RECENT RESULTS IN CEREAL PROTEIN RESEARCH*

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Introduction

OSBORNE was the first to develop scientifically systemazized concepts on cereal proteins including wheat and gluten proteins. In Hungary, extensive research in this field by KOSUTÁNY and later by VUK, former head of the Department of Food Chemistry at our University, yielded a comprehensive picture on the proteins of Hungarian wheats and established several new notions on the factors affecting the rheological properties of gluten. Over sixty years have passed since that time and our knowledge on cereal proteins has substantially increased. The application of up-to-date fractionating techniques (electrophoresis, chromatography, molecular sieves) allowed the separation of wheat proteins into many fractions and the preparation of many constituents in the pure state. We have an exact picture on the amino acid composition of the individual fractions and many data have been collected on their terminal groups. The first steps have been made in the study of the structure of gluten protein constituents (molecular weight, conformation, number of polypeptide chains, system of disulphide bonds, investigations of partial hydrolysis products etc.).

In many fields, however, no fundamentally new results as compared to the classical statements of OSBORNE have been achieved. This applies e.g. to the processes leading to wheat protein formation including gluten formation and particularly to the relationships between the chemical structure and the rheological properties of gluten proteins. In spite of many interesting partial results, we still do not dispose of a clear picture in this field.

On other cereal proteins there is much less knowledge available then on wheat. This is particularly the case for several Hungarian cereals (maize, rice, rye, triticale etc.).

Intense research on cereal proteins has been going on at our Department for over ten years, with the double objective of studying the separation of proteins, their composition and structure, and, on the other hand, of developing novel technologies for cereal processing, by investigating the relationships

* Dedicated to Prof. L. Telegdy Kováts on the occasion of his 70th birthday.

between the rheological properties and the structure of gluten proteins. By a complex application of modern biochemical, rheological and analytical methods, we succeeded in obtaining considerable results in wheat, maize and rice biochemistry and processing. The present paper will summarize some results of this research work and will briefly deal with some general problems of cereal protein chemistry.

Some results on the fractionation of wheat proteins

What we consider most important among results obtained on the fractionation of wheat proteins is the fact that we succeeded in separating several conjugated proteins and several enzyme proteins. This finding basically changes the picture on wheat proteins and their classification, and, essentially, adds a new chapter to wheat protein chemistry.

This is made clear by Table 1, summarizing the classification of wheat proteins according to OSBORNE and to our present notions. Diagrams 1 and 2 demonstrate the preparation of purothionin and wheat proteolipide, resp.

Table 1

Classification of wheat proteins

According to Osborne	According to our present knowledge
	Simple proteins
Albumin	Hystons
Globulin	Albumins
Gliadin	Globulins
Glutenin	Gliadins
Proteose	Glutelins
	Enzym proteins
	Conjugated proteins
	Nucleoproteins
	Lipoproteins
	Glycoproteins
	Enzym proteins

by way of example of the applied separation technique. Referring to the structure of conjugated proteins, Diagram 3 presents the structure of the polysaccharide part of wheat glycoprotein. A more detailed survey on conjugated proteins was given in an earlier paper [1].

Diagram 1

Preparation of purothionin

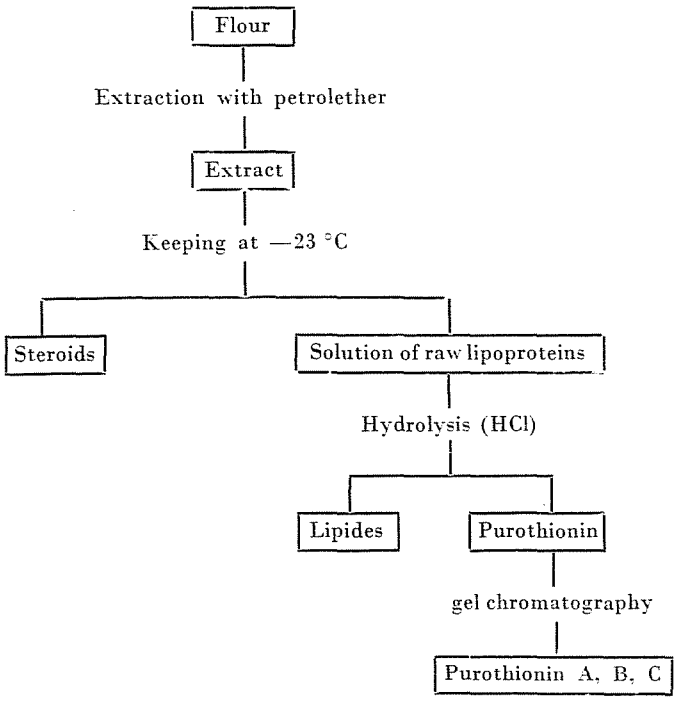


Diagram 2

Preparation of wheat proteolipide

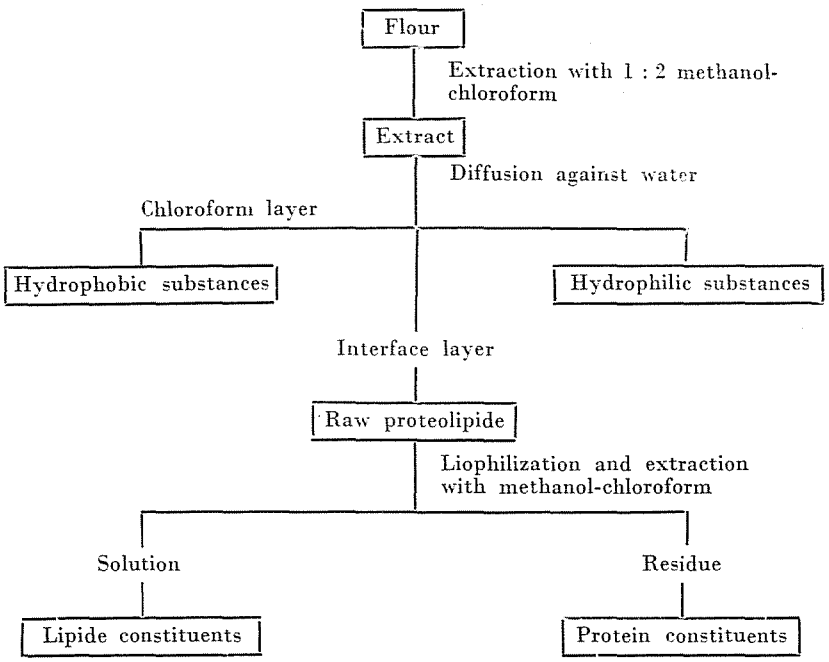
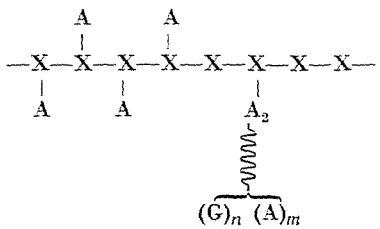


Diagram 3

Hypothetical structure of wheat glycoprotein



A = Arabinose
X = Xylose
G = Galactose
~~~ = polypeptide chain

It would facilitate a more exact elucidation of wheat protein and in general of cereal protein composition if the individual constituents (polypeptide chains) could be isolated. However, up to the present we were successful in some cases only. By a complex application of fractionating techniques, we obtained from gliadin several gamma gliadin constituents likely to be fully homogeneous. Some pure fractions of wheat albumins and globulins were also isolated.

One of the main difficulties in separation consists is the intereaction of the individual protein fractions impairing the sharpness of fractionation. To eliminate or lessen this difficulty, we developed a method for the separation of chemically modified gluten proteins. The modifying reactions and the separation techniques are presented in Diagrams 4 and 5. It has been demonstra-

Diagram 4

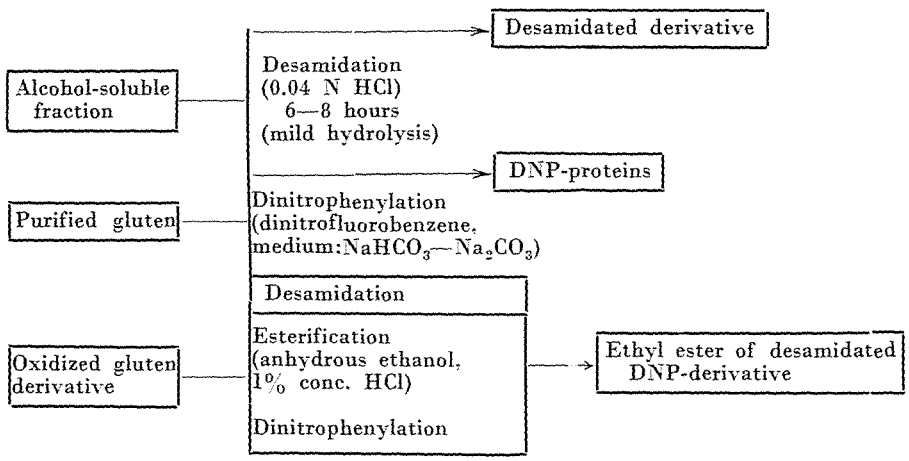

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Diagram 5

Main parameters of gel chromatography

	Gluten	Oxidized gluten	Gliadin
Column dimensions	2×60 cm 3×60 cm	2×60 cm	4×120 cm
Eluent buffer	Pyridine-glacial acetic acid-water (400 : 20 : 580) 0.1 M Na_2HPO_4	Pyridine-glacial acetic acid-water (400 : 20 : 580) Phosphate buffer solution (pH 8)	
Gel type	Sephadex G—100 Sephadex G—200	Sephadex G—100	Sephadex G—200
Swelling time	48 hours	48 hours	48 hours
Method for protein determination	Photometry 286 nm 380 nm	Photometry 285 nm	Photometry 380 nm
Sample weight	25 mg	80 mg	30 mg

ted that a chemical modification of some groups of gluten proteins favourably changed solubility conditions and reduced the interaction between proteins. Based on these facts, a more effective separation method by gel chromatography was developed.

Structure of wheat proteins

According to molecular weight determinations by ultracentrifuging gluten proteins are built up of constituents with extremely heterogeneous molecular weights, ranging from 20 000 to 3 or 8 million. By applying treatments splitting the disulphide bonds (oxidation by performic acid, reduction, sulphitolysis), a product with a much more homogeneous molecular weight distribution (20 000 to 60 000) is obtained, indicating that gluten proteins with higher molecular weight are composed of several polypeptide chains connected by disulphide bonds. Little is known on the primary structure of the individual polypeptide chains. In our Institute, we have been intensely studying the determination of N- and C-terminal amino acids for many years. A number of methodological problems could be solved and detailed qualitative and quantitative are available data. It has been found that gluten proteins contain at least 10, but probably 40 to 50 different polypeptide chains. Some data are presented in Tables 2 and 3. In addition to the rather well-known C- and N-terminal amino acids, some products of partial hydrolysis could be identified. However, from these data only a few conclusions may be drawn as yet. It can be stated e.g. that the distribution of amino acids belong-

Table 2

N-terminal amino acids of some gluten constituents

N-terminal amino acid	Gliadin	Glutenin
	micromoles of N-terminal amino acid per 1 g of protein	micromoles of N-terminal amino acid per 1 g of protein
Asparagic acid	1.10	0.73
Glutamic acid	1.30	0.89
Serine	1.00	0.97
Threonine	0.61	0.64
Glycocoll	1.57	0.30
Alanine	0.70	0.73
Valine	3.40	1.96
Leucine	1.12	0.63
Phenylalanine	0.31	0.24
Hystidine	4.30	3.5

Table 3

C-terminal amino acids of wheat gliadin
(Nedelkovits, Wöller, 1970)

C-terminal amino acid	Amount (number of end groups per 100 amino acids)
Serine	0.70
Proline	0.79
Glycine	0.65
Alanine	0.15
Leucine	0.24
Tyrozine	0.02
Phenylalanine	0.35

ing to different types (hydrophobic, polar etc.) is not uniform along the chain and therefore characteristic hydrophobic sections are formed.

Calculations based on rotational dispersion measurements indicate that the share of the helix part in polypeptide chains of gluten constituents is low and generally does not exceed 15 to 20%. The rest of the polypeptide chains is characterized by the random coil state. As to the tertiary structure, no factual experimental data are at disposal as yet.

Our present experimental results allow to outline the probable structure of low and high molecular weight constituents of the gluten complex. This assumed structure is presented in Figs 1 and 2. We have reported of the structure of high molecular weight gluten protein constituents at an international symposium in Riga [2].

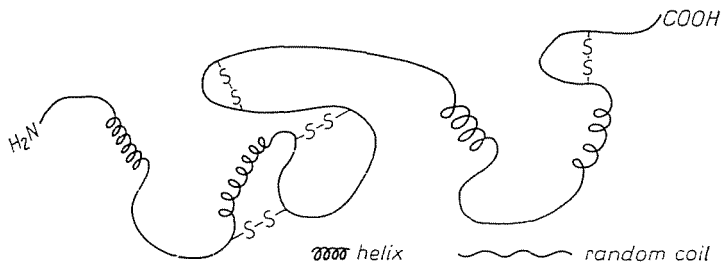

Fig. 1. Structure of low molecular weight gluten protein

Fig. 2. Structure of high molecular weight gluten protein

Rheological properties of the gluten complex

Gluten prepared from wheat flour exhibits all three characteristical types of rheology, i.e. it displays plastic, elastic and viscous properties, depending on stress conditions. Therefore, extremely complex rheological models must be used for the rheological characterization of gluten. If however, a narrower range of parameters is selected, a simplified characterization can be applied, particularly for comparative tests.

Consequently, rheological investigations of gluten are complex problems. Although the first instrumental tests were developed as early as by the turn

of the century, many problems are still open. The actually employed instruments based on empirical principles (plastometer, glutograph, valorigraph-gluten extensiometer, penetrometer [4, 5, 6, 7] primarily yield values expressed in empirical units. It is absolutely necessary for scientific purposes to develop test methods allowing to express the rheological properties in absolute physical units.

The instruments applied at present offer also this possibility. The data obtained by using a penetrometer can be applied for determining the yield value. For a conical immersed body, the following equation is valid (assuming that the tested sample is practically incompressible):

$$\tau_h = \frac{P}{h^2} \cdot K = \frac{P}{h^2} \cdot \frac{1}{\pi} \cdot \cos^2 \alpha \cdot \operatorname{ctg} \alpha$$

where τ_h — yield value
P — total load acting on the cone
h — depth of immersion.

In the case of capillary plastometers, the apparent viscosity can be calculated from the throughput by using the Poiseuille equation.

The yield value can also be calculated by applying the Buckingham—Reiner equation derived for Bingham bodies:

$$\eta_{pl} = \frac{P_k}{V}\left[1 - \frac{4\,\tau_h}{3\,P_k} + \frac{1}{3}\left(\frac{\tau_h}{P_k}\right)^4\right]$$

where η_{pl} — plastic viscosity
P_k and V — consistence variables
τ_h — yield value.

A graphical approximation is also in use. In the case of higher stresses, the above equation can be simplified to the form

$$P_k = V \cdot \eta_{pl} + 4/3\ \tau_h,$$

since the term $(\tau_h/P_k)^4$ can be neglected. By a graphical representation of the two consistence variables, a curve is obtained whose part corresponding to higher stresses is rectilinear, allowing a graphical determination of τ_h.

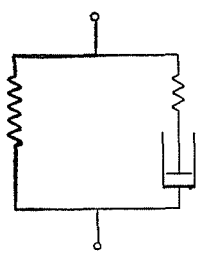

Fig. 3. Rheological model for gluten

Some approximate data can be calculated from measurements with parallel plate plastometers.

None the less, the characterization of rheological properties by absolute physical units required the development and application of further test methods.

Based on a method that we applied earlier for dough relaxation tests, a well reproducible testing method has been developed to yield reliable rheological values for the characterization of gluten. The three-element model presented in Fig. 3 was found to be a satisfactory approximation of the rheological properties of gluten [8].

Relationships between the rheological properties, and the chemical structure of gluten

The factors affecting the rheological properties of gluten were discussed in earlier comprehensive papers [9, 10]. I wish to emphasize, however, that — in addition to the relative ratios of the individual protein fractions and to their nature (amino acid composition and sequence of amino acids) — it is basically the mode of interaction between the individual fractions and their sterical arrangement that determines the characteristics of the gluten complex. Therefore, the study of covalent and non-covalent (i.e. hydrogen and hydrophobic) bonds which contribute to the build-up of protein structures suits to furnish important data which may help to solve the problem in question. Again the relationships between rheological properties and chemical structure are of a complex nature and therefore the investigation of an individual fraction or of a definite bond type cannot yield a full picture of the actual conditions.

New results were to be achieved in fields that have not yet been investigated or where open problems were left, in order to establish a coherent picture based on the findings of modern protein chemistry.

Referring to the amino acid composition, great stress has been laid on the degree of amidation, the amount of hydrophobic side chains and their effect on rheological properties. Referring to the fraction distribution, quantitative evaluation of the data by methods of mathematical statistics and application of gelchromatography considered to now for this purpose have been aimed at.

It was of special concern to elucidate the role of hydrogen bonds and hydrophobic bonds, since practically no relevant research has been carried out on gluten chemistry and rheology. Not only the role of these bonds in the control of rheological properties has been studied, but information sought for on the groups participating in these bonds.

With regard to the thiol-disulphide system, the exact extent of its action on rheological properties has been investigated.

Relationship between the amino acid composition and the rheological properties [11, 12]

A correlation was found primarily between the cystine content, the amidation degree of glutamic acid and aspartic acid and the total amount of some amino acids with hydrophobic side chains, on the one hand, and the rheological properties of gluten, on the other hand. The degree of correlation is seen from the data in Table 4.

Table 4

Correlation between amino acid composition and rheological properties of gluten

Characteristics	Correlation coefficient (ratio)
Relaxation time — degree of amidation	0.562
Relaxation time — cystine content	+0.593
Relaxation time — amount of amino acids with hydrophobic side chains	0.502

Role of gluten fraction ratios and of their nature in the formation or rheological characteristics [13]

A moderate correlation exists between rheological properties and the amount of fractions obtained by peptization with acetic acid, by paper electrophoresis and by gel chromatography (Table 5).

Table 5

Correlation data

Characteristics	Correlation coefficient (ratio)
Relaxation time — Fraction A	−0.503
Relaxation time — Fraction C	+0.603
Relaxation time — Fraction a	−0.656
Relaxation time — Fraction c	+0.544
Relaxation time — low molecular weight fraction	−0.567
Relaxation time — high molecular weight fraction	0.335

Role of disulphide bonds in the formation of rheological properties

My own research work was mainly concentrated on the conditions of the reoxidation following the reduction of gluten and on the rheological properties of the obtained products, since these studies appeared to furnish the most valuable informations for solving the problems raised in the introduction.

The procedure reported by BECKWITT et al. [14, 15] was applied for reduction and reoxidation, consisting in the following steps. A 5% solution of the gluten sample in 6 M urea solution was reduced in nitrogen medium for 12 hours, using β-mercaptoethanol as reducing agent.

The reoxidation was carried out at different urea concentrations (1 M to 8 M solutions) and pH values (3.5 to 5 and 5 to 8.5). The gluten derivative content of the solution varied between 1 and 10%, since preliminary experiments demonstrated that products comparable to native gluten are easiest to obtain in this concentration range. The reoxidation was carried out by passing oxygen through the solution for 168 hours. Subsequently, the product was purified by dialysis and centrifuged. Finally, the rest of free water was removed by manually kneading the product in a polythene bag.

The rheological properties of the gluten samples reoxidized under different conditions are presented in Table 6. From these data it appears that the reoxidation conditions have an important effect on the rheological properties of the product. The samples that approximated the properties of native gluten were obtained from 4 M urea solutions at pH 5.5 and from 6 M urea solutions at pH 3.5. At low urea concentrations, well-caking, very soft and non-

Table 6

Rheological properties of gluten derivatives reoxidized under different conditions

No.	Urea conc., M	pH	τ_h, dyn/cm²	Relaxation time, sec	Remark
1	1	3.5	150	unmeasurable	
2	1	5.5	220	unmeasurable	
3	1	8.5	350	unmeasurable	
4	2	3.5	240	30	
5	2	5.5	280	42	
6	2	8.5	1200	unmeasurable	
7	4	3.5	540	68	elastic
8	4	5.5	770	92	
9	4	8.5	1800	150	
10	6	3.5	300	42	moderately elastic
11	6	5.5	270	29	
12	6	8.5	1900	180	
13	8	3.5	320	35	
14	8	5.5	2700	unmeasurable	non-elastic
15	8	8.5	2900	unmeasurable	
	Native gluten		590	83	gluten with average elasticity and extensibility

elastic products were generally obtained. From 8 M urea solutions, no products comparable to native gluten could be obtained.

These data indicate the great importance of disulphide bonds for the physical properties of gluten. However, it is also evident that the number of disulphide bonds in itself does not unequivocally define these properties, but that the arrangement of the disulphide bonds is also essential.

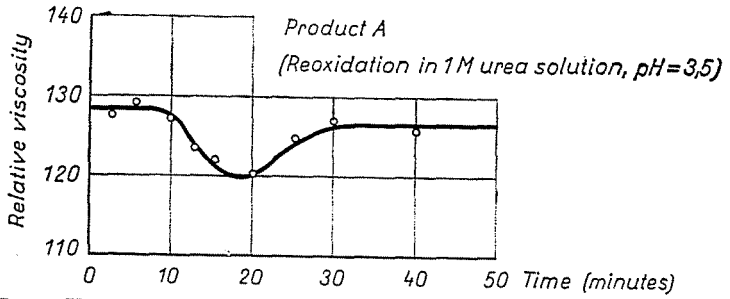

Fig. 4. Viscosity changes during the decomposition of reoxidized gluten

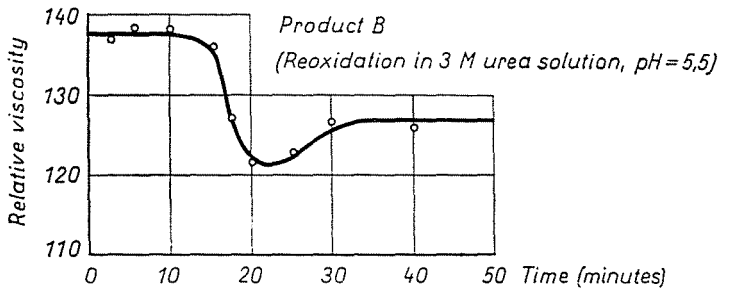

Fig. 5. Viscosity changes during the decomposition of reoxidized gluten

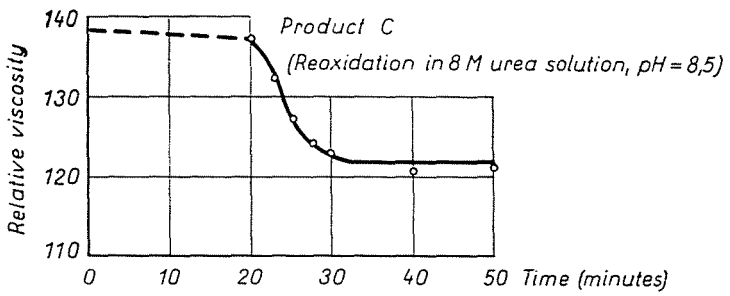

Fig. 6. Viscosity changes during the decomposition of reoxidized gluten

Disulphide bonds are either intramolecular or intermolecular. The ratio of intra- to intermolecular disulphide bonds was likely to be of great importance for the resulting rheological properties. To confirm this assumption, the viscosity changes in solutions of gluten reoxidized under different conditions during their decomposition by performic acid or sulphitolysis has been measured. The results are presented in Figs 4, 5 and 6.

These figures clearly demonstrate the differing kinetics of the viscosity changes in the case of gluten products reoxidized under different conditions. With gluten reoxidized at the highest urea concentration in alkaline solution, the viscosity — after a relatively rapid decrease — remains approximately constant during the further period of decomposition by performic acid. The reoxidized gluten product, comparable to native gluten behaves differently: after a rapid decrease in viscosity it reaches a minimum value. After passing this minimum, the viscosity increases and tends towards a limit value. The

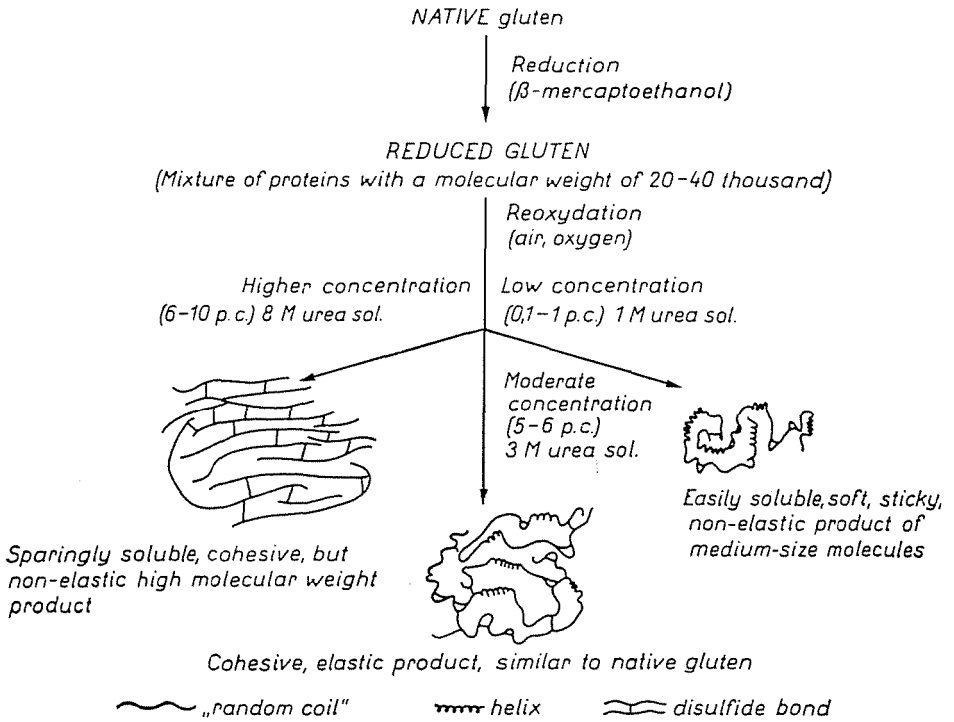

Fig. 7. Production schema of gluten with predetermined rheological properties

reoxidized product obtained at the lowest urea concentration in acid solution again behaves differently, insofar as the viscosity decrease is not so sharp and the increase after passing the minimum is relatively the highest.

The curves allow to conclude on differences between the disulphide bond systems of reoxidized glutens with differing rheological properties. The viscosity decrease which occurs with all samples in the period when the splitting of the disulphide bonds starts indicates that larger protein molecules are being decomposed into smaller units owing to the splitting of intermolecular disul-

phide bonds. The further course of the viscosity curves depends on the presence and number of intramolecular disulphide bonds within the fragments. The decomposition of such bonds leads to a change in the conformation of the molecule: the molecule unfolds to a certain extent and consequently the viscosity will increase. When the splitting of the intramolecular disulphide bonds is complete, the conformation of the molecule and therefore the viscosity of the solution will undergo no further changes. From this view, the curves indicate that the gluten sample reoxidized at the highest urea concentration in alkaline solution contains practically only intermolecular disulphide bonds, while the two other samples contain a substantial number of intramolecular disulphide bonds.

The finding that the rheological properties of the product can be influenced by the conditions of reoxidation might be of great industrial significance, since it implies the potentiality that a given gluten may yield products with differing, predetermined rheological properties (Fig. 7).

If economically justifiable methods for reduction and reoxidation can be found, gluten with rheological properties adapted to the requirements of any given cereal processing technology might be obtained by decomposing the native gluten to units consisting of the basic polypeptide chains and selecting suitable conditions for reoxidation.

Role of hydrogen and hydrophobic bond systems in rheological properties

Our extensive studies proved that hydrogen and hydrophobic bonds are of great significance in the rheological properties of gluten. The cohesive and elastic substance characteristic for gluten cannot come into being without an appropriate system of hydrogen and hydrophobic bonds.

In the formation of intermolecular hydrogen bonds, primary amino groups play the main role, while amino acids with hydrophobic side chains are of importance in the formation of hydrophobic bonds. A detailed report on our studies concerning the rheological properties of chemically modified glutens was given earlier [11, 12].

Fictitious structure of the gluten protein complex

In the model in Fig. 8 is — according to on our findings — likely to reproduce the most characteristic features of the gluten protein complex. We hope that our further research will enable us to establish a more detailed and more exact model within a short time.

Fig. 8. Hypothetical structure of gluten protein complex

Some results on maize and rice proteins

Partly on request of industrial organizations and ministerial departments, we started research on maize and rice proteins. Both of these cereals are of great importance in the national economy of Hungary. Among our results in this field, data on the amino acid composition of maize proteins (Table 7) and on the fraction distribution of rice proteins (Table 8) may be of interest.

Table 7

Chemical composition of maize flours obtained from different hybrides

Hybride	Type and particle size (μm) of flour	Lysine %	Methionine %	Tryptophane %	Vitamin B_1, γ/mg	Carotin mg%
MV 530	Full, 0—300	0.48	0.22	0.07	5.1	2.20
	Degermed, 0—300	0.30	0.19	0.06	1.0	2.10
	Full, 300—1200	0.51	0.25	0.06	5.2	2.50
	Degermed, 300—1200	0.29	0.21	0.06	0.8	1.90
MV 570	Full, 0—300	0.43	0.21	0.08	5.2	8.15
	Degermed, 0—300	0.29	0.19	0.06	0.9	7.80
	Full, 0—1200	0.46	0.22	0.09	5.0	8.70
	Degermed, 0—1200	0.28	0.18	0.07	0.7	8.40
MV 602	Full, 0—300	0.32	0.17	0.05	5.0	6.90
	Degermed, 0—300	0.25	0.16	0.05	1.1	6.95
	Full, 0—1200	0.34	0.15	0.06	4.9	7.40
	Degermed, 0—1200	0.22	0.15	0.05	0.8	7.35

Table 8

Fraction distribution of Hungarian rice proteins
(Vu Van KY, 1971)

Fraction	Crude rice	Husked rice	Polished rice
	Percentage in total protein content		
Albumin	14.0	13.0	6.5
Globulin	15.5	14.5	12.7
Prolamin	10.1	9.9	8.9
Orizenin	60.4	62.6	71.9

In our further research program, work is to be continued on the structure of wheat proteins, in order to explain the individual steps of cereal processing on a molecular level. Research program involves other cereals of Hungarian produce, to obtain a comprehensive picture of their proteins.

Summary

Recent investigations opened a new chapter in the chemistry of wheat proteins: i.e. the chemistry of complex wheat proteins. Great attention has been paid to the glycoproteins and lipoproteins of wheat. The proportion of helical parts in the gluten proteins is small, the low moleculare weight components have mostly intramolecular disulphide bonds. The high molecular weight proteins of the gluten complex contain a great number of polypeptide chains with intermolecular disulphide bonds. Their molecules have a linear character. In the determination of the rheological properties of the gluten complex the system of disulphide bonds, the intermolecular hydrogen and hydrophobic bonds are of the greatest importance.

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