The Chemistry of CEREAL PROTEINS

Second Edition

Radomir Lásztity

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Radomir Lásztity, D.Sc.
Professor
Department of Biochemistry and Food Technology
Technical University
Budapest, Hungary

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The Author

R. Lásztity, D.Sc., is Professor of the Department of Biochemistry and Food Technology at the Technical University, Budapest, Hungary. Dr. Lásztity received his M.Sc. degree in chemical engineering in 1951 and his D.Sc. degree in chemical sciences in 1968 from Technical University.

After receiving his M.Sc., Dr. Lásztity began work in the Department of Food Chemistry (later changed to the Department of Biochemistry and Food Technology) at the Technical University as an assistant. In 1963 he was promoted to Associate Professor and in 1969 to University Professor. He was Head of the Department in the period 1972 to 1993. From 1970 to 1976 he was acting Vice Rector of the University.

Dr. Lásztity's main research activities are chemistry and biochemistry of cereal proteins, rheology of food, and food analysis. The results of his research work have been published in more than 550 scientific papers in Hungarian and foreign scientific journals. He is Chairman of the Food Protein Working Group of the Hungarian Academy of Sciences, Deputy Technical Director and a member of the Executive Committee of the International Association for Cereal Science and Technology (ICC). He was Chairman of the Codex Committee on Methods of Analysis and Sampling of the FAO/WHO Food Standards Program from 1975 to 1988.

Preface

In the last decade or so since the first edition of this book appeared, the field of chemistry of cereal proteins has continued to grow. New, powerful separation techniques, such as reverse phase high performance chromatography and capillary electrophoresis, have been introduced and there has been dramatic progress in the application of achievements of molecular biology and genetic engineering of proteins. Thanks to the development in methods of study on the structure of proteins, our knowledge concerning interactions in protein systems has increased enormously.

Although the central role of investigation of wheat proteins remains the same, more rapid progress may be observed in the field of proteins of other cereals. Particularly in some industrial countries (e.g., Western Europe) — due to the standing surplus of cereal production — growing interest has focused on the nonfood uses of cereals, including the protein preparations made from cereals.

The objective of this edition is the same as the first edition. The book gives an overview on the present status of our knowledge of cereal proteins and about recent trends in this field.

The structure of the book also remains unchanged, but all the chapters are revised and additions and corrections have been made. The chapters dealing with cereals, other than wheat, are substantially enlarged, including newer results of investigations. Particular attention has been given to the progress made in molecular biology of cereal proteins and also to some aspects of nonfood uses of cereal proteins.

I would like to thank those who expressed their views and comments regarding the first edition; this has been a help in improving the second edition.

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1 Introduction

The growing of cereals plays the major role in the agricultural production of the majority of countries. This fact is connected first of all with the importance of some cereals in nutrition. The majority of wheat, rice, rye, sorghum, and millet is used for food; they contribute an important source of protein in the diet in different countries of the world. In many developing countries cereal proteins form 70 to 90% of the total protein consumption. Maize, barley, and growing parts of other cereals are used in animal feed, especially in developed countries.

The great nutritional importance of cereals, the deficiency of some essential amino acids, and the efforts to increase the efficiency of the feeding, enhanced by severe shortages of foods in parts of the world, have stimulated the investigation of the genetics and biosynthesis of the cereal proteins. In the last decade significant advances have been made in the biochemical and genetic studies of individual cereal protein components. A number of genes were isolated and sequenced. The results of these completed investigations, along with the results of the morphological investigation of cereal grains, have led to new methods and possible means of breeding and production of cereals. At the same time, these results are also interesting with regard to the cereal processing, nutrition, feeding, and industrial uses of cereals.

A relatively new field of investigation of cereal proteins involves the connection of the preparation of cereal protein concentrates with the biological (nutritive) value of proteins, as well as with the possible nonfood uses of these proteins. Recently some new results were published concerning the biological value of food proteins, especially protein mixtures. In light of these results it seems that our views concerning the nutritive value of plant proteins must be reevaluated because the role of animal proteins was in many cases overestimated. From the point of view of the nutritive value of cereals, the problem of the inhibitors of the proteolytic enzymes is also very interesting. The existence of trypsin inhibitors in soya has been known for many years, and due to the intensive study of them the primary structures of some compounds are also known. The isolation and characterization of some protein inhibitors from wheat, rye, triticale, and barley show that the problem of proteolytic inhibitors is probably a general problem of all the cereals.

Besides the relatively new fields of investigation — mentioned above — the traditional desire of cereal chemists to understand the contributions of the protein to the milling, dough forming, and baking properties of wheat and some other cereals remained unaltered.

Making a comparison between the different cereals, it can be stated that the main efforts of scientists were concentrated on wheat and that the investigation of proteins of the other cereals was not so developed. Recently an increasing interest in maize, barley, rye, triticale, rice, and sorghum protein investigation has been evident.

Although extensive reviews covering the storage proteins[1] — and particularly prolamins[2] — have been published in some monographs, and also some reviews concerning wheat[3] and maize[4] proteins as well as the chemistry of triticale,[5] rice,[6] barley,[7] oats,[8] sorghum, and millet,[9] no monograph is available giving a general review of the more recent results on the protein chemistry of all important cereal grains.

In the framework of this book, based on the analogies between the different cereal proteins, a new general approach to the chemistry and biochemistry of these proteins will be given. A very important element of this approach is the separation of the storage proteins and metabolically active proteins, which have in many cases quite different character, composition, and technological value.

Great attention was given to the interpretation of the bread-making function and other technological properties of cereal proteins at the molecular level.

It is hoped that this book will provide new information about the progress in the chemistry of cereal proteins and also on some new forms of interpretation for all who are involved in any aspect of cereal research and technology.

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2 The Importance and General Characterization of Cereal Proteins

I. INTRODUCTION

Cereals have been important crops for thousands of years. In most countries of the world the proportion of land devoted to cereals is the highest among cultivated plants. According to statistical data,[1] the world production of cereals was estimated at 1815 million tonnes (see Table 1). In the last period (1986 to 1990) both the area under cereal crops and the production increased. The most marked increases were observed in the production of wheat, maize, rice, and barley. A decreasing tendency was characteristic for oats and rye. The majority of the cereals produced is used for human consumption. The most important other forms of use are animal feed, industrial uses, and seed corn. The total protein consumption of the world population is estimated at more than 100 million tonnes. Cereal proteins provide more than half of the total protein production of the world. If we consider that the production of milk and meat is also based on feed containing cereals, it is clear that in the provision of protein requirements the cereal proteins play the most important role.

II. GENERAL CHARACTERISTICS OF CEREAL PROTEINS

The chemical composition of cereals is characterized by a high content of starch, a relatively significant protein content, and a relatively low lipid content. The differences between different cereal grains are significant, as they are between different varieties of the same cereal. The average chemical compositions of the cereals are demonstrated in Table 2.

It can be seen that among the cereals the protein content of wheat is the highest. Some Russian spring wheats may have a protein content over 20%. The lowest protein content is observed in rice.

According to the comprehensive studies of Osborne,[2] the wheat proteins were divided into four classes on the basis of solubility: albumins, soluble in water; globulins, soluble in salt solutions (10% NaCl solution was frequently used), but insoluble in water; gliadins, soluble in 70 to 90% ethanol; and glutenins, insoluble

TABLE 1
World Production of Cereals[1] (10^6 t)

Kind of Cereal	Period 1971–1975	1976–1980	1981–1985	1986–1990
Total cereals	1420	1550	1680	1815
Wheat	420	455	506	523
Rice	360	392	441	484
Maize	350	395	423	467
Barley	170	168	162	160
Rye	34	30	24	24
Oats	56	48	44	43
Sorghum	54	61	74	82
Millet	32	29	30	33
Triticale[a]	0.4	0.5	0.6	1

[a] Estimated data.

TABLE 2
Average Gross Composition of Cereal Grains (% Dry Weight Basis)

Cereal Grains	Protein	Fat	Starch	Fiber	Ash
Wheat	12.2	1.9	71.9	1.9	1.7
Rye	11.6	1.7	71.9	1.9	2.0
Barley	10.9	2.3	73.5	4.3	2.4
Oats	11.3	5.8	55.5	10.9	3.2
Maize	10.2	4.6	79.5	2.3	1.3
Millet	10.3	4.5	58.9	8.7	4.7
Sorghum	11.0	3.5	65.0	4.9	2.6
Rice	8.1	1.2	75.8	0.5	1.4

in neutral aqueous solutions, saline solutions, or alcohol, but soluble in acids and bases. The terms gliadin and glutenin are usually reserved for preparations from wheat. Osborne suggested the generic names prolamin and glutelin for the equivalent protein fractions of other cereal grains.

The fractionation of the cereal proteins on the basis of solubility has retained its importance to the present time. It gives relatively reproducible results that provide some information about the proteins. However, the fractions obtained are mixtures of different proteins. Each group has subgroups, and none of the groups consists of a single pure protein. There are also proteins that do not appear to fall into any of the four groups; e.g., some proteins remain insoluble (residue protein) after the extraction procedure of Osborne.

Thus, it is understandable that better classification schemes are needed. Biologists and biochemists suggest classification according to biological function. According to this proposition, proteins of cereal grains can be divided into two classes: metabolically active or cytoplasmic proteins and storage proteins. The former correspond roughly to the group consisting of albumins and globulins (according to Osborne's classification), and the latter are comprised of the prolamins and glutelins. However, some overlapping of properties and function is possible. In addition — due to our lack of knowledge — the biological role of many proteins is not yet known.

On the basis of the morphology of cereal grains the proteins may be divided into three groups: endosperm proteins, proteins of the aleurone layer, and proteins of the embryo (or germ). The different classification possibilities are summarized in Table 3. The cytoplasmic protein group includes the most important metabolically active proteins, the membrane proteins, nonenzymic regulatory proteins, proteins of organelles, etc.

TABLE 3
Possibilities for the Classification of Cereal Proteins

On the Basis of Morphology	On the Basis of Biological Function	According to Solubility (Osborne)	On the Basis of Chemical Composition
Endosperm proteins	Metabolically active cytoplasmic proteins	Albumins	Simple proteins
Proteins of the aleurone layer	Enzymes	Globulins	Complex proteins
Proteins of the embryo	Membrane proteins	Prolamins	Lipoproteins
	Proteins of ribosomes	Glutelins	Glycoproteins
	Regulatory proteins	Residue proteins	Nucleoproteins
	Other proteins		Metalloproteins
	Storage proteins		Chromoproteins
	Low molecular weight proteins		Phosphoproteins
	High molecular weight proteins		

Storage proteins are typically endosperm proteins. Nevertheless, smaller amounts of storage proteins also may be found in the aleurone layer and in the embryo.

From the chemical point of view the existence of complex proteins is also interesting. In Osborne's classification the complex proteins were not taken into account. The newer investigations show that the protein-lipid and protein-carbohydrate interactions and the lipo- and glycoproteins play a very important role in the properties and technological value of different cereals.

Cytoplasmic and storage proteins differ considerably in physical properties and amino acid composition. Generally the cytoplasmic proteins are easily soluble in water or salt buffer solutions, their molecular weight is relatively small, and the molecules have a globular form. The storage proteins of the endosperm are generally insoluble in water and salt solutions. It is characteristic for the endosperm storage proteins to include two types of proteins: a low molecular weight protein consisting of single polypeptide chains and having only intramolecular disulfide bonds, and high molecular weight proteins consisting of many polypeptide chains cross-linked by intermolecular disulfide bonds. Many of the polypeptides of storage proteins have repeated sequences of amino acids. This suggests that the genes encoding them evolved initially by tandem duplication of short sequences of DNA. Reviews of the structure and evolution of cereal grain proteins are given by Kreis et al.[3] and Tatham et al.[4]

The differences in amino acid composition of cytoplasmic and storage proteins are relatively great and influence the nutritive value of the two types of proteins. The storage proteins contain a large proportion of glutamic acid and proline and only a small proportion of lysine, arginine, threonine, and tryptophan. The metabolically active proteins, containing considerably lower glutamic acid and proline content and a higher proportion of lysine and arginine, have a higher biological (nutritive) value.

Concerning the distribution of metabolically active and storage proteins it can be stated that the proteins of the aleurone layer and germ belong mainly to the group of metabolically active proteins, and the storage proteins are presumably located in the endosperm. Modern milling procedures allow a comparatively clean separation of the embryo (or germ), pericarpaleurone layer (or bran), and endosperm (or flour). Therefore the differences in amino acid composition — demonstrated in Table 4 — approximately represent the differences between the storage and cytoplasmic proteins.

The protein concentration in the different morphological parts (endosperm, aleurone layer, embryo) of the cereal grains shows big variations. The protein content of the germ is the highest (about 30%); a relatively high concentration (about 20%) may be observed in the aleurone layer; and the lowest protein concentration is evident in endosperm. The ultrastructure of the endosperm of different cereal grains is similar. The storage proteins form a matrix and/or protein granules surrounding the starch granules. It has been observed by many research workers that the protein concentration varies from the inner to the outer endosperm parts. Hinton[5] investigated soft wheats and found a protein concentration range from 6.2% in the innermost endosperm cells through 8.8% in the middle endosperm to 13.7% in the outer (subaleurone) endosperm cells. In high-protein hard wheat types the uneven distribution may be even more marked. As a consequence of the differences in protein concentration and amino acid composition, the nutritive value of the whole grain is generally higher than that of the endosperm. Finally, the total content of essential amino acids depends on the mass ratio of the main morphological parts of the grain. The aleurone layer and germ have higher protein content than the endosperm and a higher ratio of essential amino acids; therefore, the higher mass ratio of the aleurone layer and germ results in a higher protein content and essential amino acid content in the grain kernal. Differences in the proportion of the different proteins (including

TABLE 4
Amino Acid Composition of Wheat Grain, Flour (Extraction Grade 72%), Wheat Germ, and Wheat Aleurone Proteins (g/100 g Protein)

Amino Acid	Wheat Grain	Flour	Whole Germ[a]	Aleurone[b]
Alanine	3.40	2.91	7.00	6.5
Arginine	4.61	3.20	8.96	12.3
Aspartic acid	4.71	4.10	10.21	9.3
Cystine	2.24	1.69	0.66	—
Glutamic acid	31.52	36.86	15.45	18.3
Glycine	3.89	3.39	6.54	7.0
Histidine	2.20	1.83	2.63	4.3
Isoleucine	3.59	3.61	3.91	3.2
Leucine	6.79	6.74	6.79	6.7
Lysine	2.52	1.95	7.76	5.9
Methionine	2.11	1.49	1.88	0.4
Phenylalanine	4.75	4.68	4.07	4.2
Proline	10.44	11.38	4.37	4.6
Serine	4.53	4.40	4.62	5.0
Threonine	2.87	2.31	4.82	3.8
Tryptophan	1.32	1.15	—	—
Tyrosine	3.20	2.31	3.12	2.8
Valine	4.22	3.94	5.65	5.5

[a] From Pomeranz et al.[6] (g/100 g amino acids).
[b] From Fulcher et al.[7]

different types of storage proteins) may have a similar effect. This is the situation in some mutants of corn and barley, where changes in the proportion of prolamins and glutelins (i.e., lower quantity of prolamins) result in a higher lysine content and higher nutritive value. The problem of the mutants with high lysine content will be discussed later in Chapters 6 and 7.

Researchers have observed the negative correlation between yield and protein content of cereals. The theoretical basis for these observations has also been considered, and generally the following explanation is accepted. The energy needed to produce proteins is approximately twice that required for starch synthesis. Therefore, an increase in protein concentration at a constant yield level will require either a higher photosynthetic rate and duration or more efficient partitioning of assimilates into grain (higher harvest index). Application of nitrogen fertilizer leads to an increase in yield and protein content. Low-protein cultivars did not necessarily give a greater protein response to nitrogen fertilizer than high-protein cultivars.

The wide variation in protein content is not only dependent on genetic factors; it may be a result of environmental effects. Protein is synthesized throughout the fruiting period of the plant. Starch synthesis, on the other hand, starts later during fruiting and accelerates as maturity approaches. If growing conditions in the late fruiting period are good, starch yield will be good and grain yields high, but protein content will be relatively low. The availability of nitrogen throughout the growing period is of course of major importance, as mentioned above. Other environmental factors that might result in high protein are drought and certain diseases. The protein content of cereals is important for two reasons. First, cereal protein is an important nutrient. Second, the amount and type of protein are important in the functional uses of the flour. When the protein content of a cereal grain changes, the relative proportions of the various protein classes also change. Generally at a low protein content the amount of metabolically active proteins (albumins and globulins), expressed as a percentage of total protein, is much higher than at a higher protein content. In contrast, a relative decrease in the ratio of storage proteins might be observed. This appears logical if we remember the biological roles of the two groups of proteins. As the plant produces more protein, less is required for physiological functions and more is available as storage protein.

In this book the proteins of the individual cereals will be discussed based on classification according to biological role. Although this classification also has disadvantages, it may be justified by the fact that the technological role, functionality, nutritive value, physical properties, etc. of the two groups differ considerably. In many cases the roles of different groups are well defined (e.g., enzymes, natural enzyme inhibitors), the individual protein components may be isolated in pure form, or, based on the knowledge of corresponding DNA sequences, their primary structure may be determined.

III. THE BIOSYNTHESIS OF CEREAL PROTEINS

In the last decade much progress has been made in determining the molecular biology of cereal proteins. This progress is based on the growing knowledge about the genomes of cereal plants, the location of protein-coding genes, the isolation, sequencing, and cloning of these genes, and the involvement of improved techniques used in the morphological study of cereal grains.

It is not the author's intention to cover this field in any detail; rather, the subject will be outlined and the reader referred to other sources from which more details may be obtained.

Different types of cereal proteins have been studied in recent years; nevertheless, research on synthesis and deposition of storage proteins has predominated.

In a review on seed storage proteins, Shewry and Miflin[8] give a good summary about synthesis of cereal grain proteins, particularly that of storage proteins. Storage proteins are tissue specific, only being synthesized in the corresponding storage organ. They accumulate relatively late in development after cessation of cell division. The amount of storage proteins is highly dependent on the nutritional status of the plant. Under conditions of low nutritient availability, the seed synthesizes mainly