolo, 1996). Food legume seeds differ significantly in their total soluble sugar content and composition (Table 1.16). Wrinkled peas may have about 10.2−15.1% total soluble sugar, whereas soybean may contain 5% total soluble sugar (Reddy *et al.*, 1984b). The individual oligosaccharides are not evenly distributed in the different fractions of legume seeds (Vaidehi and Kadam, 1989), which may demand consideration when producing or using legume-based food ingredients. As

TABLE 1.16 Soluble Carbohydrate Compositions of Food Legumes

Legume	Sucrose	Raffinose	Stachyose	Verbascose	Ajugose	Total Soluble
Winged bean seed	0.3−8.2	0.2−2.0	0.1−3.6	0.04−0.9	–	3.4
Smooth pea	2.3−2.4	0.3−0.9	2.2−2.9	1.7−2.3	0.06	5.3−8.7
Wrinkled pea	2.3−4.2	1.2−1.6	2.9−5.5	2.2−4.2	0.13	10.2−15.1
Great bean	2.0−3.8	0.3−0.7	2.3−3.8	–	–	9.9
California small white bean	3.0	0.3−0.7	2.9−3.7	0.1	–	7.7
Red kidney bean	1.6	0.3−0.9	2.4−4.0	0.1−0.5	–	8.0
Navy bean	2.2−3.5	0.4−0.7	2.6−3.5	0.1−0.4	–	5.6−6.2
Pinto bean	2.	0.4−0.6	2.9−3.0	0.1−0.2	–	6.7
Pink bean	1.4	0.2−0.4	0.2−0.4	–	–	–
Black eye bean	2.6	0.4−1.0	0.4−0.9	–	–	–
Black gram	0.7−1.5	0−1.3	0.9−3.0	3.4−3.5	–	3.0−7.1
Bengal gram	0.7−2.9	0.7−2.4	2.1−2.6	0.4−4.5	–	3.5−9.0
Mung bean	0.3−2.0	0.3−2.6	1.2−2.8	1.7−3.8	–	3.9−7.2
Red gram	2.7	1.0−1.1	2.7−3.0	4.0−4.1	–	3.5−10.2
Soybean	–	0.7−1.3	2.2−4.2	0−0.3	–	5.3
Broad bean	1.4−2.7	0.1−0.5	0.5−2.4	1.6−2.1	–	3.1−7.1
Lentil	1.8−2.5	0.4−1.0	1.9−2.7	1.0−3.1	–	4.2−6.1
Cowpea	1.8−3.1	0.4−1.2	2.0−3.6	0.6−3.1	–	6.0−13.0
Lupine seed	1.0−2.6	0.5−1.1	0.9−7.1	0.6−3.4	0.3−2.0	7.4−9.5

Values are reported in g/100 g on a dry weight basis.
Data from Reddy *et al.* (1984b); Kamath and Belavady (1980).

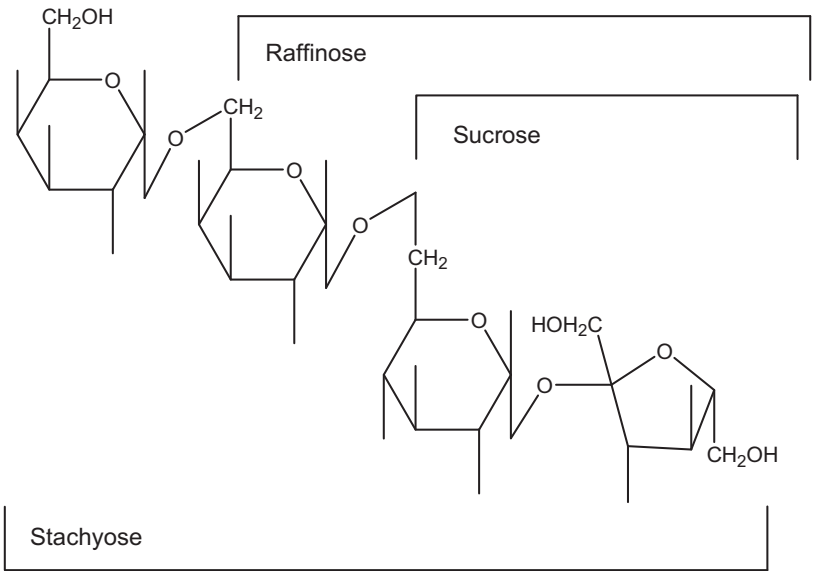


FIGURE 1.18 Structure of legume soluble carbohydrates stachyose, raffinose, and sucrose.

shown in Figure 1.18, these oligosaccharides are non-reducing sugars derived from sucrose by adding one or more galactose units via α (1→6) linkages to the glucose moiety of the sucrose unit. They are commonly known as the raffinose family or galacto-oligosaccharides. Raffinose oligosaccharides are not digested in the human small intestine owing to the absence of α-1,6-galactosidase in human intestinal mucosa, although sucrose is hydrolyzed and absorbed.

The raffinose family of oligosaccharides has been associated with flatulence and abdominal discomfort after legume consumption. These oligosaccharides tend to draw fluid into the lumen by osmosis, and lead to possible abdominal distension, cramps, and diarrhea. In the large intestine, these oligosaccharides may be broken down to monosaccharides by enzymes produced by local microorganisms. This increases the local osmolality and leads to greater water retention. The monosaccharides may also be further utilized by the microorganisms, which may produce significant amounts of gas and small molecular weight acids. The acids lower local pH, which may irritate the lining of the colon, and increase the movement of the intestinal contents. Both the fluid retention and increased movement of intestinal contents may cause diarrhea. The gas products, including carbon dioxide, hydrogen, and methane, may lead to bloating and cause problems in individuals with colonic pathologies, including irritable bowel syndrome.

The level of oligosaccharides in food may be reduced by improving food processing conditions, such as soaking the seeds or meal in water (Reddy *et al.*, 1984b; Vaidehi and Kadam, 1989; Martin-Carrejas *et al.*, 2006). Breeding efforts and genetic modification have been explored to reduce these oligosaccharides in legumes. In addition, seed germination, fermentation and α-galactosidase treatment, and irradiation are possible approaches to eliminate raffinose family oligosaccharides from legumes (Reddy *et al.*, 1984b; Rochfort and Panozzo, 2007). Completely removing the raffinose family of oligosaccharides may not eliminate flatulence from legume consumption, as dietary fiber components also contribute to flatulence. Interestingly, further studies have suggested that these oligosaccharides may provide potential health beneficial effects, such as immunomodulation and altering digestive passage speed (Parsons *et al.*, 2000; Rochfort and Panozzo, 2007).

4. Conclusion

Carbohydrates, one of the major constituents of legume seeds, may serve as an excellent source of dietary fiber and provide health benefits to consumers. Individual fractions of legume carbohydrate may contribute differently in human nutrition and food safety, and food functionality. A number of postharvest treatments, including those involved in food preparation, may affect the digestibility of legume carbohydrate and alter their nutritional values and beneficial properties, as well as undesirable properties such as flatulence production.

D. Lipids

Plant seeds store lipids in small spherical organelles with diameters ranging from 0.5 to 2.5 μm, called oil bodies (Tzen and Huang, 1992). Theses organelles, also called spherosomes, are located in the cotyledons and contain mostly triglycerides. Lipid synthesis in soybean seeds is controlled through regulation of the levels of fatty acid biosynthetic proteins, the presence of which depends on the developmental stage of the seed (Ohlrogge and Kuo, 1984).

Legume seed oils are generally good sources of polyunsaturated fatty acids, especially the essential fatty acids omega-6 linoleic acid and omega-3 linolenic acid. Soybean has by far the highest amount of linoleic acid, and good amounts of oleic and palmitic acids (Table 1.17). Peanut (*Arachis hypogaea*) has the highest monounsaturated fatty acid content on a per-weight basis, almost entirely composed of oleic acid.

Phytosterols are gaining prominence as cholesterol-lowering nutraceutical agents. They are structurally similar to cholesterol, and are found in the cell membranes of plant cells as rigidifying components. From Table 1.17, peanut has the highest amount of phytosterols per seed weight, followed by soybeans, peas, and broadbeans.

Phospholipids are another class of lipids found in legume seeds. They are a large family of polar lipids, the most prominent among which is lecithin from soybeans. Soy lecithin has many health benefits and has been utilized in many functional foods (Wang *et al.*, 2006). The major phospholipids in broad beans include phosphatidylcholine, phosphatidylehtanolamine, and phosphatidylinositol (Yoshida *et al.*, 2009). Legume seed phospholipid content diminishes as the seed matures owing to a decrease in the membrane component proportions in the developing seed (Wang *et al.*, 2006).

E. Other Components of Interest

1. Enzyme Inhibitors

Legume seeds contain low-molecular-weight proteins (Clemente and Domoney, 2006) that inhibit the activity of hydrolases such as proteases, amylases, and lipases (Lajolo and Genovese, 2002). Of these, protease inhibitors are the most important and extensively studied.

Soybean protease inhibitors are classified into two families, Bowman−Birk and Kunitz, which differ in their structure, weight, and activity. Bowman−Birk inhibitors have a molecular mass of between 6 and 10 kDa, with seven disulfide bridges, and can act against trypsin and chymotrypsin simultaneously at independent binding sites (Lajolo and Genovese, 2002; Becker-Ritt *et al.*, 2004). They are made up of two discrete polypeptide binding loops that hold a binding site each, making it possible for them to inhibit two molecules of enzymes at the same time (Clemente and Domoney, 2006). The Kunitz inhibitors are smaller, with only one polypeptide chain of molecular mass about 2 kDa (Becker-Ritt *et al.*, 2004). They have two disulfide bridges and act specifically against trypsin (Lajolo and Genovese, 2002).

TABLE 1.17 Fatty Acid and Phytosterol Composition of Some Major Food Legumes

	Fatty Acids of Interest (g)							
Legume	**16:0**	**18:1**	**18:2**	**18:3**	**Saturated**	**Monounsaturated**	**Polyunsaturated**	**Phytosterol (mg)**
Glycine max	2.116	4.348	9.925	1.330	2.884	4.404	11.255	161
Cicer arietinum	0.501	1.346	2.593	0.101	0.626	1.358	2.694	35
Arachis hypogaea	5.154	23.756	15.555	0.003	6.834	24.429	15.559	220
Pisum sativum	0.125	0.232	0.411	0.084	0.161	0.242	0.495	135
Vicia faba	0.204	0.297	0.581	0.046	0.254	0.303	0.627	124
Lens culinaris	0.133	0.180	0.404	0.109	0.156	0.189	0.516	–
Phaseolus vulgaris	0.343	0.123	0.332	0.278	0.366	0.123	0.610	–

Amino acid values are per 100 g edible portion.
Data from USDA (2008).

The effect of these protease inhibitors on digestion and metabolism is well documented. In a study by Grant *et al.* (1995), the contents and effects of long-term dietary exposure to protease inhibitors and lectins in four major food legumes (soybean, cowpea, kidney bean, and lupin) in rats were investigated. In terms of content, soybean had the highest amount of 24.6 g trypsin inhibited/kg and 12.0 g chymotrypsin inhibited/kg, while lupin had the lowest of 1.1 g trypsin inhibited/kg and 1.4 g chymotrypsin inhibited/kg. Consumption of the soybean diet, which was high in protease inhibitors and low in lectins, over a 700-day period, resulted in extensive enlargement of the pancreas in the rats, with some exhibiting macroscopic nodules on the pancreas. In an earlier study by Jaffe and Lette (1968), growth was severely hampered in rats fed beans (*P. vulgaris*) low in hemagglutinin and high in trypsin inhibitor activity.

It has been shown that aqueous heat treatment of soybean at 80°C for 40 minutes significantly reduced protease inhibitor activity and improved growth in rats (Armour *et al.*, 1998). Not withstanding the established anti-nutritive activities of legume protease inhibitors, recent studies suggest possible health beneficial applications for these seed components. Soybean protease inhibitors, especially the Bowman−Birk family, have been shown to possess anticarcinogenic properties (see review by Kennedy, 1998). A study by Lin and Ng (2008) revealed a dimeric Kunitz-type trypsin inhibitor in black soybean (*Glycine max* cv. 'Dull Black') that stimulates nitric oxide production by macrophages and inhibits human immunodeficiency virus-1 (HIV-1) reverse transcriptase. It also showed promise as an inhibitor of cell proliferation in liver and breast cancer cells. More research is needed to confirm and elucidate the mechanisms behind these novel biological activities of legume protease inhibitors.

α-Amylase inhibitors are characterized as oligomeric proteins made up of glycopeptide subunits (Berre-Anton *et al.*, 1997). They are synthesized on the endoplasmic reticulum as preproteins, glycosylated, and transported to the protein storage vacuoles (Pueyo *et al.*, 1993). Two isoforms, α-AI1 and α-AI1′, have been isolated from kidney beans (*P. vulgaris*), with both showing inhibition of mammalian α-amylase by a mixed non-competitive inhibition mechanism (Berre-Anton *et al.*, 1997). The same study found that the α-AI1 form has a low optimum activity pH of 4.5 at 30°C, suggesting that this enzyme may exhibit specificity for pancreatic α-amylase, whose optimum activity pH falls within a similar range. In the common bean, molecular level control of α-amylase inhibitors appears to be exerted by a gene that closely resembles the one responsible for coding lectins in both structure and amino acid composition (Moreno and Chrispeels, 1989). Inhibition of starch digestion by α-amylase inhibitor has been shown to decrease dietary protein and fat utilization in experimental rats, resulting in retarded growth (Pusztai *et al.*, 1995).

2. Lectins

Legume lectins are a group of homologous glycoproteins found mostly in the seeds (Loris *et al.*, 1998). The protein's affinity for sugar moieties is specific and reversible (Lajolo and Genovese, 2002; Hamelryck *et al.*, 1996), and this specificity has been used to explain their role in rhizobia−host plant recognition during the initiation of nitrogen fixation (Bohlool and Schmidt, 1974). In the experiment by Bohlool and Schmidt (1974), soybean lectin, labeled with fluorescein isothiocyanate, selectively bound to only soybean nodulating strains of *Rhizobium japonicum*. They concluded that the soybean lectins must be interacting with specific polysaccharides on the surface of the appropriate *Rhizobium* cell, leading to initiation of nodulation. Legume lectins show considerable similarity in their primary, secondary, and tertiary structures, but small variations in amino acid sequence result in vast differences in quaternary structure (Srinivas *et al.*, 2001) and carbohydrate specificities (Loris *et al.*, 1998).

Lectins are agglutinating by binding to sugar moieties in cell membranes, disrupting membrane structure. This activity, when it takes place in the gut, interferes with digestion and absorption, making lectins anti-nutritional factors. Lajolo and Genovese (2002) reported that purified soybean lectins impaired growth, induced small intestine enlargement and damage, and stimulated hypertrophy and hyperplasia of the pancreas in experimental rats. Phytohemagglutinin, the seed lectin from *P. vulgaris*, is made up of two polypeptide subunits (E and L), which have been found to be erythroagglutinating and leucoagglutinating, respectively (Hamelryck *et al.*, 1996). Legume lectins are usually inactivated by thermal processing, as cooking at atmospheric pressure for 15 minutes was found to be adequate to curtail their anti-nutritive effects.

Despite their well-known anti-nutritive properties, research is beginning to show possible health beneficial effects of some legume lectins. Raw kidney beans high in lectins were found to reduce lipid accumulation in obese rats (Pusztai *et al.*, 1998). From that study, Pusztai and co-workers concluded that it may be possible to develop dietary adjunct or therapeutic agent from the bean lectin to stimulate gut function and reduce obesity.

3. Lipoxygenase

Lipoxygenase is a non-heme, iron-bearing, monomeric enzyme that catalyzes the dioxygenation of fatty acids containing (1Z,4Z)-pentadiene systems (Schilstra *et al.*, 1994). In legumes, lipoxygenases catalyze the hydroperoxidation of lipids, leading to the development of off-flavors. This usually takes place in the seeds postharvest. The physiological role of lipoxygenases in the plant is still not clear, but they have been found to be capable of oxidizing plant pigments (chlorophyll and carotenoids) and cholesterol (Sessa, 1979). It appears that the activity of the enzyme is influenced by the presence of its hydroperoxide products (Smith and Lands, 1972). Enzyme activity also increases during processing when the cell walls are ruptured and cellular control mechanisms are no longer effective (Sessa, 1979). Off-flavor development depends on the fatty acid composition, and results from the formation of volatile short-chain aldehydes, ketones, and alcohols (Kobayashi *et al.*, 1995; Yuan and Chang, 2007).

IV. EFFECTS OF GERMINATION

Germination has long been seen as a possible method of enhancing the nutritive value and esthetic qualities of legume seeds (Chen *et al.*, 1975). The uses of germinated seeds vary: they can be dried and ground into flour for uses similar to those of ungerminated seed flours. The fresh germinated seed is eaten as a vegetable, particularly in Eastern cultures, while a growing awareness has made these 'sprouts' a more common diet component in Western culture as well. Conversely, germination can also be a hindrance to legume purveyors. Whereas the shelf-life of dried, mature seeds is lengthy under proper storage conditions, fresh sprouts are relatively perishable, requiring stricter temperature and humidity control. Changes to the seed composition during germination are discussed here, but it should be noted that comparison of germination studies is difficult owing to protocol variations in relative humidity, temperature, seed age, seed species, and time of germination, among others. In addition, publications analyzing germinated seeds that do not provide results on a dry weight basis make it difficult to draw conclusions: the increased water weight of the seedling inherently gives the appearance of falling levels of the macronutrients and kilocalories on a per-weight basis. The body of research has become too complex to thoroughly discuss each of these variables here. Thus, an overall discussion of the major trends of germination is presented. Please refer to the literature for specific details.

A. Carbohydrates

Of particular interest when discussing legume carbohydrates in germination are raffinose and stachyose, two α-galactosides that are major offenders in the production of flatulence. Aman (1979) notes that these molecules generally decrease during the early days of germination, coinciding with an increase in fructose (Viana *et al.*, 2005). Other research supports this assertion in various legumes, including most notably soybeans, black beans, and chickpeas [El-Adawy, 2002 (chickpeas); Martin-Cabrejas *et al.*, 2008 (soybeans); Donangelo *et al.*, 1995]. Donangelo *et al.* (1995) suggests that α-galactosidase activity during germination may be responsible for such changes. Viana *et al.* (2005) confirmed this assertion both by chemical substrate reactions and by partially purifying the enzyme. The carbohydrate composition of germinating soybean seeds is shown in Figure 1.19.

The magnitude of the decrease in α-galactosides varies across species of legumes, as do the amount and type of fiber present in the seeds (Donangelo *et al.*, 1995; Vanderstoep, 1981; Martin-Cabrejas *et al.*, 2008). Of these works, Martin-Cabrejas *et al.*, (2008) and one other (Bau *et al.*, 1997) report a decrease in total dietary fiber in soybean germination, whereas Donangelo *et al.*, (1995) report a slight increase. Aman (1979) found no difference in mung beans and a small decrease in fiber in chickpeas, while El-Adawy (2002) reported a small increase in fiber when chickpeas germinated. Nonetheless, differences in fiber content of chickpeas are in the range of $\pm 2\%$ weight, indicating little dietary significance. Germinated soybeans, however, lost fiber equivalent to 10% of the dry matter in 24 hours (Martin-Cabrejas *et al.*, 2008).

B. Lipids

It is well established that lipids decrease in legume seeds during germination, on a dry weight basis [Bau *et al.*, 1997 (triglycerides); Chen *et al.*, 1975 (triglycerides); El-Adawy, 2002 (fat); Mostafa and Rahma, 1987 (oil content)], while the extent of the decrease varied, the apparent decrease may be due to an increase in other dry matter in the

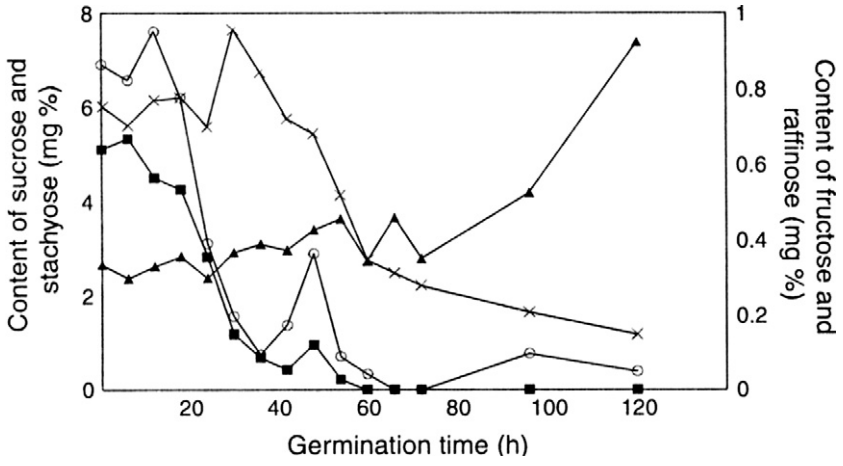


FIGURE 1.19 Contents (mg %) of raffinose (○), stachyose (■), fructose (▲), and sucrose (×) in germinating soybean seeds. *(From Viana* et al.*, 2005. © Elsevier.)*

seedling (Liu, 1997). It appears that the degradation of lipids, in addition to carbohydrates, is used to fuel the growth of the seedling and the accompanying processes (Bau *et al.*, 1997).

C. Proteins

Many studies have shown an increase in total protein contents in various legumes by dry weight after germination (El-Adawy, 2002; Bates *et al.*, 1977; Kakade and Evans, 1966; Martin-Cabrejas *et al.*, 2008; Palmer *et al.*, 1973). This may be attributable to (1) the use of carbohydrates and lipids as energy sources for the germinating seeds, thus allowing for protein to contribute a greater proportion of the remaining weight, and (2) the production of enzymes. Fewer studies have shown only slight increases in protein or no increase during germination (Ahmad and Pathak, 2000).

Several groups have studied the impact of germination on the composition of free amino acids, including non-protein amino acids. Kuo *et al.* (2004) reported that germination affected the free protein and non-protein amino acids of beans, peas, and lentils differently, with no specific trend across all three legumes. They also noted that light during germination affected the amino acid contents of the species differently. Rodriguez *et al.* (2008) and Urbano *et al.* (2005) both showed a decrease in protein nitrogen as germination progressed in beans, peas, and lentils, coinciding with a similar magnitude increase in non-protein nitrogen. Changes in individual amino acids were again species specific. A more complete discussion of amino acids and protein turnover in soybeans specifically can be found in Bau *et al.* (1997).

D. Vitamins and Minerals

While there is much variation in legume seed compositional changes during germination, the least disputed topic may be that of vitamin C. The contents of vitamin C, or ascorbic acid, increased during germination in soybeans (Ahmad and Pathak, 2000; Bau *et al.*, 1997) and various peas and beans (Sangronis and Machado, 2007; Chen *et al.*, 1975; Vanderstoep, 1981; Khattak *et al.*, 2007; Fernandez-Orozco, 2008). The respiration process is said to be triggered by ascorbic acid (Sangronis and Machado, 2007).

The B vitamins are next, although there is a less clear trend. Riboflavin has been shown to increase in soybeans under some conditions (Ahmad and Pathak, 2000; Bau *et al.*, 1995), as well as in chickpeas (El-Adawy, 2002) and peas (Urbano, 2005). Minor increases in thiamine have also been shown in germinated soybeans and white, black, and pigeon beans (Ahmad and Pathak, 2000; Sangronis and Machado, 2007), while a decrease (El-Adawy, 2002; Urbano *et al.*, 2005) or no change (Vanderstoep, 1981) was reported in chickpeas and peas. Results on niacin are similarly mixed and more limited (Bau *et al.*, 1997; El-Adawy, 2002).

The data on Vitamins E and A are limited (Bau *et al.*, 1997). Chen *et al.* (1975) reported low values in peas and beans, although their values for germinated seeds increased over their dry seeds. Fernandez-Orozco *et al.* (2008) showed differences in tocopherol content of two varieties of soybeans, although both showed an overall

increase in all four tocopherol isomers and vitamin E activity after germination. Mung bean seeds, in contrast, showed an overall decrease in tocopherol contents and vitamin E activity, despite a slight increase in the α-tocopherol isomer.

The effect of germination on minerals is also varied in the literature. Calcium showed increases in soybeans (Bau *et al.*, 1997; Donangelo *et al.*, 1995) and black, white, and pigeon peas (Sangronis and Machado, 2007). Iron content appeared to decrease across various legumes (Chen *et al.*, 1975; Donangelo *et al.*, 1995; Sangronis and Machado, 2007; Vanderstoep, 1981). Although iron content drops, the increased vitamin C in germinated seeds may make it more bioavailable. Other minerals do not appear to have as clear trends in germination or as much evidence. Worthy of note, it has been suggested that the mineral content of the water used for germination has the potential to significantly affect the mineral content of the germinated legumes.

E. Anti-Nutritional Factors

A reduction in anti-nutritional factors of legumes has been one of the driving forces behind legume germination research, as destruction of these compounds without the use of heat processing may afford an easier solution to their removal. Anti-nutritional factors were discussed earlier. The majority of research has been focused on the effect of germination on trypsin inhibitors and phytate.

1. Trypsin Inhibitor Activity

Trypsin inhibitors prevent the action of trypsin in the gut, which breaks down proteins into absorbable amino acids and small peptides. Thus, a reduction in trypsin inhibitors would theoretically improve protein absorption. There are many reports of a reduction in trypsin inhibitor activity (TIA) during germination, including peas (Urbano *et al.*, 2005), various beans and soybean (Sangronis and Machado, 2007; Donangelo *et al.*, 1995; Mostafa and Rahma, 1987), and chickpeas (El-Adawy, 2002). However, some researchers have reported little or no change in TIA (Vanderstoep, 1981; Kakade and Evans, 1966) while others found an increase in TIA (Palmer *et al.*, 1973). Kakade and Evans (1966) showed that soaking the seeds for longer periods decreased TIA, which may help to explain differences among studies if the germination protocols were not standardized with respect to their soaking/rinsing methods.

Despite accurate measurements of TIA, the ultimate measure of interest is still the utilization of protein by live animals, for which feeding studies and protein efficiency ratio (PER) are often the instruments of choice. Germination for 2 and 4 days with and without light showed increased PER values for peas, but decreased after 6 days (Urbano *et al.*, 2005). A rat feeding study showed improved nutritive value of kidney beans after germination, despite increases in TIA mentioned above (Palmer *et al.*, 1973). *In vitro* protein digestibility tests have also indicated better digestibility in chickpeas (El-Adaway, 2002), various beans (Sangronis and Machado, 2007), and soybeans (Mostafa and Rahma, 1987).

2. Phytic Acid

Phytates, although possessing antioxidant capacity, are still often viewed as anti-nutritional factors owing to their ability to bind and prevent absorption of minerals. Therefore, decreases in phytate, or phytic acid, are considered a desirable outcome of processing. Khattak *et al.* (2007) reported that germination of chickpeas under blue light produced the greatest decrease in phytic acid, while Urbano *et al.* (2005) showed a similar decrease in phytates in peas regardless of the presence of light during germination. Decreases were also seen in various beans (Sangronis and Machado, 2007) and chickpeas (El-Adawy, 1997), whereas no decrease in phytic acid activity was seen in soybeans and black beans after 2 days' germination (Donangelo *et al.*, 1995). The breakdown of phytates during germination is thought to occur because of a surge in phytase activity (Bau *et al.*, 1997).

F. Nutraceutical Components

Research on the antioxidant components in germinated legumes is recent and growing. Isoflavones in soy are of particular interest because of the connection between their consumption and decreased incidence of chronic diseases. Therefore, it was questioned whether germination would positively or negatively affect the contents and composition of isoflavones in soy seeds. Zhu *et al.* (2005) studied the isoflavone contents of two soybean varieties grown to

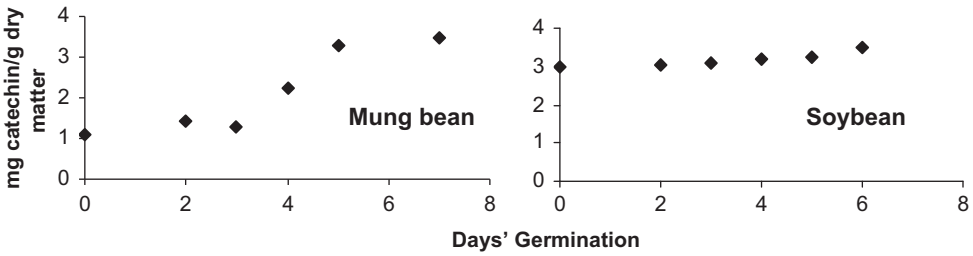


FIGURE 1.20 Total phenolic contents of germinated soybean and mung bean seeds. *(Adapted from Fernandez-Orozco* et al.*, 2008.)*

specific hypocotyl lengths. Although there were differences between the two varieties, they concluded that total isoflavone concentration in mg/g dry ground seed increased at 1 day of germination, then decreased until the end of the study. However, this increase and decrease coincided with an increase and decrease in the malonyl derivatives of isoflavones, which have a significantly higher molecular weight than the aglycone forms, though similar antioxidant activity on a molar basis. For this reason, it is difficult to interpret the antioxidant effect of these changes and is recommended that isoflavone values be reported on a molar basis.

Fernando-Orozco *et al.*, (2008) studied both the phenolic contents and various measures of antioxidant capacity. Soybean seeds and mung beans both increased in total phenolic contents during several days' germination time. The increase in phenolics was accompanied by increases in antioxidant capacity of the germinated seeds, as increases were also seen in Trolox equivalent antioxidant capacity, peroxyl radical-trapping capacity, and inhibition of lipid peroxidation. Total phenolic content results can be seen in Figure 1.20.

G. Esthetic Food Quality

Germinated legume seeds are commonly thought to have improved organoleptic qualities. Ahmad and Pathak (2000) showed a decrease in 'beany flavor' and overall improved sensory scores after 3 days' germination of soybeans. Germination also enhanced the sensory scores of a soy-breadfruit product in Nigeria (Ariahu *et al.*, 1999). Chen *et al.*, (1975) investigated the acceptability of bean and pea sprouts, although not specifically in comparison to their dry seeds, and all varieties were found to be acceptable to the sensory panel.

V. EFFECTS OF FERMENTATION

Fermentation of legumes has been used by humans for millennia as a method to preserve food, introduce variation into the diet, and decrease cooking times. The detailed history of fermentation is well covered elsewhere (Hesseltine, 1965; Deshpande *et al.*, 2000). In particular, the FAO Agricultural Services Bulletin 142 from the year 2000 is an excellent resource on both the scientific and societal rationale for fermentation (Deshpande *et al.*, 2000). It addresses major crop legumes in addition to some that are more locally produced, including the biochemical changes that occur in fermentation using different methods and a variety of microbial cultures. Thus, only a short review of fermentation is given here.

The function of fermentation depends somewhat on the locale where it is being performed. In remote or developing areas, where refrigeration is not readily available, fermentation mainly serves as a food preservation technique through the generation of products such as alcohol and lactic and acetic acids. Food preservation is less of a concern in modern countries, and the reasons for choosing to ferment a food shift towards achieving desirable flavor and consistency, among other esthetic qualities. Fermentation also reduces cooking time in legumes by enzymatically breaking down structural components, so products require less energy input to be cooked, a desirable outcome in both developing and modern parts of the world.

The above benefits are seemingly obvious to the cook and perhaps explain why early humans chose to ferment foods, but there are other functions of fermenting foods that have only become apparent through careful observation and science. The anti-nutrients in legumes discussed previously, including trypsin inhibitors, phytates, and gas-producing oligosaccharides, are reduced, often to insignificant levels. This reduction is seen both because of enzymatic action, but also because soaking and boiling (often steps in the fermentation process) leach or destroy these components. Partly because of this reduction of anti-nutrients and partly because the microbial enzymes

'predigest' carbohydrates, proteins and triglycerides, nutrient availability in the products increases. The presence of the microbes may also add protein, amino acids, and/or vitamins to the fermented food.

Concern over aflatoxins arises when discussing legumes and fungi, but the FAO reports that they have generally not been of large concern in fermentation products, particularly in modern processing, but also largely in traditional processing (Deshpande *et al.*, 2000).

Since the FAO report, several articles have been published on the effect of fermentation on the isoflavone content and antioxidant properties of legumes. Fermentation of soybean with *Rhizopus oligosporus* showed increases in total phenolic contents and scavenging activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (McCue and Shetty, 2003). Fermentation of black bean with various filamentous fungi showed similar increases in total phenolic contents (Lee *et al.*, 2008). Pyo *et al.*, (2005) showed an increase in antioxidant activity against both 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and DPPH radicals after fermentation, and also the bioconversion of the glycoside forms of isoflavones to their aglycone form. Other groups have also shown similar results: the sugar side-chains are cleaved off the glycoside isoflavones, leaving behind aglycone forms in higher amounts (Ikeda *et al.*, 1995; Chun *et al.*, 2007).

VI. STORAGE

The storage of legumes has received considerable attention, particularly because of the potential for immense loss of food through biological aging in addition to microbial, insect, and rodent infestation. Physical factors such as moisture, temperature, and oxygen concentration affect the rates of deterioration and infestation. The amount of water in the seeds and the environment seems to play the dominant role in determining the rate of seed deterioration during storage (Liu, 1997). An older, but still relevant, discussion of the topic can be found in Salunkhe, Kadam, and Chavan's *Postharvest Biotechnology of Food Legumes* (1983). A more recent discussion of soybean seed aging during storage can be found in Liu's *Soybeans: Chemistry, Technology, and Utilization* (1997).

A. Respiration, Moisture, and Temperature

Respiration plays a major role in storage stability of legumes, as discussed previously for cereals. The rate of respiration is higher in oilseeds than in cereals (Salunkhe *et al.*, 1985). In addition, seeds of higher moisture content have a higher rate of respiration. Respiration of the stored seeds increases moisture, thus encouraging the growth of mold. It is obvious that it becomes critical to dry seeds before storage to prevent the cycle from beginning. Temperature also affects the rate of respiration of legume seeds, halving it for every decrease of 10°C (Salunkhe *et al.*, 1985). For soybeans, a moisture content of 13% or below has been suggested to be adequate for ambient storage in the USA (Liu, 1997), although lower temperature and moisture contents down to 5°C and 11% have been suggested to be 'ideal' (Liu, 1997).

Ambient conditions also need to be considered in storage of legumes. A similar moisture equilibrium exists in legumes, as was discussed previously for cereals. Legume seeds stored at a relative humidity of 70% will equilibrate to about 14% moisture content (Dobie, 1982). *Codex Alimentarius* (1995) standards recommend two levels of seed moisture content for storage based on climate and length of storage. Selected values are shown in Table 1.18. For those seeds stored without a seed coat, the moisture content should be 2% below what is shown.

B. Seed Aging and Food Quality

Of most interest is not only *that* seeds age, but *what* happens in the process and how it affects the seeds' intended uses. A decrease in protein extractability or solubility with increased storage time has been shown in multiple studies (Saio *et al.*, 1980, 1982; Liu *et al.*, 2008; Narayan *et al.*, 1988a). Extractability of the 11S protein decreased more rapidly than that of the 7S (Saio *et al.*, 1982). Decreases were exacerbated by adverse storage conditions of ≥ 30°C, ≥ 80% relative humidity, and/or excessive storage times. Saio and Baba (1980) showed that poor storage of soybeans resulted in deformations of protein bodies and loss of starch granules. Whole beans have been shown to be more resistant to degradation than milled flours (Saio *et al.*, 1982). The color of soybeans changed from an initial creamy yellow to light brown after 9 years of ambient storage (Narayan *et al.*, 1988a).

Degradation of the protein in legumes, particularly soybeans, is of interest because of their food uses. Improperly stored soybeans produce a lower yield of tofu (Narayan *et al.*, 1988b; Hou and Chang, 2004), softer tofu, and fewer

TABLE 1.18 Selected Suggested Moisture Contents of Legume Seeds

	Recommended Moisture Content (%)	
Legume	Tropical Climate, Long term	Moderate Climate, Short Term
Bean	15	19
Lentil	15	16
Chickpea	14	16
Pea	15	18

Data from *Codex Alimentarius* (1995).

solids in soybean milk (Saio *et al.*, 1980). Hou and Chang (2004) suggested that assessing the color change of soybeans in storage may be a rapid method for predicting the quality of tofu made from those soybeans. Organoleptic scores for soymilk, tofu, and soynuts decreased significantly with an increase in storage time of the beans up to 9 years (Narayan *et al.*, 1988b). Improper storage also resulted in decreases in other functional properties, including emulsifying activity, emulsifying stability, thermal stability, and the protein disperse index (Liu *et al.*, 2008).

Other changes have been noted in soybeans during storage, including hydrolysis of neutral fats to free fatty acids (Yanagi *et al.*, 1985); a decrease in free sugar (Hou and Chang, 2004), available lysine, trypsin inhibitor activity, and lipoxygenase activity; and an increase in non-protein nitrogen and peroxide values (Narayan *et al.*, 1988a).

C. Effect on Isoflavones

Because of the ability of isoflavones to mitigate chronic disease, research on the effects of storage has expanded. Several groups have shown that isoflavones in stored soybeans (Hou and Chang, 2002; Lee *et al.*, 2003), soy protein isolate and soy flour (Pinto *et al.*, 2005) saw reductions in the malonylglucoside forms and increases in the glucoside and aglycone forms. Total isoflavones tended to remain at about the same level during study periods of 1 and 3 years (Lee *et al.*, 2003; Pinto *et al.*, 2005). The rate of conversion was enhanced by increased temperature and humidity and decreased to non-significant levels with refrigeration (Hou and Chang, 2002; Pinto *et al.*, 2005).

Further discussion of isoflavones as affected by soybean storage and processing, including a discussion of the kinetics of isoflavone degradation, can be found in a review article by Shimoni (2004).

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