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CORN PROTEINS

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PREFACE

One of the most interesting aspects of plant metabolism is the transformation that occurs with all nitrogen compounds within the cell. Made by amino acids, the protein is among those compounds. Above all, the proteins are source of energy for the plants, besides being catalysts and structural elements for the cells. Those functions show its biological significance. Thus any scientific work - research or review - involving a deep knowledge of its nature and function is justified.

The present work which we are introducing to the scholars interested in the protein problem, is a review of the methods for studying corn proteins. Its author, an Englishman, has a very good knowledge of the subject, backgrounded by several years of research in England and United States, making it very easy for us to recommend this work, which in the words of the author, "the investigator should find this article a useful handbook providing up-to-date practical information on the subject".

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1. INTRODUCTION

1.1. General Considerations

The study of corn proteins is almost as old as the study of proteins themselves. When Thomas B. Osborne presented his classification scheme of proteins around 1900, much of the ground work had been done with corn.

Since the pioneer work of Osborne, interest in corn proteins has filled the literature with a wealth of information about these seed proteins. Nevertheless, it is not the aim of this article to present a comprehensive literature review on the subject. Not only would this be an arduous task, but the reader would probably find himself lost in a mountain of information without being able to grasp the more important points. In the pages that follow, it is hoped to present the reader with a basic understanding of the subject through discussion of the more significant papers in the field. For the purpose of initiating research on corn proteins, the investigator should find this article a useful handbook providing up-to-date practical information on the subject.

1.2. Nutritional Aspects

The study of corn proteins, like those of other cereal proteins, has been greatly stimulated by their nutritional and economic importance. Cereal crops are the principal source of protein for livestock and humans in most parts of the world. Corn itself is grown principally in the Americas where it has been cultivated for many centuries. The value of corn as a source of food is apparent from its composition. It is rich in all three of the major classes of food: carbohydrate, fat, and protein, 100 grams dry weight of whole kernel corn for example contains:

carbohydrate	83.0 g
fat	4.9 g
protein	10.6 g
ash	1.5 g

These components are not, however, evenly distributed within the

kernel. The different organs contain quite different proportions of these components, though all three are present in each of the organs. The embryo or germ (the names commonly used for the waxy region of the kernel which is actually the scutellum with the embryo embedded in it) is rich in fat and protein (see Table 1.2). The endosperm comprises mostly of carbohydrate with only about 9.5% protein and 1% fat.

Table 1.2 - Approximate composition of the germ and endosperm of corn
Germ is in the order of 11% of the kernel dry weight.

Component	Germ	Endosperm
carbohydrate	35%	89.0%
fat	35%	1.0%
protein	20%	9.5%
ash	10%	0.5%

The nutritional aspects of corn show us why their proteins are especially important. Of the three major components only protein is nutritionally deficient. But why? The problem lies in the fact that animal tissues are unable to synthesize certain of the amino acids needed as building blocks for the synthesis of protein. For this reason an adequate intake of these "essential" amino acids is needed in the diet. Where corn is the only source of protein in the diet, the individual obtains insufficient amounts of the essential amino acids lysine and tryptophan. If we look at the amino acid composition of the germ and endosperm protein (Table 9.6 appendix) we see that the endosperm protein is responsible for the low level of lysine in corn. Endosperm protein contains only 1.7% lysine which is far below the value of 4.2% estimated as the lysine requirement for man by the FAO (Food and Agricultural Organization of the United Nations, 1957). The germ protein actually contains more than the required level of lysine* and is also more balanced in essential

* Tryptophan values for germ protein are not available. Since endosperm contains an average of 0.45% tryptophan and whole kernel corn about 0.7%, it may be assumed that the germ protein is also much richer in tryptophan than endosperm protein.

amino acids overall. For this reason whole kernel corn is more nutritious. Thus, although corn is not lacking in quantity of protein it is in quality, and a state of malnutrition results where corn is the sole source of protein. This is particularly evident in children as a failure to grow. They become lethargic and have little resistence to disease. This condition, known as "KWASHIORKOR", is common among the poorer people of underdeveloped countries. Although such people may be restored to normal physical health when given adequate diets, they may have suffered permanent brain damage and never recover intellectually.

It is evident then that any improvement in the quality of corn protein would be of immense benefit. Corn mutants with higher proportions of lysine and tryptophan in the protein have been discovered in recent years, and their superior nutritional quality has been confirmed by feeding trials. As we shall see, these mutants have provided a great stimulus in the study of corn proteins. Our state of knowledge on this subject today undoubtedly owes a great deal to the nutritional and economic importance of these mutant corns.

2. CLASSIFICATION OF PROTEINS

2.1. General Considerations

When it became clear in the latter half of the nineteenth century that there existed many kinds of proteins, the problem then arose as to how to classify them. Simply using their chemical composition was inadequate since all proteins are polymers of some 20 amino acids in varying proportions. Some proteins have non-protein molecules attached to them such as carbohydrate (glycoproteins), lipid (lipoproteins), nucleic acid (nucleoproteins), and pigments (chromoproteins). Such proteins may be distinguished in this way from simple proteins which are nothing more than a chain of amino acids.

The most successful classification of proteins has been on the basis of solubility in certain solvents. This arose from an extensive study of proteins from diverse sources, one in particular being corn, by Osborne and co-workers. This study culminated in the presentation of his classification scheme of proteins at the turn of the century.

2.2. The Osborne Classification Scheme of Proteins

Corn proteins, like all known proteins, can be classified according to the scheme of Osborne and co-workers (Chittenden and Osborne, 1891; Osborne, 1894; Osborne and Mendel, 1914). The scheme classifies proteins on the basis of their solubility in four well-defined solvents; water, salt solution, 70 - 80% ethanol, and dilute sodium hydroxide (Table 2.2). It is important to realize that the scheme is sequential. In other words, albumins, the water-soluble proteins are also soluble in salt solutions and dilute sodium hydroxide. Globulins, the saline-soluble proteins are not soluble in water, but soluble in dilute sodium hydroxide.

Table 2.2 - The Osborne Classification Scheme of Proteins

The name of the corresponding protein found in corn is given in the third column.

Solvent	Protein	Nomenclature of Corn Protein
WATER	ALBUMIN	ALBUMIN
SALT SOLUTION	GLOBULIN	GLOBULIN
70-80% ETHANOL	PROLAMINE	ZEIN
DILUTE SODIUM HYDROXIDE	GLUTELIN	GLUTELIN
INSOLUBLE	SCLEROPROTEIN	-

Outside the plant kingdom the vast majority of proteins are either albumins or globulins. In plants too, this is true of proteins found in many organs. The enzymes, it may be noted, would fall into these categories. The prolamines and glutelins are mainly restricted to the cereal grains (the endosperm in particular), where aqueous insoluble proteins are ideal as storage proteins.

The prolamines are perhaps the most interesting of the protein classes. These proteins are soluble in 70% - 80% ethanol, a solvent which in many biochemical procedures is used to precipitate protein. This unusual property clearly suggests that this class of protein has physical properties very different from other proteins. When we look at the amino acid composition, we can begin to understand this peculiar property. Prolamines have a very high proportion of hydrophobic amino acids and few amino acids with charged groups. A high ratio of non-polar to polar groups would favour solubility in organic solvents. Finally, it may be noted that the name prolamine is derived from the fact that this class of proteins contains high levels of proline and the amide amino acids, glutamine and aspargine.

2.3. Protein Composition of the Corn Kernel

The corn kernel contains all four of the Osborne classes of protein. However, these proteins are not distributed evenly throughout the grain. The endosperm protein is principally prolamine and glutelin as may be seen from the proportions of the various protein classes shown in Table 2.3. In contrast to the endosperm, the germ protein comprises mostly of albumin and globulin with no prolamine (Mertz, Lloyd and Bressani, 1958; Paulis and Wall, 1969). Thus it is important in studies on corn proteins to distinguish data obtained from isolated endosperm and the whole kernel. Throughout the text this distinction will

always be made since some workers use the whole kernel while others prefer the isolated endosperm material.

Table 2.3. - Proportions of Protein Classes in the Corn Endosperm *

Fraction	% Total Nitrogen **
ALBUMIN	3
GLOBULIN	2
ZEIN	40
GLUTELIN	35

* Adapted from several studies

** The remaining nitrogen is unidentified protein and some amino acids and peptides.

Because of their relative high levels, zein and glutelin are usually referred to as the storage proteins of corn.

Examinations of the ultrastructure of corn endosperm with the electron microscope (Christianson et. al., 1969) show that zein is located in granular structures (diameter 2 μ) called "protein-bodies". The protein-bodies appear embedded in a protein matrix comprising of the other storage protein glutelin. Electron micrographs give the impression of beads (protein-bodies) scattered about in a mass of cotton wool (glutelin).

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3. OPAQUE-2 CORN

Before going into further details about corn proteins, it would be useful to introduce the reader to opaque-2 corn at this point. The nutritional importance of opaque-2 corn greatly stimulated the study of corn proteins, and in the pages that follow, the reader will just how much it has helped to increase our understanding of corn proteins.

Opaque-2 corn is the result of a single gene mutation. The opaque-2 gene is recessive, so cross pollination with normal corn results in the repression of the character in the F I seed. Only when the plants of the F I seed are self-pollinated or crossed with an opaque-2 plant does the character reappear in the F2 seed. Opaque-2 corn has what might be described as a floury endosperm in contrast to the horny translucent endosperm of normal corn. It is the dull, opaque appearance of the endosperm that gave rise to its name.

The importance of opaque-2 was discovered in 1964 by Mertz, Bates and Nelson (1964), when it was found that its protein contained nearly twice as much lysine as the protein of normal corns. As was mentioned above, lysine is the limiting amino acid in corn and therefore the principal reason for the inadequate nutritional quality of its protein. Feeding trials (Mertz et al., 1965) with rats soon confirmed the increased nutritional quality of the opaque-2 protein.

The big question that arose for the biochemists was how does a single gene mutation so drastically alter the amino acid composition of the protein? A group of French workers (Mossé et al., 1966) found that the principal effect of the opaque-2 gene was to depress zein synthesis. In Table 3 a typical example of the relative proportions of the Osborne classes of protein in opaque-2 endosperm is presented.

Table 3 - Protein Composition of Opaque-2 Endosperm

The lysine content of each fraction, in moles %, is given in the last column.

Fraction	% Total Nitrogen	% Lysine
ALBUMIN	5	5.5
GLOBULIN	4	5.1
ZEIN	22	0.1
GLUTELIN	45	3.8

If one compares the data with that for normal corn (see Table 2.3) it is apparent that opaque-2 has much less zein and somewhat more of the other proteins than does normal corn. Since the level of protein in opaque-2 endosperm (about 9%) is similar to that of normal, this drop in proportion represents a substantial reduction in zein synthesis. It should be noted that the opaque-2 gene has no effect on the germ proteins (Mertz et al., 1965) which, in any case, do not contain zein.

Now, it is possible to explain the increased level of lysine in opaque-2 protein. Since the proportion of zein is substantially reduced, and zein contains no lysine, the total proportion of lysine is increased through the higher proportions of other proteins which possess higher levels of lysine. Thus we find opaque-2 endosperm contains about 3.6% lysine (grams lysine per 100 grams protein) and normal about 1.7% (see Table 9.7 appendix).

Another important point with regard to the action of the opaque-2 gene is that the amino acid composition of each of the protein classes is the same as that of the corresponding protein in normal corn. We may conclude then that the opaque-2 gene does

not alter the amino acid composition of any of the individual proteins. In the following pages we shall see how, in order to reach this conclusion, opaque-2 played a large part in leading us to a better understanding of corn proteins.

4. FRACTIONATION OF CORN PROTEINS

4.1. General Considerations

All procedures for the fractionation of corn proteins are based on Osborne's classification of proteins. Usually corn proteins are separated by sequential extractions with solvents in which the protein classes are soluble. Thus it is important to realize that in these procedures the proteins are not separated so much by their solubility in these solvents, but rather by extractability. A solvent may extract a protein in which it is not truly soluble or may fail to extract proteins which are truly soluble in it. This should always be borne in mind when extraction procedures are used to fractionate proteins. For example, water extracts of corn meal on standing produce a protein precipitate which is only soluble in salt solutions. The protein is therefore by definition a globulin but was extracted by a solvent in which it is not truly soluble.

Perhaps the only method which truly fractionates corn proteins according to their solubility is that of Mertz and colleagues. Details of the method are given towards the end of this section. In this method, virtually all the protein is first extracted from the meal, and the extract subjected to fractionation with various solvents. However, as we shall see, this method has not proven to be very popular. The problem being that the proteins may not be suitable for further study due to the harsh extraction procedure, and the ill-defined fractions produced.

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4.2. Osborne Extraction Procedure

The sequential extraction procedure of Osborne and his colleagues was first developed around the beginning of the century, and is still widely used today. This procedure arose from a study of corn proteins made by Osborne and co-workers, which played a great part in the formulation of his classification scheme of proteins.

The procedure utilizes the sequential extraction of corn meal with water, salt solution, 70% ethanol and finally dilute sodium hydroxide. This may be summarized in the following table.

Table 4.2 - Osborne Extraction Procedure for Corn Protein *

Solvent	Protein Extracted
WATER	ALBUMIN
0.5 M NaCl	GLOBULIN
70% ETHANOL	ZEIN
0.2% NaOH	GLUTELIN

* for practical details see Flow-Sheet I, 9.1.

Due to the fact that water extracts proteins truly soluble only in salt solutions (pseudo-albumins) and some albumins can only be extracted by salt solutions (pseudo-globulins), many workers have preferred to first extract all the albumins and globulins with salt solution and then separate them by fractionation (for practical details see Flow-Sheet Ia.9.2). In order to separate the components, the saline extract is dialyzed against water which causes globulins to precipitate. Amino acids are also removed and appear in the dialisate. The globulins can then be separated from the albumins (which remain soluble) by centrifugation or filtration of the

dialysis bag contents. This procedure results in a better separation of albumins, globulins and amino acids, and is recommended when the proteins are to be studied further by electrophoresis or chromatography.

Separation of the remaining proteins, zein and glutelin, presents an even more serious problem by the Osborne method. Electrophoretic studies especially led to the belief that the Osborne procedure does not separate zein and glutelin very well. Electrophoretic analysis of these two fractions always revealed that characteristic slow-moving bands of zein were present in glutelin fractions (see figure 5.I). An interesting discussion is presented by Dimler (1966) in a paper which summarizes the evidence that Osborne glutelin is contaminated with zein.

This problem, together with the fact that about 20% of total nitrogen fails to be extracted, are the principal defects of the Osborne procedure for fractionating corn proteins.

It is at this point that the advent of opaque-2 corn proved very useful. As was mentioned earlier the principal characteristic of opaque-2 corn is a reduced level of zein. When opaque-2 endosperm is fractionated by the Osborne procedure, the amino acid composition of the protein fractions was found to be similar to that of corresponding fractions of normal corn except for glutelin (Mossé et al., 1966). The amino acid composition of normal and opaque-2 glutelin is shown side by side for easy comparison (Table 9.8). It may be seen that the glutelin of opaque-2 contains more lysine and less leucine than does normal glutelin. Looking at the data from a different viewpoint one may notice that normal glutelin has an amino acid composition shifted from the opaque-2 type towards zein. From what has just been said above, it could be that opaque-2 glutelin is less contaminated with zein than normal glutelin, due to the lower level of zein in opaque-2.

However, an alternative interpretation might be that the opaque-2 mutation somehow changed the amino acid composition of the protein. This was a crucial point in the elucidation of the mode of action of the opaque-2 gene. Could a single mutation not only change the proportions of proteins syntesized, but drastically change the amino acid composition of a protein as well? This question stimulated the work which led to the development of several modified methods aimed at resolving this problem.

4.3. Modified Osborne Extraction Procedures

The real breakthrough to the glutelin problem came in 1968 when Moureaux and Landry published a preliminary report of a modified Osborne procedure (Moureaux and Landry, 1968). The significance of their work was difficult to evaluate at the time because of the lack of amino acid composition data. Nevertheless, an interesting point made by the French workers was that following the extraction of zein by alcohol one could extract more protein with a further alcohol extraction provided that 2-mercaptoethanol* was present. The protein extracted was reported to be low in lysine and somewhat like zein in amino acid composition, but no details were given. It was evident that this protein might be the zein that was thought to contaminate glutelins.

The author (Sodek and Wilson, 1971a) took up this study in order to verify this point. The isolated endosperm was fractionated by the regular Osborne procedure except that an extra extraction step with alcohol containing 2-mercaptoethanol was included between the extraction of zein and glutelin. Practical details of the procedure are given in the appendix (Flow-Sheet II, 9.3). It should be noted that the terms "zein-1" and "zein-2" have been adopted to distinguish the alcohol-soluble proteins extracted

* 2-mercaptoethanol reduces cystine to cysteine, thereby breaking the disulfide bonds responsible for protein aggregation.

in the presence (zein-2) and absence (zein-1) of 2-mercaptoethanol. Zein-1 is therefore equivalent to the zein described by Osborne.

The amino acid composition for each of the protein fractions obtained from opaque-2 and normal endosperm are shown in Tables 9.9 and 9.10 (appendix). The results show the very similar amino acid composition of corresponding protein classes from both normal and opaque-2. The new fraction, zein-2, has an amino acid composition similar to that of zein-1 but differs notably in its higher methionine and glycine contents and lower leucine and aspartic acid. The important point to note, however, is that the glutelins from the normal and opaque-2 endosperms are virtually identical. We can now say that the Osborne procedure really does not separate zein and glutelin very well, leaving variable amounts of zein as a contaminant of the glutelin fraction. Furthermore the opaque-2 gene does not affect the amino acid composition of any of the proteins.

Now that the main problem of the Osborne procedure has been resolved, it is of interest to examine the effect of the opaque-2 gene on the size of these fractions. In Table 4.3 may be found the data for hybrid varieties (WF9 x Ml^4 normal and R802 x R75 opaque-2) and inbreds R802 normal and opaque-2. It should be noted that in the case of the inbreds the normal and opaque-2 kernels were obtained from the same ear (R802 plants segregating 1:1 for the opaque-2 gene) and therefore extremely close genetically.

In each case the principal effect of the opaque-2 gene is to reduce the level of zein-1. In the hybrids the zein level is reduced from 41.9% in normal to 17.8% in opaque-2, while in the inbred opaque-2 the zein level is almost zero. Zein-2 is much less affected by the opaque-2 gene; its level being about 14% as compared to about 19% in normal. The questions arise whether zein-2 is under separate genetic control or is a mixture of zein (opaque-2 controlled) and some other low-lysine protein (not controlled by opaque-2).

The levels of the other proteins increased in absolute amount as well as in proportion. The level of amino acids (a fraction ignored by many workers) is also remarkably high in opaque-2 endosperm. This could be a secondary effect resulting from the lower demand for amino acids due to repressed zein synthesis. The increased level of amino acids may, in turn, be responsible for the higher absolute levels of the other proteins which synthesis has less competition from zein for amino acids.

Table 4.3 - Protein Composition of Normal and Opaque-2 Corn Endosperm

R802 is an inbred line; WF9 x M14 and R802 x R75 are hybrids. The normal and opaque-2 versions of R802 were obtained from the same ear, i.e. an R802 plant segregation for the opaque-2 gene.
(from Sodek and Wilson, 1971a)

Fraction	R802 normal %	R802 opaque-2 %	WF9 x M14 normal %	R802 x R75 opaque-2 %
Free amino acid	4.4	19.7	2.9	9.1
Albumin	0.9	3.3	1.7	3.6
Globulin	1.5	4.7	3.2	5.2
Zein-1	36.9	2.9	41.9	17.8
Zein-2	18.5	13.6	18.8	13.9
Glutelin	22.9	37.2	18.3	35.3
Total recovered	85.1	81.4	86.8	84.9

4.4. Landry-Moureaux Extraction Procedure

Landry and Moureaux, who originally noted (Moureaux and Landry, 1968) the advantage of including 2-mercaptoethanol in the Osborne extraction procedure, have now reported their method in detail (Landry and Moureaux, 1970). Working with whole kernel corn rather than isolated endosperm, they present a method which fractionates glutelin into three components, G1, G2 and G3 (for practical details see Flow-Sheet III, 9.4). Briefly, their innovation of the Osborne procedure is the following. After zein has been extracted, further extractions with alcohol containing 2-mercaptoethanol are made to bring out the G1 fraction. The remaining protein is extracted successively with 0.6% 2-mercaptoethanol in sodium borate buffer pH 10 with sodium chloride at ionic strength 0.5 (G2 fraction), followed by 0.6% 2-mercaptoethanol in sodium borate buffer pH 10 containing 0.5%sodium laurylsulfate (G3 fraction). The amino acid composition of the three glutelin subfractions are given in Table 9.11 (appendix). The component glutelin G1 is equivalent to zein-2 (see 4.3) both in the method of extraction and amino acid composition. The amino acid composition of the G2 and G3 fractions are also very characteristic, justifying the subdivision of glutelin into three components. The G2 fraction is characterized by very high levels of histidine and a low level of aspartic acid. In some of its characteristics (high proline and glutamic acid and low aspartic acid) it approaches the prolamines, but in others (high histidine and arginine) it is like the aqueous soluble proteins. These characteristics together with a low lysine level (about 1%) place G2 between the prolamines and aqueous-soluble proteins. Obviously a high proline level can no longer be used to characterize prolamines since the proline level of G2 is even higher than that of zein.

The G3 fraction has an amino acid composition very close to that of albumins and globulins (compare Table 9.9). It is noteworthy that if one calculates the amino acid composition of a

mixture of G1, G2 and G3 in the proportions in which they are found, the result is extremely close to that of Osborne glutelin (see Landry and Moureaux, 1970).

When normal and opaque-2 kernels are analysed for protein content (Landry, 1972) we note that only the G3 fractions of glutelin components are affected (Table 4.4.I). This is believed to occur not through direct action of the opaque-2 gene, but through an increase in saline-soluble proteins (albumin + globulin). As we saw above, the G3 fraction resembles the saline-soluble fraction in amino acid composition, and this together with other evidence (see 7.2) suggests that the G3 fraction may be made up of aggregates of saline-soluble proteins. The high proportion of saline-soluble proteins, it should be noted, is due to the fact that whole kernel material was being studied here.

It may be added that the Landry-Moureaux procedure has very recently been used to obtain data on the protein composition of the endosperm, including several high-lysine mutants. (Misra et al., 1972). This data largely confirms the data of Landry presented in Table 4.4.I, although unfortunately free amino acids were not separated from the saline-soluble proteins in the work with endosperm.

The Landry-Moureaux procedure is clearly a very useful and valid method. Confidence in the procedure stems from the fact that the amino acid compositions of the G1, G2 and G3 components are similar for normal and opaque-2 corn (Moureaux and Landry, 1972b). Moreover, these three components show little variation in amino acid composition during development of the grain (Moureaux and Landry, 1972a).

Table 4.4.I - Protein Composition of Normal and Opaque-2 Kernels

After the Landry-Moureaux extraction procedure (Landry, 1972)

Fraction	% Total Nitrogen	
	Normal	Opaque-2
Free amino acid	5.9	16
Saline-soluble *	17.2	20.1
Zein	41.5	9.9
G1	7	7.2
G2	10	10.3
G3	12.1	19.5
Residue	6.3	7.9

* Albumin + Globulin.

Thus we reach a point where a considerably improved method of fractionation of corn proteins is presented. This does not detract from the classical procedure of Osborne. His method has been the most widely used since its presentation around the beginning of the century, and only now in 1970 do we find it has been most significantly improved. In the author's opinion the Landry-Moureaux method is the best extraction procedure available to date, and to be recommended for use in any study on corn proteins. By this method, corn proteins may be classified as shown in Table 4.4.II.

4.5. Other Modified Osborne Procedures

Now that the reader is aware of the principal deficiencies of the Osborne extraction procedure and how these were overcome, we can now discuss some other significant papers on this problem

Table 4.4.II - Classification of Corn Proteins by the Landry-Moureaux Extraction Procedure

For practical details see Flow-Sheet III (9.4)

STEP	SOLVENT	FRACTION
	Without 2-Mercaptoethanol	
I	0,5M NaCl	Albumin, Globulin Amino Acids
II	55% isopropanol	Zein
	With 2-Mercaptoethanol	
III	55% isopropanol	Glutelin "G1"
IV	pH 10 buffer, μ = 0.5	Glutelin "G2"
V	pH 10 buffer + detergent	Glutelin "G3"

in the light of what is known today. Unfortunately, in the past, several laboratories have introduced modified extraction procedures which were difficult to evaluate in the light of knowledge at the time. These papers presented what appeared to be promising improvements but only now can we really try to understand what was achieved.

One of the earlier innovations introduced to improve the separation of zein and glutelin was the use of hot alcohol. Instead of simply extracting zein with 70% ethanol at room temperature (the normal Osborne procedure) Mossé and his group (Mossé et al., 1966) also included an extraction step with 70% ethanol at 60°C. The amino acid composition of "cold" zein and "hot" zein (extracted after "cold" zein) is shown in Table 9.12. (appendix). If one compares the G1 or zein-2 data (see Table 9.11 or 9.10) it may be seen that "hot" zein has an amino acid composition between zein and G1 (zein-2). Thus it seems probable that the use of hot alcohol removes some of the zein that contaminates glutelin, but obviously not all.

Yet another attempt to obtain a better separation of zein and glutelin was made by Paulis, James and Wall (1969). They tried using 0.5% sodium acetate in the alcohol extraction step. This had been reported many years ago to extract more zein (Nagy et al., 1941). This fact was confirmed by Paulis et al. who went on to show that such zein was somewhat different in amino acid composition from the simple alcohol-extracted type (Table 9.13 appendix). The acetate-alcohol zein contained higher proportions of glycine and lower aspartic acid suggesting the presence of some G1 protein (methionine, which also characterizes G1, showed no difference but the values are unreliable due to the hydrolysis procedure used). The higher histidine values suggest the presence of some G2 protein too, though the proline values were not high as might be expected.

Despite the fact that an additional 8% or so protein was extracted by alcohol-acetate, the remaining glutelin fractions from opaque-2 and normal endosperm were still sufficiently different to suggest that normal glutelin was more highly contaminated with zein (Table 9.14, appendix). Nevertheless, Paulis et al. were able to obtain glutelin preparations from opaque-2 and normal which were almost identical in amino acid composition. Unfortunately the procedure is rather laborious and does not lead itself to routine studies. Briefly, their procedure is as follows. After zein extraction, Paulis et al. digested all the starch in the residue with α-amylase leaving a protein residue (glutelin). This protein was still different in amino acid composition when obtained from opaque-2 and normal corn (Table 9.14). However, after reduction with 2-mercaptoethanol followed by alkylation to prevent reformation of disulfide links, a protein could be removed with 70% ethanol which left glutelin fractions from normal and opaque-2 endosperm very similar in amino acid composition (Table 9.14). This procedure is analogous to that of Landry and Moureaux in that reduction of the alcohol-insoluble proteins allows the removal of an alcohol-soluble protein component (G1).

4.6. Mertz Fractionation Procedure

One cannot fail to mention the fractionation procedure of Mertz and his colleagues, since it appears frequently in the literature though its use has been largely restricted to his group. Perhaps the method has not proven to be popular because of the harsh methods of extraction used. In this respect it may not be suitable further studies when such techniques as electrophoresis are to be used. Nevertheless, the method is interesting in that it presents a different approach to the fractionation of corn proteins.

The method was introduced in 1957 by Mertz and co-workers (Mertz and Bressani, 1957; Mertz, Lloyd and Bressani, 1958; Lloyd and Mertz, 1958; Bressani and Mertz, 1958). The principle of the method is to first extract virtually all the protein using an alkaline copper reagent (for practical details see Flow-Sheet IV, 9.3). The exctract is adjusted to pH 3 to precipitate zein and glutelin leaving "acid-soluble" proteins in the supernatant. The precipitate is dissolved in 66% ethanol at pH 11.5. On dropping the pH to 6, glutelins precipitate out leaving zein in the supernatant.

Fractionation of endosperm and germ samples give values in the ranges shown in Table 4.6.I. The levels of nitrogen in the supposed zein and glutelin fractions are not unlike those obtained by Osborne procedures.

Table 4.6.I - Size of Protein Fractions of Corn after the Mertz Procedure

data taken from Concon (1966)

Fraction	% Total Nitrogen	
	Endosperm	Germ
Residue	2-11	0-11
Acid Soluble	16-26	30-40
Zein	41-60	5-10
Glutelin	17-31	49-54

When Mertz and colleagues used their method to compare the size of these fractions in normal and op aque-2 endosperm, the data

shown in Table 4.6.II was found. Again, the levels are not unlike those found by the Osborne procedure. However, big differences are seen when the amino acid composition of the Mertz fractions (Table 9.15, appendix) are compared with those of Osborne. Thus the lysine contents of the various Mertz fractions (g/100 g protein) were as follows:

acid-soluble, 5.9 (opaque-2) and 1.8 (normal);
zein, 1.0 (opaque-2) and 0.3 (normal);
glutelin, 3.7 (opaque-2) and 3.6 (normal).

This led Mertz, Bates and Nelson (1964) to erroneously conclude that the opaque-2 gene increases the lysine content of the endosperm by increasing the lysine content of the acid-soluble and zein fractions as well as by increasing the glutelin fraction and decreasing the amount of zein.

Clearly, the Mertz fractions are different from their corresponding Osborne fractions. However, it is noteworthy that glutelin is well separated from zein as may be observed in the similarity in amino acid composition of normal and opaque-2 glutelin. The effect of the alkaline copper reagent is to reduce the disulfide bonds (together with a great deal of destruction of the same, see Concon, 1966) and in this way a good separation of zein and glutelin is achieved, just as the case when Osborne glutelin is reduced with 2-mercaptoethanol.

In conclusion, the Mertz procedure has the advantage over the classical Osborne procedure in that virtually all the protein is extracted and a good separation of zein from glutelin is achieved. In some other respects, however, it presents certain disadvantages. The zein and acid-soluble fractions are not clear cut, with variable amounts of cross-contamination with proteins of other classes.

Table 4.6.II - Protein Composition of Normal and Opaque-2 Endosperm Fractionated by the Mertz Method

from Mertz, Bates and Nelson (1964)

	% Total Nitrogen	
Fraction	Normal	Opaque-2
Acid-soluble	34	35
Zein	37	26
Glutelin	29	39

4.7. Sucrose Density Fractionation Procedure

Yet another fractionation procedure that is completely different from the others in approach has been presented by Christianson et al. (1969). The results obtained are not only interesting in themselves but can be related to knowledge of corn proteins from sequential extraction procedures.

The prime objective of Christianson and his colleagues work was to isolate and identify protein bodies and other particles of storage protein using the sucrose density gradient technique. In section 2.3 the reader was given a brief description of the subcellular structure of corn endosperm, as to the belief that zein is located in "protein-bodies" which are surrounded by a matrix of glutelin protein.

Because of technical difficulties with the hard mature endosperm, Christianson and colleagues used the softer material obtained at 24 days after pollination. At this period of

endosperm development, synthesis of storage protein is well under way and occurring rapidly. A brief description of the method is as follows. The isolated endosperm was homogenized in buffer and the starch granules left to settle out. The supernatant, which contained 90% of the total nitrogen, was layered onto a sucrose density gradient (15% - 70% w/v). After centrifugation it was observed that several light-scattering zones were present along the tube. These zones corresponded to protein, as determined by nitrogen assays of fractions taken from the tube. This may be seen more clearly from the figure (4.7).

Peaks (or zones) I and II did not contain any visible particular matter. From solubility and electrophoretic properties, zone I was judged to contain albumins and globulins. On a similar basis, zone II had the properties of glutelin.

The large peak near the bottom of the tube was divided into two parts; zone III the leading edge of the peak, and zone IV the peak itself. Microscopic studies of zone III revealed the presence of free protein-bodies, whereas zone IV contained the same type of protein granules but embedded in a protein matrix. The protein-bodies of zone IV were soluble in 70% ethanol and could be removed from the matrix protein this way. The matrix protein, being soluble only in dilute sodium hydroxide, is clearly glutelin.

Amino acid analysis of the four zones confirms their identity in terms of protein class (Table 9.16, appendix). The free protein-bodies of zone III present an amino acid composition that is clearly zein. Similarly, the alcohol-soluble fraction of zone IV is zein. Note the characteristic low values of lysine and glycine and high leucine. The alcohol-insoluble fraction of zone IV (glutelin) has an amino acid composition different from that of Osborne glutelin extracted from the same corn (c.f. columns 4 and 6). A similar observation may be made for zone II protein, which is also a glutelin as judged by its solubility properties.

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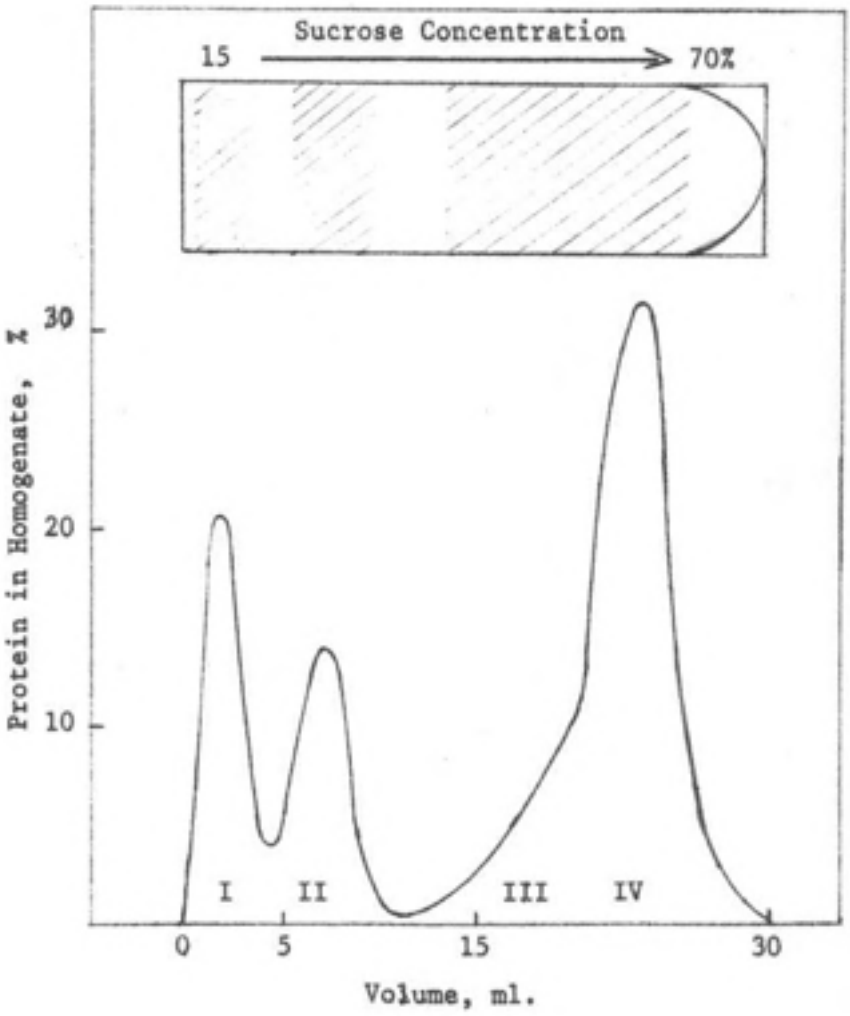
Figure 4.7 Fractionation of Corn Proteins on a Sucrose Density Gradient

from Christianson et al., 1969.



Better interpretation of the data is possible in the light of Landry and Moureaux's work (1970) discussed earlier (see 4.4.). Clearly, zone II glutelin resembles the G3 fraction (Table 9.11) of Landry and Moureaux in amino acid composition. Note the characteristic high relative values for lysine, aspartic acid and low proline and glutamic acid values. This comparison may be extended further. Christianson and colleagues report that zone II proteins do not penetrate the gel upon electrophoresis. Only after reduction and alkylation of the disulfide bonds did the protein migrate into the gel. In this respect, it is interesting to recall Landry and Moureaux's hypothesis (Landry and Moureaux, 1970; Moureaux and Landry, 1972a) that the glutelin G3 fraction is an aggregate of saline-soluble proteins; aggregation occurring through the formation of disulfide bonds.

The zone IV glutelin fraction may be compared with the G2 fraction (Table 9.11) of Landry and Moureaux in amino acid composition. Both these glutelin fractions present similar characteristics, such as high relative values for histidine, proline and low values for aspartic acid and alanine.

It is difficult to find where the G1 fraction of Landry and Moureaux might be located. This fraction is characterized by its high methionine content, but due to problem with methionine analysis (see 8.1.) low values may be incorrect, making this an unreliable criterion. Nevertheless, taking into account its other characteristics, such as (relative to zein) high glycine, low aspartic acid and leucine, one might suggest it is present in the protein-body fractions (c.f. amino acid composition with Osborne zein, column 5) or possibly mixed with the zone IV glutelin.

The beauty of the density gradient fractionation procedure is that with a completely different approach to

endosperm protein fractionation Christianson and colleagues succeeded in isolating characteristic fractions, recognizable as protein fractions isolated by sequential extraction procedures. It should be noted that their procedure does not use a preliminary treatment with an organic solvent (i.e. to remove lipid), and it avoids the interaction of proteins that probably account for the problems encountered with the Osborne procedure.

4.8. A Note on Preliminary Lipid Extraction

Very little attention has been given to the importance of a preliminary lipid extraction of the ground corn before the extraction of proteins. When dealing with whole kernel corn, extraction of lipid is essential, at least from the point of view of easier handling of the material. The high lipid content of the germ (about 35%) results in a coarse somewhat sticky powder which is not ideal for protein extraction. Removal of lipids results, after further grinding, in a fine dry powder. A fine powder allows a greater efficiency of protein extraction due to the accessibility of solvents to the solid material. A preliminary lipid extraction is always made therefore, when dealing with whole kernel corn.

The lipid content of the isolated endosperm is generally low (about 1%), and proteins can be extracted successfully without a preliminary lipid extraction (Sodek and Wilson, 1970). Nevertheless, lipid extraction is recommended for endosperm material as well for the following reasons. First, removal of lipids permits a finer powder. Second, there does not appear to be any problem of the organic solvent changing any characteristics of the proteins subsequently extracted (protein denaturation). Thirdly, a preliminary lipid extraction step may be necessary for a good separation of glutelin fractions G1 and G2 (Landry, 1972) for instance. In other words, there appear to be no

disadvantages of a preliminary extraction lipids, but on the contrary several advantages.

There is no standard method for lipid extraction; and a variety of solvents have been used. Thus we find acetone (Baudet, et al., 1966; Subramanian, et al., 1970), ether (Baudet et al., 1966), hexane (Lloyd and Mertz, 1958), butanol (Concon, 1966), pentane (Concon, 1966), pentane-hexane (Boundy et al., 1967) for example. Often, low temperatures have been employed during extraction, probably due to fears of protein denaturation.

Some of the more commonly used methods are briefly as follows. One method is to reflux the sample in a Soxhlet apparatus with the organic solvent, such as hexane (Lloyd and Mertz, 1958) for 24-48 hours. Another is to place the ground sample in a glass column (chromatography-type) and percolate the organic solvent through. Baudet et al. (1966) used this method, running the column at 10° and passing successively acetone (500 ml) and ether (250 ml). Alternatively, one can homogenize the samples with the organic solvent at a reduced temperature, collect the powder by filtration, and wash the powder on the filter with the solvent, preferably a different one (Concon, 1966). The use of two different solvents leads to a more efficient extraction of lipids. After lipid extraction, a finer powder can be obtained using a ball-mill, for example.

5. METHODS OF FURTHER FRACTIONATION

Various methods have been used to study and further fractionate the protein classes. These include electrophoresis, ion-exchange chromatography, gel filtration and ultracentrifugal analysis. Electrophoresis is the technique most commonly used because it is rapid, gives excellent resolution, and several samples can be run simultaneously. Since the other techniques are rarely found in the literature and have achieved very little anyway, the discussion that follows will be largely restricted to electrophoresis.

Electrophoresis is useful in that it can show the investigator what he has in a particular fraction, whether it be a single protein or a heterogeneous mixture of proteins. The band patterns obtained present a useful "picture" of the fraction which can readily be compared with those of other fractions when similar experimental conditions are used. For this reason, electrophoresis is frequently used as an adjunct to amino acid analysis as a means of "assaying" the fractions.

The desirability of running experiments under identical conditions, whether by electrophoresis or other methods, presents certain problems. The most serious problem is the insolubility of zein and glutelin in the buffer solutions used in these techniques. This problem may be overcome by including 6-8 M urea in the buffers, since zein and glutelin are soluble in strong urea solutions. It should be noted, however, that when comparisons are to be made with albumins and globulins these too should be run with urea-containing buffers to standardize experimental conditions.

A second problem with zein and glutelin is that these proteins readily form aggregates through disulfide cross-linking.

Once these proteins are in solution, the disulfide bonds may be continuously breaking and re-forming. Since there is no distinction between one sulfhydryl (-SH) group and another, reformation can occur at random resulting in an interchange of disulfide links ("disulfide interchange"). Through disulfide interchange, one may find a whole series of polymers of different size depending on the degree of cross-linking. Even if the subunits were identical this would result in a large number of electrophoresis bands, for example. The situation becomes even worse if the conditions of electrophoresis etc. (buffer, pH) favour disulfide interchange, since the formation and breaking down of aggregates during the run will result in loss of resolution.

Clearly, the best way out of this problem is to study the subunits under conditions that they cannot aggregate. This may be done by reduction and alkylation of the proteins beforehand; a technique used with success by Wall and his associates in the United States (Boundy et al., 1967; Christianson et al., 1969; Paulis et al., 1969). In this process, disulfide bonds are reduced with 2-mercaptoethanol (100-fold excess over protein cystine) in 0.01 M phosphate pH 8 buffer containing 8 M urea for 16 hours at room temperature (Cavins and Friedman, 1968). The reduced sulfhydryls are then alkylated by reaction with a twofold excess (over total sulfhydryl) of acrylonitrile for 1 hour at room temperature. The advantage of alkylation is that an uncharged group is added to the sulfhydryl group and thereby prevents reformation of disulfide bonds without altering the electrophoretic properties (Wiel and Siebles, 1961).

Unfortunately, the literature is full of seemingly meaningless electrophoretic patterns and chromatography peaks. What has been achieved with these techniques is as yet very little, only a general idea of what might be expected will be mentioned here.

Albumins and globulins generally present a number of bands of high mobility with a major band of similar mobility common to both fractions. The electrophoretic patterns of zein, glutelin, and globulin may be represented schematically as in figure 5.I.

Zein is generally characterized by two slow moving bands, with little else. Glutelin, on the other hand, presents many bands, faster moving than those of zein, except for two bands which correspond to those of zein. This is indicative of the poor separation of zein and glutelin by the Osborne method. It is noteworthy that some of the glutelin bands correspond with those of globulin. Again, the reader is reminded of the Landry-Moureaux hypothesis that part of the glutelin fraction (namely G3) is made up of globulin aggregates.

Unfortunately, we must await electrophoretic analysis of the Landry-Moureaux glutelin components. Hopefully, the subdivision of glutelin into three fractions should reduce the number of bands and thereby facilitate the identity and verification of the origins of these components. Now that there is a better understanding of corn proteins, better and more meaningful work can be expected in the future with electrophoretic analysis and other techniques.

Figure 5.1 Schematic Electrophoretic Patterns of Zein, Glutelin and Globulins

Proteins fractionated by the Osborne procedure, then reduced and alkylated before electrophoresis on starch gel in aluminium lactate-lactic acid buffer pH 3,5 in 8 M urea (Dimler, 1966).

6. SPECIFIC FRACTIONS AND PROTEINS

The purpose of this section is to provide the reader with a "dictionary" of specific fractions and proteins of corn, so that he may find readily all the pertinent information regarding a particular fraction without having to read the whole article.

6.1. Non-Protein Fraction

extraction solvent: water
amino acid composition: Table 9.9 and 9.17
remarks: mostly free amino acids.

This fraction would include the free amino acids. Together with albumin it forms part of the fraction extracted by water in Osborne-type procedures. Separation from albumin can be achieved by precipitation of the latter with 5% trichloroacetic acid leaving the non-protein elements soluble. Dialysis may also be used but a different proportion of larger molecules such as peptides may be obtained.

This fraction surprisingly has received very little attention. In mature normal corn it represents only a few percent of the total nitrogen in the kernel and for this reason many workers have not bothered to separate it from proteins in aqueous extracts. However, in more recent years it has been shown (Mossé et al, 1966; Sodek and Wilson, 1971a) that in opaque-2 corn it represents between 10-20% of the total nitrogen and therefore an important fraction in this case.

When 5% trichloroacetic acid-soluble water-extracts have been examined for amino acids, it was found that free amino acids can account for most of the nitrogen in this fraction. The remainder may be low molecular weight peptides and other nitrogenous components.

The (free) amino acid composition of this fraction from endosperm is given in Table 9.9. It is noteworthy that the amino acids with long biosynthetic pathways are present in very small quantities, such as histidine, valine, leucine, phenylalanine and tyrosine. On the other hand, amino acids close in metabolic relation to carbohydrate metabolism, such as glutamic acid and aspartic acid are present in high quantities. In fact glutamic and aspartic acids and their amides can account for 55% of the total free amino acids in this fraction.

In the case of opaque-2 endosperm, where this fraction represents a large fraction of the total nitrogen, the (free) amino acid composition is similar in proportions to that of normal corn (Table 9.9). Noteworthy is the higher proportion of free lysine in opaque-2 endosperm (4.2% vs. 2.5%).

6.2. Albumin

extraction solvent: water
amino acid composition: Table 9.9; see also Paulis and Wall, 1969.

Albumin, by definition, is protein soluble in water. This class of proteins is also soluble in salt solutions and dilute sodium hydroxide.

In mature corn endosperm it is found in small amounts where it forms about 3% of the total protein. Albumin is more abundant in the germ where, together with globulin, it forms a large part of the protein in that organ (Mertz, Lloyd and Bressani, 1958, Paulis and Wall, 1969). Most of the enzymes of the kernel would fall into this class. There is some evidence that the albumin fraction undergoes turnover during endosperm maturation (Sodek and Wilson, 1970, 1971a), which might be expected of an enzyme fraction.

Albumin is a heterogenous mixture of proteins producing many electrophoretic bands (e.g. see Paulis and Wall, 1969; Paulis, James and Wall, 1969).

6.3. Globulin

extraction solvent: 0.5 M NaCl
amino acid composition: Table 9.9; see also Paulis and Wall, 1969.

Proteins soluble in salt solutions are, by definition, termed globulins. Globulins are also soluble in dilute sodium hydroxide but not in water.

In mature endosperm they form about 2-3% of the total protein. They are considered a minor storage protein, though in some other seed crops, such as legumes, they are the major storage protein. The germ contains large amounts of globulin which, together with albumin, account for most of the protein in this organ (Mertz, Lloyd and Bressani, 1958; Paulis and Wall, 1969)

Globulins are heterogenous by electrophoretic analysis (e.g. see Paulis and Wall, 1969; Paulis, James and Wall, 1969).

6.4. Zein

Zein is the name given to the prolamine fraction of corn proteins. According to Osborne's classification, prolamines are protein soluble in 70-80% ethanol. However, as will be apparent below, zein or zein-like proteins are extracted more efficiently when this solvent is modified.

Zein is restricted to the endosperm where it forms about 40% of the total protein. This major storage protein

is localized in protein-bodies, the name given to granular structures of protein observed in the electron microscope (Christianson et al., 1969). The corn mutant opaque-2 contains much less of this protein (from 0-20%) and consequently has fewer and much smaller protein-bodies (Wolf et al., 1967).

6.4.1. Zein (Osborne)

extraction solvent: 70% ethanol
amino acid composition: Table 9.12 ("cold zein")

In order to distinguish zein extracted by 70% ethanol from zein extracted by modified solvents, the term Osborne zein has been applied in this article. However, in the literature the word zein is invariably used.

Osborne zein has a high proportion of hydrophobic amino acids and few basic amino acids (see Table 9.12) which accounts for the unusual solubility of this protein. Zein is especially characterized by the virtual absence of lysine in the protein. The prolamines of other seeds such as gliadin of wheat and hordein of barley have similar amino acid compositions but are not so low in lysine as is zein.

Starch gel (Dimler, 1966) and polyacrylamine gel (Sodek and Wilson, 1971a) electrophoresis of zein results in two slow-moving bands. Several peaks are obtained after gel filtration, however (Landry, 1965; Ivanov and Ganchev, K.D., 1969).

6.4.2. α-Zein and β-Zein

solubility characteristics:
90% ethanol (α-zein);
60% ethanol (β-zein).

Zein, extracted by the Osborne method, may be separated into two components by alcohol fractionation. After precipitation of zein by dialysis against water, the residue is extracted with 95% ethanol. The soluble component is α-zein and represents about 80% of the total prolamine fraction. The insoluble component is β-zein and may be solubilized in 60% ethanol.

Gel electrophoresis studies by Turner et al. (1965) show that when the disulfide links of these proteins are reduced the components α- and β-zein produce very similar patterns. However, if disulfide bonds are not broken β-zein fails to migrate into the gel. Thus β-zein is a disulfide-linked aggregate of smaller components. These smaller components are released when the disulfide links are broken.

Although there is no amino acid data available for these two components, Mossé et al. (1966) remark that α- and β-zeins can be differentiated on their methionine, glycine and cystine content, suggesting that β-zein may have an amino acid composition shifted to the zein-2 type.

6.4.3. Zein-1

extraction solvent: 55% isopropanol
amino acid composition: Table 9.10
remarks: essentially the same as Osborne zein.

When 55% isopropanol is used instead of 70% ethanol to extract zein, the amino acid composition is very similar to Osborne zein (c.f. Tables 4.IV and 4.IX). This solvent is used in extraction procedures II and III given in the appendix. In the case of extraction procedure II, the authors applied the name zein-1 to distinguish this fraction from zein-2, a zein-like protein extracted subsequently. The authors of procedure III use the term zein, simply, because the zein-like

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protein extracted subsequently, they classed as a glutelin component.

55% isopropanol appears to extract just as much, if not more, zein as 70% ethanol (Sodek, unpublished) and for this reason is to be preferred. Landry and Moureaux (1970) found this solvent extracted as much zein from corn kernels as did hot and cold 60% ethanol combined.

On electrophoresis, 2 slow-moving bands are presented (Sodek and Wilson, 1971a).

6.4.4. Zein-2

extraction solvent: 55% isopropanol +
0.6% 2-mercaptoethanol
amino acid composition: Table 9.10
remarks: is the same as glutelin G1.

After removal of zein by alcohol solutions, more zein-like protein can be extracted provided 2-mercaptoethanol is included in the alcoholic solvent. This extraction step is used in procedures II and III (appendix). The authors of procedure III chose the name glutelin G1 since, although they recognized the protein to be like zein, it is a contaminant of glutelin when the classical Osborne procedure is followed. The authors of scheme II, since they did not further fractionate the remaining glutelin, used the term zein-2 because of its zein-like properties.

Zein-2 should prove interesting for workers in this field. Although its general amino acid composition is like that of zein (abundant in hydrophobic amino acids and deficient in basic amino acids) it is sufficiently different and characteristic to warrant a distinctive name. Particularly interesting is the

very high methionine level (about 5.5%). Methionine is an essential amino acid and sufficiently low overall in corn protein to make any increase in level desirable from a nutritional point of view. Other notable features of the amino acid data are the higher levels of glycine and cysteine and the lower levels of aspartic acid and leucine. The lysine content appears to be as low as in zein (Table 9.10). When higher lysine levels are found (Sodek and Wilson, 1971a) this may be due to the omission of a preliminary lipid extraction of the corn meal resulting in contamination of the zein-2 fraction with glutelin-2 (Landry, 1972).

The interesting question with regard to zein-2 is its composition. Is it a mixture of zein and some other protein or peptide rich in methionine and glycine? Or, is zein-2 a modified zein with perhaps many leucine and aspartic acid residues substituted by methionine, cystine and glycine? Although the opaque-2 gene, which drastically reduces the level of zein, has little effect on zein-2, this does not help us to decide between these alternatives. The first alternative could arise, for example, through the association of a certain quantity of zein with glutelin peptides through disulfide cross-linking as an artefact of extraction.

6.5. Glutelins

This group of proteins are, by definition, soluble in dilute alkali. They are insoluble in water and salt solutions. Glutelin forms the order of 35% of the endosperm protein and may be regarded as a major storage protein. In the cell these proteins are present in the form of a protein matrix. The dough-like properties of corn flour may be attributed to these proteins.

Glutelins of corn have proven to be the most difficult of the proteins to study. The problem may be that they consist of several proteins joined together through disulfide linkages, some of which may arise as artefacts of extraction. As we shall see below only when the disulfide bonds are broken do we find means of putting these proteins in order.

6.5.1. Glutelin (Osborne)

extraction solvent: 0.2% NaOH
amino acid composition: Table 9.8
remarks: contains zein-2 (glutelin-1).

Since it has been possible to fractionate glutelin into three distinct components (see below) the term Osborne glutelin is used here to define the corn proteins soluble in 0.2% sodium hydroxide when the Osborne extraction procedure is followed. The amino acid composition (Table 9.8) is much more balanced than that of zein, the other major storage protein, and consequently of higher nutritive value. Although this fraction presents a high degree of disulfide cross-linking, a high cystine value is not found in the analysis. This is due to the destruction of sulfide groups by sodium hydroxide (Concon, 1966; Boundy et al., 1967), note the high cystine values of glutelin isolated without the use of sodium hydroxide (Table 9.16).

6.5.2. Glutelin G1

extraction solvent: 55$ isopropanol +
0.6% 2-mercaptoethanol
amino acid composition: Table 9.11
remarks: equivalent to zein-2.

This fraction is identical to zein-2, though it was extracted from the whole kernel (Landry and Moureaux, 1970) rather than the isolated endosperm as for zein-2 (Sodek and

Wilson, 1971a). It appears to be a contaminant of Osborne glutelin, perhaps arising as an artefact of extraction. For further information, see zein-2.

6.5.3. Glutelin-G2

extraction solvent: 0.6% 2-mercaptoethanol in sodium borate, pH 10 + NaCl, μ = 0,5

amino acid composition: Table 9.11

remarks: sometimes referred to as saline-soluble glutelin.

Glutelin G2 was extracted from whole kernel corn according to procedure III (appendix) by Landry and Moureaux (1970). This fraction is a component of Osborne glutelin. In amino acid composition (Table 9.11) this fraction has certain characteristics of both the prolamines (high proline, glutamic acid; low aspartic acid) and the albumins and globulins (high histidine, arginine). Glutelin G2 is perhaps most characterized by its very high histidine content, but also noteworthy are the very high level of proline and low level of aspartic acid and lysine.

6.5.4. Glutelin G3 - Zeanin

extraction solvent: 0.6% 2-mercaptoethanol in sodium borate, pH 10 + 0.5% sodium laurylsulfate

amino acid composition: Table 9.11

This protein fraction was extracted from whole kernels according to procedure III (appendix) with the solvent indicated. The authors of this work have subsequently indicated zeanin as a name for this fraction (Landry and Moureaux, 1970).

Its amino acid composition is very similar to that of albumins and globulins. It differs from other glutelin components in its high levels of lysine, arginine, aspartic acid and low levels of glutamic acid and proline.

Glutelin G3 is believed to originate, at least in part, from the albumin-globulin proteins through aggregation of the latter proteins by disulfide bridges (Moureaux and Landry, 1972a). In support of this hypothesis we may note the similarity in amino acid composition. Furthermore, this fraction, which forms one of the protein peaks after sucrose density gradient fractionation (Christianson et al, 1969), can only enter the gel upon electrophoresis after the disulfide bonds are broken. This suggest the presence of high molecular weight proteins composed of aggregates of smaller components through disulfide linkages.

Landry (1972) has suggested this fraction may also contain proteins associated with membrane structures such as mitochandria. Thus further fractionation of this component seems possible.

6.6. Residue

The residue is the protein fraction remaining after the last extraction step. Its size and amino acid composition depends on the extraction procedure used. The classical Osborne procedure leaves about 20% of the total nitrogen unextracted (residue fraction) with an amino acid composition like that given by Dalby (1966).

When modified extraction procedures using 2-mercaptoethanol are employed, only a few percent of the total nitrogen remains insoluble with an amino acid composition

like that shown in Table 9.11. The amino acid composition data suggest that the residue fraction (in this case) is not a random mixture of unextracted proteins but mainly of unextracted G2 and G3 proteins.

7. FORMATION OF PROTEINS DURING KERNEL DEVELOPMENT

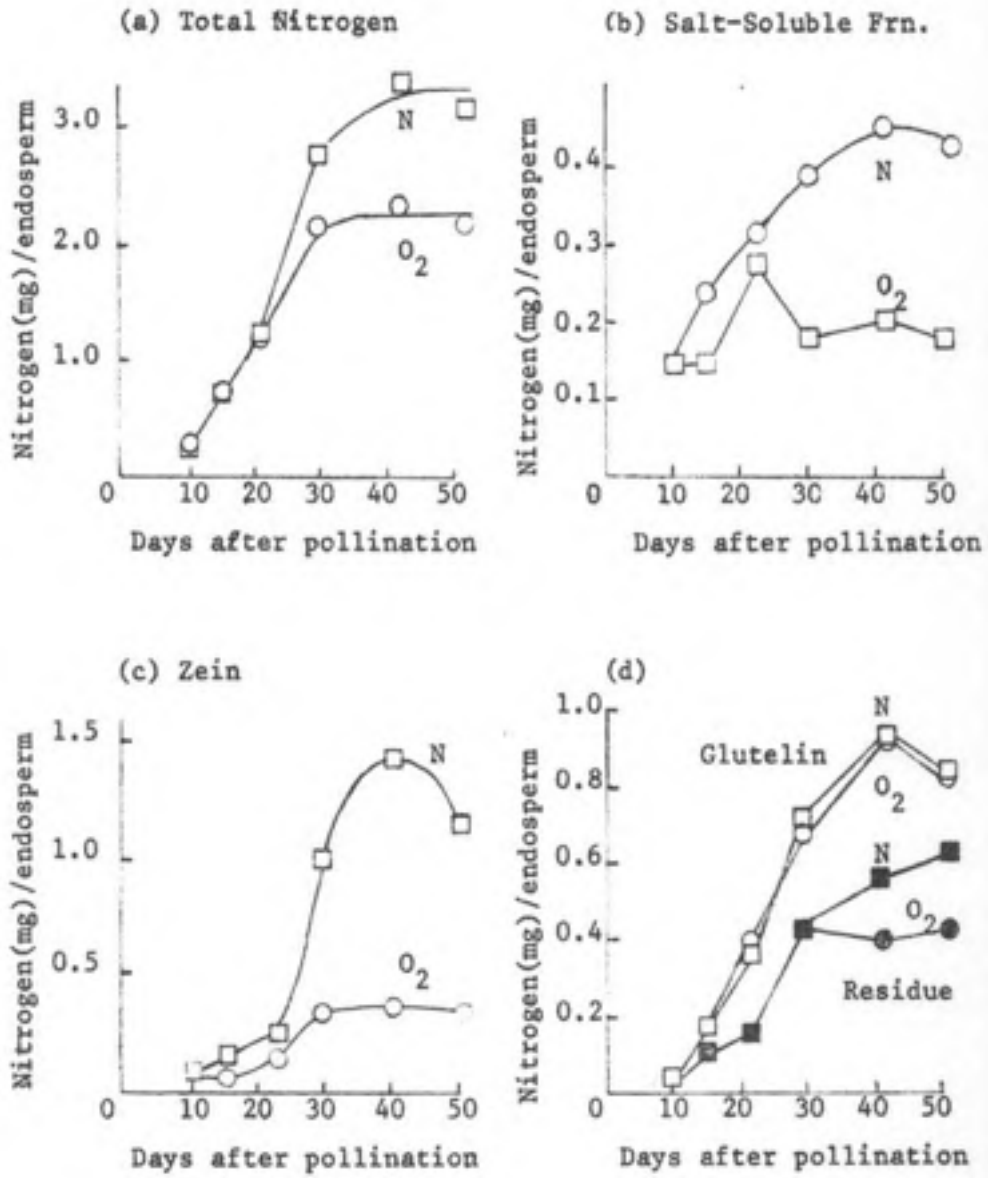
Although the proteins of the mature grain have received considerable attention, surprisingly little interest has been shown in the formation of these proteins during kernel development. Zeleny (1935) in 1935 and more recently Bressani and Conde (1961) examined the changes which occur in the proportions of various nitrogen fractions during development of the grain. However, to present the reader with an up-to-date picture of protein formation in the developing grain, the discussion will be restricted to two of the most important papers on this subject. The most recent of the two was done in great detail and employed the most up-to-date extraction procedure (Moureaux and Landry, 1972a). The earlier paper (Dalby, 1966; Murphy and Dalby, 1971) is also of interest in that it presents a comparison between normal and opaque-2 endosperm. This paper will be discussed first.

7.1. Formation of Endosperm Proteins During Grain Development of Normal and Opaque-2 Corn.

This experiment was carried out by Dalby and Murphy (Dalby, 1966, Murphy and Dalby, 1971) in the United States. They fractionated normal and opaque-2 endosperm proteins at different stages of development using the classical Osborne technique. The material used was the homozygous normal and opaque-2 versions of an inbred line, W64A.

In figure 7.1 it may be seen that the total nitrogen content of opaque-2 endosperm only reaches about two-thirds the level of that in normal.

Figure 7.1 Changes in total nitrogen (a), saline-soluble nitrogen (b), zein (c) glutelin and residue (d) nitrogen during development of normal (N) and opaque-2 (O_2) corn endosperm (Dalby, 1966)



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The levels of nitrogen in both endosperm types increase from 10 days after pollination to about 35 days after which it reached a plateau. During the period of rapid protein synthesis, between 20 and 30 days, the curves for normal and opaque-2 diverge due to the more rapid synthesis in normal endosperm.

The remaining figures (7.1.b,c,d.) present the data for the individual protein fractions. The saline-soluble fraction (note: in this case = globulins + albumins + amino acids) shows a marked increase in level in opaque-2 in contrast to an apparent steady level in normal. Since the free amino acids, albumins and globulins of this fraction were not studied separately, it is difficult to know what underlies these changes. It should be remembered that opaque-2 endosperm contains considerably more free amino acid than the normal at later stages at least (see Table 4.3).

Turning to the zein fraction (fig. c) one finds another sharp contrast between normal and opaque-2. In normal endosperm, zein synthesis starts more or less after 20 days and continues rapidly up to about 40 days after pollination. Zein synthesis in opaque-2, on the other hand, is slow and plateaus at a low level after 30 days. Clearly the opaque-2 gene considerably depresses the synthesis of zein.

The glutelin fraction (fig. d) is interesting in that it shows a steady increase from 10 to 40 days, the levels being identical for both opaque-2 and normal. This may, however, be simply coincidental since the amino acid analysis revealed much lower levels of lysine for normal glutelin than opaque-2 glutelin. In opaque-2 the lysine content of glutelin dropped from 5.7 to 3.8% (15 and 52 days) whereas that of normal dropped from 4.7 to as low as 1.1%. Thus the normal glutelin is probably more heavily

contaminated with zein than that of opaque-2; a deficiency of the classical Osborne extraction procedure discussed in Section 4.

Despite the drawbacks of the techniques used, the experiment clearly demonstrates the depressed synthesis of zein opaque-2 corn.

7.2. Formation of Kernel Proteins During Grain Development

This experiment, carried out by Moureaux and Landry in France, differs from the former in two important ways. First, the whole kernel is used rather than the isolated endosperm. Since the germ proteins are chiefly of the albumin-globulin type (Mertz, Lloyd and Bressani, 1958; Paulis and Wall, 1969) any divergences from the isolated endosperm most probably occur in these classes. Second, the extraction procedure used is superior and more meaningful (see section 4). Furthermore, no comparison was made with opaque-2 corn* but the amino acid composition of individual fractions taken at different stages of kernel maturation are given. This should give us an insight as to whether any qualitative changes take place in the fraction during kernel development.

In figure 7.2 are shown the changes in absolute level of individual nitrogen fractions during development of the corn kernel. The accumulation of total nitrogen in the grain starts shortly after pollination reaching a maximum about 9 weeks or so after pollination. This is much longer than found by Dalby for endosperm, suggesting that the American-grown corn matures more rapidly than the French-grown variety.

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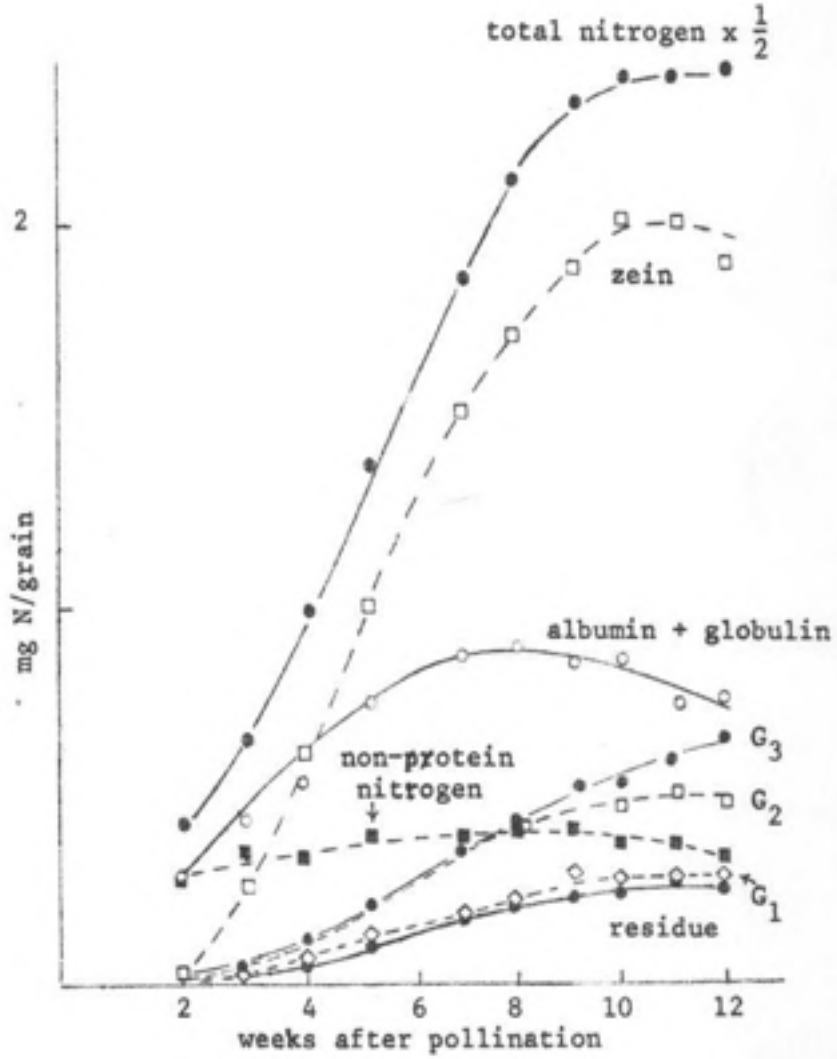
* preliminary data for opaque-2 corn is now available (see Moureaux and Landry, 1972b).

Figure 7.2 Changes in level of the Landry-Moureaux nitrogen fraction during the development of the corn kernel (Moureaux and Landry, 1972a)



The major storage proteins, zein and the glutelin are synthesized from about 2 weeks after pollination. The synthesis of zein and the glutelin components G1 and G2 falls to a low rate at about 8-9 weeks, whereas synthesis of the G3 fraction continues at a similar rate. Overall, each of the major storage proteins are synthesized at a rate approximately proportional to their final size in the mature kernel.

Synthesis of the combined albumin-globulin fraction has already started before 2 weeks, and continues to about 8 weeks after which there is a fall in level. Moureaux and Landry feel that the fall in level in the albumin and globulin fraction is the result of an aggregation of a part of this fraction to form part of the G3 fraction. In support of their idea, Moureaux and Landry show that the curve for the sum of the G3 and saline-soluble fraction is similar to other fractions in that it reaches a maximum near maturity. Furthermore, the G3 fraction is very similar in amino acid composition to the saline-soluble fraction.

From these results Moureaux and Landry put forward the following hypothesis. Saline-soluble proteins (albumin and globulin combined) and zein are synthesized directly from free amino acids. These proteins then provide, at least in part, the precursors of the glutelin proteins G3 and G1, respectively. In other words, polypeptides of the saline-soluble group aggregate to form the G3 fraction, and zein peptides are used in the formation of the G1 class. As for the G2 fraction, which has an amino acid composition in between that of zein and saline-soluble proteins, Moureaux and Landry feel it is not inconceivable that the precursors are provided by both of these groups.

Another important conclusion reached by Moureaux and Landry was that the amino acid compositions of each of the

protein fractions remained reasonably constant throughout the period of development, suggesting that the same proteins are being synthesized at the beginning of development as at the end. Furthermore, this finding strengthens the value of their extraction procedure because it shows that the solvents used are selective for a distinct class of protein.

Although the amino acid compositions of the protein fractions showed negligible variations during kernel development, that of the free amino acid fraction varied tremendously (Table 9.17, appendix). However, the total nitrogen content of this fraction remained fairly constant during development (fig. 7.2). Noteworthy is the fall in levels of alanine and glutamic acid (+ glutamine), the constant level of serine and valine, and the increase in all the others especially the basic amino acids, glycine, proline, and aspartic acid (+ aspargine). The latter represents 25% of the total amino acid residues in the mature grain. A similar amino acid composition has been noted for the mature endosperm (Table 9.9).

A further interesting point concerning the free amino acid fraction is that all amino acids closely related to carbohydrate metabolism are present in high amounts whereas those distant (i.e. with long biosynthetic pathways) are present in low amounts. It is not known whether amino acids are actually synthesized in the developing kernel or transported there preformed. If they were synthesized, then by analogy with bacterial systems it seems possible that amino acids with long biosynthethic pathways are under feedback control*, which would account for their low level. The case for leucine may be taken

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* feedback control is a system whereby the product of a metabolic pathway inhibits the activity of the first enzyme of that pathway, thereby controlling its own rate of biosynthesis and preventing unnecessary production.

to illustrate this point. Leucine is one of the most abundant amino acids in endosperm protein yet the pool size of the free amino acid is one of the smallest. It has been calculated (Wilson, unpublished) that during the period of rapid protein synthesis 4 times the amount of free leucine present is incorporated into protein per day. Since there is no evidence that leucine is transported to the grain at such a rate, it is reasonable to postulate that it may be synthesized in the grain and that its synthesis is under feedback control. The nature of nitrogen transport to the grain poses an interesting problem for the future.

8. AMINO ACID ANALYSIS

It may be apparent from the work on corn proteins discussed in this article that, whatever type of fractionation procedure is employed, amino acid composition is invariably used as a "finger-print" of the protein. Although amino acid analysis of proteins must be considered a crude method for characterizing a protein, the technique has been in fact very useful as far as the study of corn proteins is concerned. If we look back at some of the amino acid composition data on different corn proteins, we see that most of the fractions have some special characteristic in amino acid content to distinguish it easily from other fractions. For example, zein contains virtually no lysine and is high in non-polar amino acids. Zein-2 has high levels of the sulfur amino acids, cystine and methionine. High levels of histidine characterize the glutelin G2 fraction and lysine and arginine the glutelin G3, albumin and globulin fractions.

The technique of amino acid analysis will not be discussed here in any detail. Instead, the reader will be given a general idea of how an amino acid analysis is carried out, together with some of the pitfalls to watch out for.

8.1. Routine Amino Acid Analysis

In order to carry out routine analyses of amino acid mixtures, automated equipment is essential. There are two basic processes in use for the automatic separation and quantitative determination of amino acids; ion-exchange and gas-liquid chromatography. The latter was developed more recently and has the advantage of being cheaper, more rapid and more sensitive. An excellent up-to-date review of this technique may be found in the Journal of Agricultural and Food Chemistry,

written by Gerhke, one of the most prolific workers in this field (Gerhke, Zumwalt and Kuo, 1971). The classical ion-exchange technique, on the other hand, was developed by Moore and co-workers culminating in the design of the automatic amino acid analyzer (Spackman, Stein and Moore, 1958).

In order to analyse protein samples for amino acid content they must first be hydrolised to liberate the amino acids. This is usually done by incubating the sample with an excess of 6N HCl in an evacuated or nitrogen-filled sealed tube for 24 hours at 110° C. This process destroys tryptophan, which must be assayed separately*, leads to small losses of treonine and serine which can be corrected for if high precision is necessary. Cystine and methionine will also be destroyed if oxygen is present during hydrolysis. If care is taken the values for these amino acids may be taken as correct, but for high precision and certainty they should be analysed separately (method by Moore, 1963). It should be noted that the vast majority of publications involving corn proteins do not present data for tryptophan; moreover methionine and cystine are not usually analysed separately, and therefore should be regarded with caution.

Two other amino acids affected by the acid hydrolysis are glutamine and aspargine. The labile amide group is hydrolysed leaving glutamic acid and aspartic acid. Thus the values given in an amino acid analysis for aspartic and glutamic acids are actually a sum of aspartic acid/aspargine and glutamic acid/glutamine, respectively. Since the hydrolysis of the amide group also liberates ammonia, an estimation of the

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* methods may be found in a review by Friedman and Finley, 1971; Two of the better methods are by Spies, 1968 and Gaitonde and Dovey, 1970.

amide content (total only) can be made from the ammonia content of the hydrolysate. However, a suitable blank must be run since the buffers (for elution by ion-exchange) and hydrochloric acid contain significant amounts of ammonia. Very few investigators bother with this estimation and even when ammonia values are given these should be considered as overestimated unless the authors specifically mention the accuracy of their data.

After hydrolysis, the samples are dried on a rotary evaporator. If ion-exchange is to be used, the sample is simply taken up in a suitable volume of the appropriate buffer and an aliquot placed on the column. When gas-liquid chromatography is used, the sample must first be cleaned (e.g. to remove humin*) and the amino acids converted to certain volatile derivatives (Gerhke et al., 1971) before injection onto the column.

During chromatography of the samples, both systems will automatically record the elution of an amino acid as a peak on a moving chart. At the end of the run, the chart will present a sequence of peaks corresponding to the different amino acids. The identity of the peak is determined by comparison with an authentic amino acid on the basis of position or elution time.

The quantitative determination of each amino acid is made by measuring the area under the peak comparing it with that produced by a known amount of the standard amino acid. The process is simplified if the instrument is equipped with a peak integrator which prints out a number proportional to the area of the peak.

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* when samples contain significant amounts of carbohydrate, a brown substance called humin will form during hydrolysis.

8.2. Expressing the Results

The presentation of the results is a matter of choice since there is no standard way of expressing the data. In the literature one will find the following:

a) mole % - number of molecules or residues of each amino acid per 100 residues of all amino acids (recovered).

b) g % - grams of each amino acid per 100 grams of protein in the sample*.

c) mMoles/16g N - number of residues in millimoles per 16 grams of nitrogen in the sample**.

d) g/16g N - grams of each amino acid per 16 grams of nitrogen in the sample**.

e) g/g N - grams of each amino acid per gram of nitrogen in the sample.

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* sometimes taken to mean grams per 100 grams of amino acid. If so the data would total 100. Note that 100 grams of protein is not equivalent to 100 grams of amino acid, but to about 118 grams due to the molecule of water incorporated during hydrolysis.

** 16 grams of nitrogen is the approximate weight of nitrogen in 100 grams of many proteins (16g N x 6.25 = 100 g protein).

It should be noted that only the mole % unit is a true percentage, since the data is based on the total number of moles of amino acids recovered after chromatography. In each of the remaining cases the values are based on a nitrogen determination of the original sample. It is preferable to use "16g N" rather than "100 g protein", since the factor of 6.25 (x16g N = 100 g protein) used for corn proteins in general may not be very accurate for individual proteins. Thus, when the unit "per 100 grams protein" is used this really means "per 16 g N". A further point is that the "g/16g N" unit (or g/100 g protein) should theoretically add up close to 100. If the average molecular weight of the amino acids (minus a molecule of water when protein-bound) were 100 and the sample truly contained 100 grams of protein, then the values should total 118 due to the molecule of water added to each amino acid during hydrolysis. Similarly 100 grams of protein would contain 1000 mMoles of amino acids, so data in 'mM/16gN' should theoretically add up to 1000. Since the average molecular weight of the amino acids (which will vary for different proteins) is generally more than 100, then the totals usually fall short of 118 grams and 1000 mMoles.

Finally, it may be noted that the term "$\frac{1}{2}$ - cystine" is used in the amino acid analysis because, although this amino acid is determined in the form of cystine, the data is preferably expressed in terms of cysteine residues (cystine consists of two cysteine residues joined by a disulfide bond).

9. APPENDIX - FLOW-SHEETS OF EXTRACTION PROCEDURES

9.1. Flow-sheet I - Osborne procedure

STEP	SOLVENT	TEMP.	TIME OF EXTRN.	FRACTION
I	1 g defatted powder + 10 mls water	0° C	½ - 1 hr	Combined supt.
	"		½ - 1 hr	AMINO ACIDS * + ALBUMIN
	"		½ - 1 hr	
	"		wash	
II	residue+10 mls 0.5 M NaCl	0° C	½ - hr	
	"		½ - 1 hr	Combined supt.
	"		½ - 1 hr	GLOBULINS
	+ 10 mls water		wash	
III	residue+10 mls 70% ethanol	room°t	½ hr	
	"		2 hr	Combined supt.
	"		1 hr	ZEIN
	"		wash	
IV	residue+10 mls 0.2% NaOH	room°t	½ hr	
	"		1 hr	Combined supt.
	"		1 hr	GLUTELIN
	+ 10 mls water		wash	
	RESIDUE			

Stirr occasionally or continually with solvent. Centrifuge at 1,000 x g for 15' at the appropriate temperature.

* Amino acids and albumin may be separated by dialysis against water, or overnight precipitation of albumin with 5% trichloroacetic acid.

9.2. Flow-sheet Ia - Osborne procedure

- for better separation of albumin and globulin, e.g. in electrophoretic studies. Adapted from Paulis and Wall, 1969 and Paulis, James and Wall, 1969.

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1 g defatted powder

↓

3 extractions with 10 mls 0.5 M NaCl at 0º C for 1 hour each. Wash with water and combined supernatants.

(= GLOBULIN + ALBUMIN + AMINO ACIDS)

↓

for ZEIN and GLUTELIN continue as Flow-sheet I.

NaCl extract (= GLOBULIN + ALBUMIN + AMINO ACIDS)

- dialyse against several changes of water at 0 - 4º C.

Amino acids will be dialysed out. Centrifuge dialysis bag contents: precipitate = GLOBULINS
supernatant = ALBUMINS

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9.3. Flow-sheet II - Osborne, modified procedure

From Sodek and Wilson, 1971a

STEP	SOLVENT	TEMP.	TIME OF EXTRN.	FRACTION
I	1g defatted powder			
	+ 10 mls water	0°C	½ - 1 hr	Combined supt.
	"		½ - 1 hr	AMINO ACIDS + ALBUMIN
	"		½ - 1 hr	
↓	"		wash	
II	residue+10 mls 0.5 M NaCl	0°C	½ - 1 hr	
	"		½ - 1 hr	Combined supt.
	"		½ - 1 hr	GLOBULIN
↓	+10 mls water		wash	
III	residue+10 mls 55% iso-propanol	room°t	½ hr	
	"		2 hr	Combined supt.
	"		1 hr	ZEIN-1
↓	5 mls "		wash	
IV	residue+10 mls. 55% iso-propanol containing	room°t	½ hr	
	0.6% 2-mercaptoethanol		½ hr	Combined supt.
	"		½ hr	ZEIN-2
↓	5 mls 55% isopropanol		wash	
V	residue+10 mls 0.2% NaOH	room°t	½ hr	
	"		2 hr	Combined supt.
	"		1 hr	GLUTELIN
↓	10 mls water		wash	
	RESIDUE			

Footnotes: as in Flow-sheet 1.

9.5. Flow-sheet IV - Mertz Procedure

from Mertz and Bressani, 1957; Mertz, Lloyd and Bressani, 1958; Lloyd and Mertz, 1958; Bressani and Mertz, 1958.

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1 g GROUND, DEFATTED CORN

↓

Step I - suspend in 15 ml copper reagent*, stirr in 1 M NaOH to pH 11.5, continue stirring for 1-2 hours at room temperature,

centrifuge

↓ Supernatant — ↓ RESIDUE

Step II - adjust to pH 3.0 with 4N HCl,

centrifuge

↓ Supernatant — ↓ precipitate

ACID-SOLUBLE FRACTION

Step III - wash with HCl solution pH 3, followed by water

↑ washings — ↓ washed precipitate

Step IV - dissolve in dilute NaOH to pH 11.5, bring to 6 ml with water, add 12 ml ethanol, adjust to pH 6 with 10% acetic acid, leave at 4° C overnight,

centrifuge

↓ supernatant → ZEIN — ↓ precipitate → GLUTELIN

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* copper reagent: solution of 0.45% $CuSO_4.5H_2O$ and 0.06% Na_2SO_3.

Table 9.6 - Amino Acid Composition of the Germ and Endosperm Proteins

from Sodek (unpublished)

	mole %	
	ENDOSPERM	GERM
x Lysine	1.7	6.3
Histidine	1.9	2.7
Arginine	1.7	7.6
Aspartic Acid	5.7	8.2
x Threonine	3.5	4.3
Serine	5.9	5.8
Glutamic Acid	19.1	13.8
Proline	11.0	4.6
Glycine	4.9	11.5
Alanine	12.3	10.0
$\frac{1}{2}$ Cystine *	-	1.1
x Valine	5.5	7.2
x Methionine *	1.7	1.2
x Isoleucine	3.9	3.5
x Leucine	15.2	7.0
Tyrosine	2.0	1.8
x Phenylalanine	4.0	3.4

* values may be underestimated, since oxygen was not excluded during hydrolysis.

x essential amino acids.

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Table 9.7 - Amino Acid Composition of Normal and Opaque-2 Endosperm (g/100 g protein)

From Bates, 1966

	NORMAL	OPAQUE-2
Lysine	1.7	3.6
Histidine	2.8	3.7
Arginine	3.1	5.1
Aspartic Acid	7.4	9.9
Threonine	3.3	3.7
Serine	5.1	4.4
Glutamic Acid	23.7	19.6
Proline	10.0	9.5
Glycine	2.6	4.4
Alanine	8.9	6.3
$\frac{1}{2}$ Cystine	1.0	2.4
Valine	5.0	5.7
Methionine	1.6	2.1
Isoleucine	4.3	3.8
Leucine	16.7	10.5
Tyrosine	5.0	4.6
Phenylalanine	5.7	4.5

Table 9.8 - Amino Acid Composition of Normal and Opaque-2 Glutelins (Osborne)

From Jimenez, 1966

AMINO ACID	g/100 g protein NORMAL	OPAQUE-2
Lysine	3.43	4.73
Histidine	4.40	3.46
Arginine	5.42	7.76
Aspartic Acid	6.45	8.53
Threonine	3.69	4.13
Serine	4.94	4.97
Glutamic Acid	19.83	15.92
Proline	8.71	6.54
Glycine	4.08	4.67
Alanine	7.12	6.38
$\frac{1}{2}$ Cystine	1.02	0.88
Valine	5.88	7.42
Methionine	2.26	2.33
Isoleucine	3.97	4.54
Leucine	11.53	10.28
Tyrosine	2.48	2.17
Phenylalinine	4.80	5.30

Table 9.9 - Amino Acid Composition of the Free Amino Acid, Albumin, and Globulin Fractions of Normal and Opaque-2 Endosperm (mole %)

From Sodek and Wilson, 1971a.

	Free Amino Acid		Albumin		Globulin	
	Normal	Opaque-2	Normal	Opaque-2	Normal	Opaque-2
Lysine	2.5	4.2	5.4	5.6	5.0	5.2
Histidine	0.6	0.8	1.9	1.8	2.0	1.9
Arginine	1.1	1.5	5.3	4.9	5.5	6.0
Aspartic Acid	24.8	25.9	8.2	9.2	8.6	9.3
Threonine	1.2	1.0	5.0	5.4	4.9	4.7
Serine	4.6	4.0	5.6	5.6	5.6	5.3
Glutamic Acid	17.4	21.3	12.3	10.6	11.6	10.9
Proline	19.6	8.6	6.9	6.6	7.4	6.9
Glycine	4.4	1.9	10.1	10.5	11.3	12.2
Alanine	5.9	2.2	11.0	10.4	10.4	10.7
$\frac{1}{2}$ Cystine	-	0.3	0.2	1.4	1.2	0.3
Valine	1.1	1.4	7.1	7.6	7.0	7.1
Methionine	-	-	1.3	1.3	1.2	1.3
Isoleucine	0.5	0.6	4.4	4.7	4.1	4.0
Leucine	0.6	0.8	9.3	8.6	8.1	7.6
Tyrosine	1.7	1.5	2.5	2.6	2.6	3.0
Phenylalanine	1.0	0.9	3.3	3.3	3.6	3.7
Aspargine	7.9	7.2				
Glutamine	4.9	15.9				

Table 9.10 - Amino Acid Composition of Zein-1, Zein-2, Glutelin and Residue Fractions of Normal and Opaque-2 Endosperm (mole %)

From Sodek and Wilson, 1971a.

	ZEIN-1		ZEIN-2		Glutelin		Residue	
	Normal	Opaque-2	Normal	Opaque-2	Normal	Opaque-2	Normal	Opaque-2
Lysine	0.1	0.1	0.1	0.1	4.7	4.7	4.5	6.0
Histidine	0.7	0.9	1.6	1.7	3.5	3.2	2.7	2.9
Arginine	1.0	1.4	1.6	1.3	4.2	4.3	4.2	4.7
Aspartic Acid	5.4	5.0	3.6	3.6	7.3	7.9	8.1	7.9
Threonine	2.9	2.8	2.9	3.2	4.3	4.5	4.4	4.6
Serine	5.7	6.0	5.0	5.1	5.2	5.5	5.4	5.7
Glutamic Acid	21.0	20.3	19.2	18.9	14.1	13.2	12.0	11.0
Proline	10.2	11.6	12.6	13.6	9.6	9.5	9.1	7.7
Glycine	2.1	2.6	5.2	5.6	8.5	8.8	8.9	10.2
Alanine	14.2	12.8	12.1	10.9	8.6	8.6	10.4	9.9
$\frac{1}{2}$ Cystine	0.1	0.5	1.7	1.7	1.4	0.8	-	-
Valine	4.8	3.5	3.8	4.1	7.4	7.7	7.7	8.0
Methionine	0.3	0.4	5.6	5.5	1.7	1.8	2.0	2.0
Isoleucine	4.2	4.1	2.7	2.8	4.2	4.4	4.3	4.3
Leucine	19.5	19.2	14.6	14.0	9.1	9.0	10.4	9.0
Tyrosine	2.9	3.4	4.2	3.4	2.8	2.8	2.2	2.1
Phenylalanine	4.8	5.4	3.5	4.2	3.3	3.4	3.8	3.8

Table 9.11 - Amino Acid Composition of the G1, G2 and G3 Components of Osborne Glutelin and the Residue Fraction of the Corn Kernel (mole %)

From Landry and Moureauz, 1970.

	GLUTELIN G1	GLUTELIN G2	GLUTELIN G3	RESIDUE
Lysine	0.1	1.1	5.1	4.2
Histidine	1.0	6.2	2.5	3.6
Arginine	1.4	3.0	5.0	3.8
Aspartic Acid	4.0	2.1	8.0	6.6
Threonine	3.2	4.6	5.0	6.1
Serine	6.4	5.2	6.4	5.6
Glutamic Acid	19.4	19.8	12.2	11.1
Proline	11.5	19.5	6.8	12.8
Glycine	4.1	7.8	9.2	11.4
Alanine	12.8	6.9	10.6	8.6
$\frac{1}{2}$ Cystine	-	0.2	-	0.7
Valine	3.7	6.5	7.0	7.0
Methionine	5.4	0.8	2.2	1.3
Isoleucine	2.8	2.2	4.0	3.1
Leucine	15.9	9.2	9.5	8 2
Tyrosine	4.3	2.6	2.5	2.9
Phenylalanine	4.1	2.1	3.8	3.0

Table 9.12 - Amino Acid Composition of Zein

'Cold' zein = zein extracted at room temperature (25° C)

'Hot' zein = zein extracted at 60° C after extraction of the 'cold' zein (from Mossé et al., 1966)

	mole %	
	'Cold' Zein	'Hot' Zein
Lysine	0.1	0.1
Histidine	0.6	1.1
Arginine	1.1	1.2
Aspartic Acid	4.8	4.2
Threonine	3.0	3.0
Serine	6.7	6.4
Glutamic Acid	20.5	20.4
Proline	10.4	11.2
Glycine	2.5	3.5
Alanine	13.6	12.8
$\frac{1}{2}$ Cystine	1.0	1.8
Valine	3.9	3.9
Methionine	1.4	2.4
Isoleucine	3.3	2.8
Leucine	18.5	17.3
Tyrosine	3.6	3.3
Phenylalanine	5.1	4.4

Table 9.13 - Amino Acid Composition of Zeins Extracted by 70% Ethanol and 70% Ethanol with 0.5% Sodium Acetate

From Paulis, James and Wall, 1969.

	mMoles/16g N			
	70% Ethanol		70% Ethanol-Acetate	
	Normal	Opaque-2	Normal	Opaque-2
Lysine	0.6	1	1	0.7
Histidine	8	8	28	16
Ammonia	148	183	200	197
Arginine	10	11	12	12
Aspartic Acid	41	38	36	36
Threonine	24	24	29	28
Serine	52	52	52	52
Glutamic Acid	166	157	180	174
Proline	94	102	70	117
Glycine	17	20	33	32
Alanine	110	108	100	95
Valine	31	25	31	32
Methionine	10	5	10	9
Isoleucine	31	31	30	29
Leucine	151	157	148	149
Tyrosine	31	30	32	31
Phenylalanine	43	44	43	41

Table 9.14 - Amino Acid Composition of Various Glutelins from Normal and Opaque-2 Endosperm (mM/16 g N) - From Paulis, James and Wall, 1969.

	NaOH- soluble (Glutelin)		After Enzymic Removal of Starch					
			Native		Extracted Alk-Red**			
	Normal*	Opaque-2*	Normal	Opaque-2	Normal	Opaque-2	Normal	Opaque-2
Lysine	18	24	21	35	27	32	29	31
Histidine	18	18	21	26	30	32	27	29
Ammonia	202	214	198	136	127	119	124	127
Arginine	30	27	28	35	31	33	31	33
Aspartic Acid	49	53	54	66	48	51	49	49
Threonine	34	33	35	39	29	40	35	37
Serine	47	44	47	48	46	43	44	45
Glutamic Acid	131	113	130	121	128	122	122	118
Proline	73	72	76	72	90	87	96	100
Glycine	62	62	61	70	72	74	67	73
Alanine	79	69	76	74	74	68	70	67
Valine	45	52	44	55	51	54	57	62
Methionine	25	14	25	17	17	19	16	13
Isoleucine	29	27	32	34	31	32	29	30
Leucine	85	71	87	79	82	78	74	74
Tyrosine	31	23	30	25	25	23	21	21
Phenylalanine	27	25	24	26	26	25	23	23

* Previous extraction, alcohol; all the rest alcohol-acetate.

** Enzyme-treated glutelin reduced and alkylated and then 70% ethanol-soluble components removed.

Table 9.15 - Amino Acid Composition of Mertz Copper Fractions of Normal and Opaque-2 Corn (g/100g protein)

From Mertz et al., 1966

	ACID-SOLUBLE		ALCOHOL-SOLUBLE		ALKALI-SOLUBLE	
	Opaque-2	Normal	Opaque-2	Normal	Opaque-2	Normal
Lysine	5.9	1.8	1.0	0.3	3.7	3.6
Histidine	6.5	1.2	2.1	1.6	3.8	4.2
Ammonia	2.8	3.1	4.5	2.5	1.9	2.2
Arginine	10.0	2.8	3.1	2.5	5.8	5.3
Aspartic Acid	7.4	4.8	5.8	6.0	6.8	6.9
Threonine	2.3	2.4	3.4	3.4	3.3	3.4
Serine	3.7	3.9	5.6	6.5	3.7	3.7
Glutamic Acid	15.9	15.7	23.5	26.0	12.9	14.6
Proline	9.9	10.9	10.5	10.5	8.7	10.1
Glycine	3.1	2.6	2.6	2.1	3.3	3.8
Alanine	4.8	5.4	8.8	10.2	4.5	4.7
Cystine	-	-	-	-	-	-
Valine	2.4	2.4	4.2	4.5	5.4	5.6
Methionine	0.8	1.4	1.6	2.5	1.4	1.1
Isoleucine	2.2	3.0	4.2	4.2	3.4	3.4
Leucine	7.4	10.1	18.8	20.3	8.1	8.6
Tyrosine	2.8	3.4	4.8	5.4	2.9	3.1
Phenylalanine	3.2	3.2	7.1	7.1	3.8	3.8

Table 9.16 - Amino Acid Composition of Endosperm Proteins Fractionated on a Sucrose Density Gradient (mM/16g N). From Christianson, Nelsen, Khoo, Wolf and Wall, 1969. Zones II, III and IV correspond to protein peaks in the gradient, see fig. 4.7

			Matrix and Protein Bodies Zone IV		Osborne extraction procedure	
	Zone II	Free protein bodies Zone III	Ethanol-soluble	Ethanol-insoluble	Zein	Glutelin
Lysine	51	5	1	22	1	17
Histidine	17	19	15	41	9	29
Ammonia	72	137	129	116	191	132
Arginine	48	16	13	30	11	29
Aspartic Acid	80	36	37	31	47	41
Threonine	42	27	24	29	27	36
Serine	50	51	50	40	52	53
Glutamic Acid	88	172	182	131	190	150
Proline	58	146	152	155	108	121
Glycine	70	29	19	50	19	61
Alanine	79	98	109	61	128	86
$\frac{1}{2}$ Cystine	-	18	14	34	4	16
Valine	66	32	28	41	38	46
Methionine	21	16	13	14	3	16
Isoleucine	41	28	31	23	42	25
Leucine	72	144	175	85	178	99
Tyrosine	24	31	33	26	33	31
Phenylalanine	34	39	47	23	57	31

Table 9.17 - Amino Acid Composition of the Free Amino Acid Fraction at Different Stages of Development of the Corn Kernel

(mole %)

From Moureaux and Landry, 1972

	Weeks after pollination			
	2	5	9	12
Lysine	1.6	5.5	6.0	5.2
Histidine	0.8	1.1	1.2	1.6
Arginine	0.6	1.4	2.5	4.5
Aspartic Acid *	12.7	12.6	14.6	23.9
Threonine	2.4	3.5	3.6	3.5
Serine	6.5	7.0	6.4	6.3
Glutamic Acid *	34.3	29.1	21.4	13.1
Proline	8.4	8.7	12.4	13.2
Glycine	4.1	6.3	6.6	8.9
Alanine	21.8	15.5	15.7	7.6
Valine	2.3	2.5	1.8	2.3
Methionine	-	-	-	-
Isoleucine	0.7	1.3	1.3	1.8
Leucine	0.9	1.1	1.1	1.5
Tyrosine	0.8	1.4	1.7	1.7
Phenylalanine	0.2	1.0	0.9	1.0

* includes amides

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