CORRELATION BETWEEN THE CHEMICAL STRUCTURE AND RHEOLOGICAL PROPERTIES OF GLUTEN

By

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Received April 1, 1981

1. Introduction

The prominent position of wheat and wheat flour, among the raw materials of the baking industry is strongly connected with its unique proteins. Amongst cereals only bread wheats — and to a lesser extent triticale — posses reserve proteins which interact with water to yield doughs having the necessary cohesivity and elasticity for making leavened bread. The reasons for this, and the structure of gluten and its protein constituents have intrigued cereal chemists since the days of Osborne. It is evident that the factors playing a role in defining the rheological properties of gluten are of a complex character. Based on theoretical considerations and also on all the studies which have been carried out it would appear that the following two groups of factors are the most important:

— the quantity and quality (solubility, amino acid composition, molecular mass distribution etc.) of the protein components of the gluten complex,
— the interactions (disulphide bonds, hydrogen bonds, hydrophobic interactions, electrostatic interactions etc.) between the protein fractions.

In the framework of the research carried out in our laboratory the correlation between the chemical structure and the rheological properties of glutens was investigated. The effect of amino acid composition, molecular mass distribution of proteins and as most important, the interactions of proteins were studied. The results of this investigation are discussed in this paper and compared with the conclusions of other authors.

2. Materials and methods

Glutens were prepared from different varieties of wheats and wheat flours (Bezostaya, Fertődi 279, San Pastore, Kavkaz, Yubileynaya, MV-4) by washing and additional purification. The method was described earlier [1, 2].

The rheological properties of the gluten were characterized by a three-element model (combined from the Maxwell and Hooke model; H/H—N). From the experimental data the relaxation time and the relaxation constant were calculated. The stress relaxation was measured by a modified Neolaborograph instrument. The details of the measurement and calculations were published in an earlier paper [2].

The amino acid composition of the gluten proteins was determined after hydrolysis with hydrochloric acid using an automated amino acid analyzer (AAA 881 Mikrotechna, Praha).

The protein fractions of the gluten complex were separated by gel chromatography. For peptization, acetic acid solutions were used. The details of the methods were also described earlier [3a, c].

Chemically modified glutens (desamidated, acetylated, esterified glutens, N-ethyl maleic imide derivatives) were prepared with the methods described by LÁSZTITY [3], BECKWITH et al. [4], BARNAY et al. [5], HOLME and BRIGGS [6]. For splitting disulphide bonds and their reoxidation the method of BECKWITH et al. [7, 8] was used.

3. Results and discussion

3.1. *Amino acid composition*

The amino acid composition of the glutens prepared from different wheats shows slight differences which may, in many cases, be statistically significant. Nevertheless the amino acid composition of the gluten is relatively stable and may be characterised as follows:

- high glutamic acid content
- relatively high proline content
- low amount of basic amino acids (lysine, arginine, histidine)
- high degree of amidation nearly equivalent to the aspartic and glutamic acid content
- remarkable cystein and cystine content.

The mathematical-statistical evaluation of the results of the overall amino acid composition and the rheological characteristics does not exhibit significant correlation in most cases. The total disulphide bond (cystine) content shows a significant correlation with the rheological properties. This is characterised by a linear correlation coefficient varying from 0.3 to 0.6. A strong positive correlation between the cystine content and wheat flour baking value was reported earlier by WÖSTMANN [9]. The lower degree of correlation may be explained by the assumption that not only the absolute number of disulphide bonds but also their distribution is important from the view of rheological

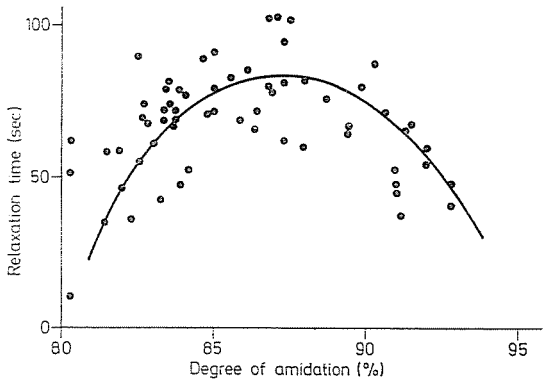


Fig. 1. Correlation between the degree of amidation and the relaxation time of gluten

properties. The problem of the disulphide bonds will be discussed in Chapter 3.3 of this paper.

The correlation between the degree of amidation (calculated on the basis of aspartic and glutamic acid content and ammonia content, resp.) and the rheological characteristics is shown in Figure 1.

The results indicate that an optimum degree of amidation exists. The observation may partly be explained by the work of BRIGGS and HOLME [6], CUNNINGHAM et al. [10] and BECKWITH et al. [4]. They postulated that an increase in the number of the amido groups increase the possibility of the formation of secondary bonds in the gluten structure which improve the rheological properties of the gluten. However, this hypothesis does not explain the negativ effect of the highest degrees of amidation.

An interesting correlation was found between the amount of amino acids with hydrophobic side chain (leucine, isoleucine, proline) and relaxation time. The correlation can be described by a second-order equation.

Based on the results mentioned above a new "Quality index" was proposed serving a preliminary evaluation of the wheat's baking value from the data of the amino acid composition of the gluten.

$$(1)\quad QN = 10\,C + 0.15\,A - K_A + 0.4\,H - K_H$$

where C = cystine content (%)
A = degree of amidation (%)
H = proline, leucine and isoleucine content
K_A = (87 — A) (absolute value)
K_H = (22.5 — H) (absolute value)

3.2. *Distribution of protein fractions*

The readily dispersed and undispersed fraction of the gluten complex (in 0.05 mol/dm^3 acetic acid solution) was measured and the correlation between the quantities of protein fractions and relaxation time were calculated statistically. The results are shown in Figures 2 and 3. The results indicate that the amount of the readily dispersible components is in negative correlation with the rheological quality. The increase in the quantity of non-peptisable fractions has an improving effect. These observations are in good agreement with the statements of many authors and with some methods for determining wheat baking value based on the swelling or dispersibility of gluten proteins in dilute organic acids (lactic acid, acetic acid).

The effect of the ratio of the high molecular mass and low molecular mass fractions (separated by gel chromatography) was also investigated. In

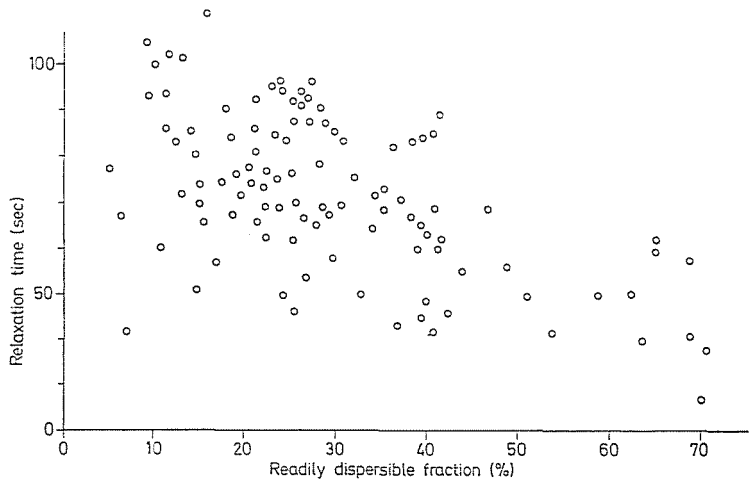


Fig. 2. Correlation between the quantity of readily dispersible protein fractions and the relaxation time of glutens

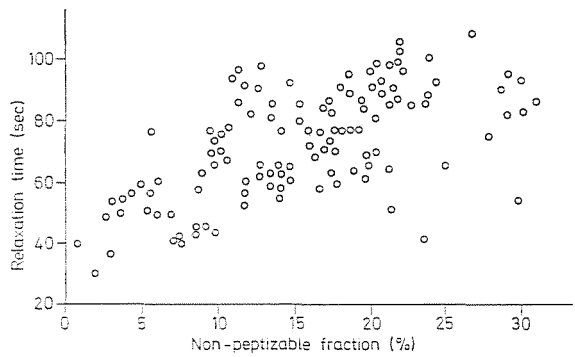


Fig. 3. Correlation between the quantity of non-peptisable protein fractions and the relaxation time of glutens

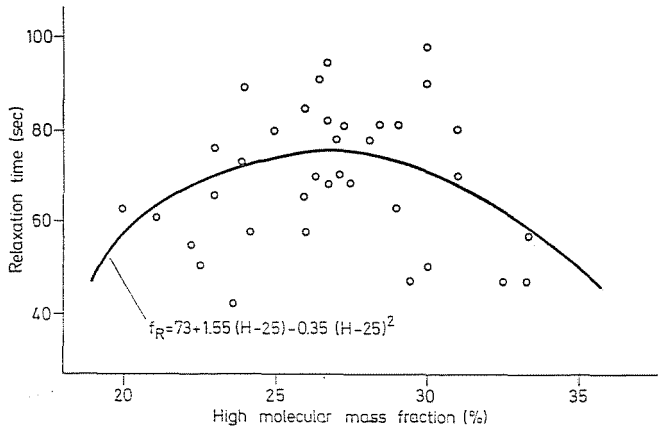


Fig. 4. Correlation between the ratio of high molecular weight protein fractions and the relaxation time of glutens

Fig. 4 the correlation between the quantity of high molecular mass proteins and the relaxation time of the gluten is demonstrated. The correlation can be expressed by a second order equation and a corresponding curve having a maximum value. The importance of the ratio of the higher molecular mass glutenin to the lower molecular mass gliadin fraction was confirmed by the investigations of many authors. Summarized results are given by KASARDA et al. [11], SIMMONDS [12], LÁSZTITY [13, 14].

3.3. *Interactions between the protein fractions*

3.3.1. *The role of disulphide bonds*

Many examples are known about the important role of disulphide bonds concerning the protein structure and its mechanical properties. The gluten proteins contain relatively small quantities of cystine and cysteine, (in average 2 to 3%). Primarily their importance was observed in relation with problems of flour improvers. In consequence, very extensive research work was started in this domain, especially in the last two decades. Nevertheless there are still many unsolved problems. To clear up the role of disulphide bonds needs further comprehensive investigations. Some useful data may be obtained by studying the changes occurring in the rheological properties due to the decomposition of the disulphide bonds. Among the procedures used in protein chemistry to break down disulphide bonds, reduction seems to be particularly promising, because of the possibility to reconvert the thiol groups formed into disulphide bonds, after the removal of the reducing agent.

Earlier tests of BECKWITH et al. [7, 8] on wheat gliadin and glutenin showed that gliadin reduction (breakdown of the S—S bonds) involves no

perceptible change in molecular mass. Though ferograms obtained by gel electrophoresis show minor deviations in mobility, these can be explained by changes in conformation. Reduced and alkylated gliadin contain practically no helical structures, according to optical dispersion tests, whereas in active gliadin their computed ratio is about 15%.

In the case of reoxidation in a diluted solution, investigations of the above-mentioned authors show that the native gliadin is practically recovered. Their finding has been confirmed by ultracentrifugal, electrophoresis and rotatory dispersion tests. No data have been published about the rheological properties of the reoxidized product. The statement according to which reoxidation in a more concentrated solution (5%) yields products with higher molecular masses and less soluble than the original gliadin is very interesting. Presumably intermolecular disulphide bonds are being formed in this case.

The decomposition of the disulphide bonds in glutenin by reduction results in the disintegration of the large gluten component molecules. In the case of reoxidation in solutions of higher contentration, a product with properties very close to those of native glutenin may be obtained. According to the cited authors, the physical properties of the product formed in the course of reoxidation are much influenced by the reoxidation conditions, primarly by pH and by urea concentration. No numerical data on the rheological properties of the glutenins produced under different conditions are reported; probably no such measurements have been carried out.

We therefore conducted studies on the conditions of reoxidation following gluten reduction and on the rheological properties of the products obtained, to provide the necessary information.

To reduce and reoxidize the gluten samples, we adopted the method applied by BECKWITH et al. [7, 8] its rough outlines being as follows: 5% solutions of the gluten samples in a 6 mol/dm^3 urea solution were prepared. The reduction was subsequently carried out with β-mercaptoethanol under nitrogen circulation for 12 hours. Part of the reduced gluten was alkylated with acrylonitrile, yielding, after dialysis and lyophilization, the S-cyanoethyl-gluten derivative. Reoxidation was carried out in different urea concentrations (solutions of 1 to 8 mol/dm^3) and at different pH values (3.5—5.5—8.5). The gluten content of the solution ranged from 1 to 10% since preliminary tests showed this concentration range to be the most favourable for producing a product similar to native gluten.

Reoxidation was performed by oxygen circulation for 168 hours. By the end of the reoxidizing process the product was purified by dialysis, compacted in a centrifugal apparatus and finally free water was removed from the agglomerating mass by hand kneading in a polyethylene bag. The samples were tested for stress relaxation by the method of LÁSZTITY [3a, 3c].

Table 1 summarizes the rheological properties of reoxidized glutens produced under different conditions.

Table 1

Rheological properties of reoxidized glutens

N	Urea conc. (mol/dm³)	pH	τh N/m²	t_r (sec)	Remarks
1	1	3.5	15	not measurable	—
2	1	5.5	22		—
3	1	8.5	35		—
4	2	3.5	24	30	—
5	2	5.5	28	42	—
6	2	8.5	120	not measurable	—
7	4	3.5	54	68	elastic
8	4	5.5	77	92	—
9	4	8.5	180	150	—
10	6	3.5	30	42	slightly
11	6	5.5	27	29	elastic
12	6	8.5	190	180	—
13	8	3.5	32	35	—
14	8	5.5	270	not measurable	non-elastic
15	8	8.5	280	not measurable	non-elastic
16	native gluten (control sample)		59	83	gluten of average elasticity and extensibility

Data in Table 1 show that the rheological properties of the products obtained are highly influenced by the reoxidation conditions. Products best approaching the properties of native gluten are produced with a (3 mol/dm³ urea) solution at pH 5.5 and with a 6 mol/dm³ urea solution at pH 3.5. In general, with low urea concentrations cohesive products without elastic properties are obtained, whereas in the case of 8 mol/dm² urea concentration, the reoxidized gluten is tougher, but has no appropriate elasticity. In alcaline media (pH 8.5) no product with properties similar to native gluten could be obtained at any urea concentration. It should be noted that reoxidation was essentially more rapid in alcaline media, and in general, the reoxidized product yielded a practically inelastic, cohesive mass when mixed with water. Its properties were largely similar to gluten extensively denaturated by heat.

The present study of the rheological properties distinctly shows the important role of disulphide bonds on the physical properties of gluten. At the

same time it is apparent that the absolute number of disulphide bonds alone does not unambiguously define these properties. In this respect the site of the disulphide bonds is also essential.

The influence of pH and urea concentration on the properties of the reoxidized products can be explained as follows: depending on pH and urea concentration, changes may occur in the conformation of the peptide chains, in the steric position of the individual groups, their dissociation conditions, their reactivity, defining the type and site of the disulphide bonds formed. The effects of urea, of pH and of ion concentration on the protein conformation are generally known.

The disulphide bonds formed might be either intramolecular or intermolecular bonds. The ratio of inter- and intramolecular disulphide bonds is presumably very important in the development of rheological properties. To check this assumption, the changes in the viscosity of the solution during decomposition by performic acid of reoxidized glutens obtained in different ways have been followed. Characteristic curves are presented in Figs 5, 6 and 7.

It can distinctly be seen that the viscosity versus time graphs are different for reoxidized gluten products having different rheological properties. The simplest curve was obtained for a gluten reoxidized in alcaline medium at a high urea concentration (Fig. 5). After a relatively rapid viscosity decrease, it remains at an approximately constant value during further oxidation with performic acid. The reoxidized product, with properties approximating those of the native gluten, follows a different course (Fig. 6). After a rapid viscosity decrease a minimum is reached. Viscosity then rises to reach a limit value. The solution of a reoxidized gluten product prepared at a small urea concentration at acid pH (Fig. 7) is subject to a smaller viscosity decrease during the performic acid oxidation, the viscosity graph shows a sharp minimum, and the subsequent increase in viscosity is higher than in Fig. 6.

Disulphide bonds of reoxidized glutens have also been subjected to decomposition by sulphitolysis. The character of the curves obtained is closely similar to the above-discussed curves.

The results of both series of experiments clearly show the differences between the disulphide bond systems of the reoxidized glutens with different rheological properties. The viscosity decrease occurring in all cases at the beginning of the disintegration of the disulphide bridges indicates that owing to the rupture of intermolecular disulphide bonds, larger protein molecules will break down to smaller units. The subsequent course of the viscosity curve will depend on whether the smaller units formed contain intramolecular disulphide bonds at all, or to what extent, since the disintegration ot these intramolecular disulphide bonds will result in a change of the conformation of the molecule, and the molecule, "opening" to a certain extent, will increase viscos-

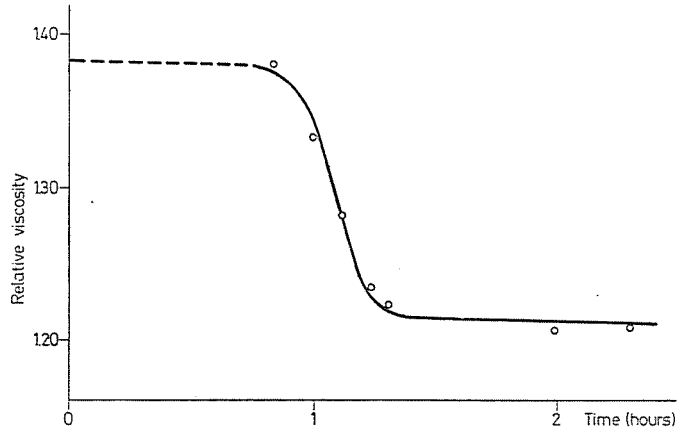


Fig. 5. Changes in the viscosity of a reoxidized gluten solution during the splitting of S—S bonds by performic acid (Product C: reoxidation in 8 mol/dm³ urea, pH 8.5)

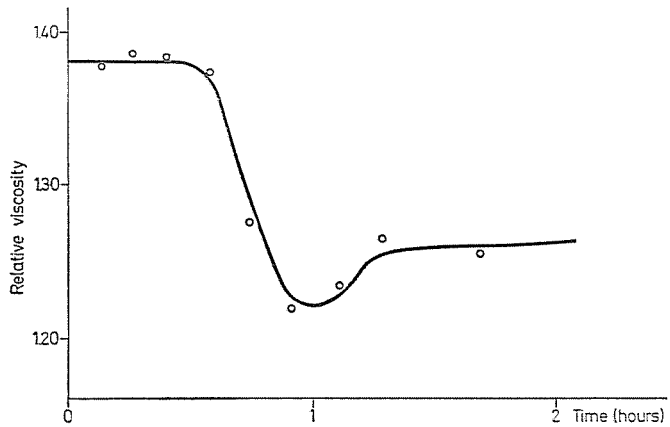


Fig. 6. Changes in he viscosity of a reoxidized guten solution during the splitting of S—S bonds by performic acid (Product B: reoxidation in 3 mol/dm³ urea, pH 5.5)

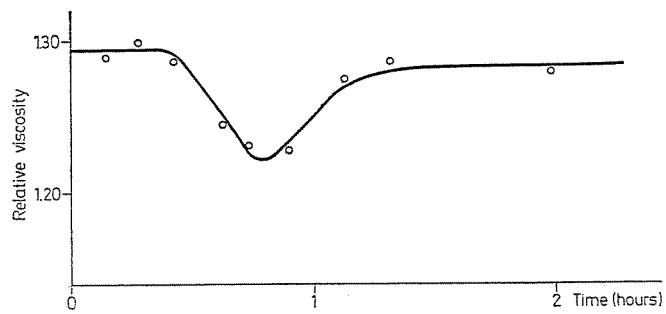


Fig. 7. Changes in the viscosity of a reoxidized gluten solution during the splitting of S—S bonds by performic acid (Product A: reoxidation in 1 mol/dm³ urea, pH 3.5)

ity. When disintegration of the intramolecular disulphide bonds comes to an end, both the conformation of the molecule and the viscosity will attain constant values. Studying the curves from this point of view, it may be seen that the reoxidized gluten prepared in an alcaline medium at a high urea concentration practically contains intermolecular disulphide bonds only. In the product close to native gluten, the number of intramolecular disulphide bonds is high and the same applies also to reoxidized gluten obtained at acid pH at a low urea concentration.

On principle the viscosity recovery after a minimum could also be ascribed to new aggregates forming secondary bonds, after the breakdown of the primary disulphide bonds. This assumption is, however, improbable, because no such increase is recorded for the reoxidized gluten prepared at alcaline pH in high urea concentration.

A good example for the slower reaction of the intramolecular disulphide bonds is presented by extensive investigations with insulin.

These studies indicated that only one of the three disulphide bonds present could be reduced by thioglycolate. Two disulphide bonds could be disintegrated with sodium sulphite, but completely disintegration of all disulphide bonds was only possible in the presence of urea, guanidine and phenylmercuric hydroxide, resp. More detailed studies revealed that it was the intramolecular disulphide bond which was the slowest and most reluctant to react.

Though there is no knowledge available about more complex proteins and about the system of disulphide bonds in gluten proteins, the consideration of some general relationships and analogies allows to presume that — at least in the case of the larger gluten protein molecules — the intramolecular disulphide bonds react slower owing to steric inhibition, and become accessible only after a certain "loosening" of the molecular conformation.

The observation of BECKWITH and WALL (1966) is of interest: the amperometrically determined disulphide content of glutenin obtained by sulphite breakdown does not exceed about two thirds of the true quantity present, up to a urea concentration of 3 mol/dm^3. The total disulphide content can be determined only in a urea solution of 6 mol/dm^3.

Finally the work of BERNACKA and KACZOWSKI [15] should be mentioned. Based on reduction dynamics, they divided the S—S bonds of the gluten complex in to four groups (three intermolecular and one intramolecular).

Reduction and reoxidation studies of gluten indicate the importance of the disulphide bonds in the structure of gluten molecules and also in its rheological properties. According to these studies high molecular mass gluten protein fractions consist of polypeptide chains connected by disulphide bridges. In addition to these, the number of disulphide bonds within the molecule is also of importance. During the reduction of the gluten, all disulphide bonds will break down, and the obtained product lacks the rheological properties of the

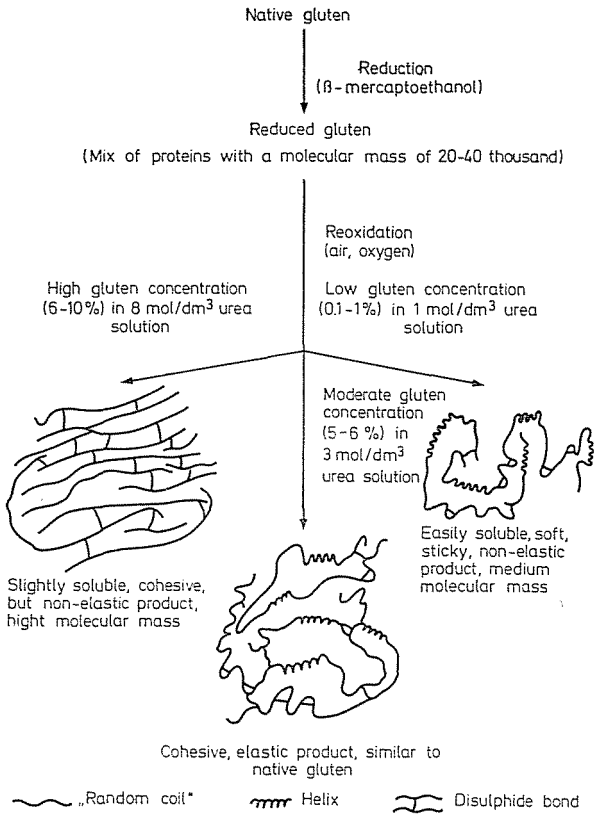


Fig. 8. Diagram of the process of producing gluten possessing rheological properties as desired

original gluten. When reoxidized, the reversion is practically quantitative. However, the site of the newly formed bonds and the proportion between inter- and intramolecular disulphide, bonds will depend on reoxidizing conditions. This fact is reflected in the rheological properties of the reoxidized product.

It follows from the above that the absolute number of the disulphide bonds alone does not unambiguously define the structure and the rheological properties. It becomes clear why, in general, no closer correlation could be established between the disulphide content and the rheological properties. It explains also why the correlation is stronger in gluten of the same wheat variety: it may be assumed that in a given wheat variety the protein biosynthesis proceeds similarly, and hence the distribution of the disulphide bonds will also be similar. The looseness of the correlation is likely to be attributed to the many other factors involved in the development of the rheological properties.

The finding that products with differing rheological properties can be obtained, depending on the conditions of reoxidation after the reduction ot a given gluten, could be of paramount importance for practice. It implies the possibility to develop an economically feasible method of reduction and reoxidation for breaking down the native glutens to units consisting of the fundamental polypeptide chains and subsequently, by selecting the appropriate conditions for reoxidation to produce glutens with rheological propertics as required by the particular grain-processing technology in question.

The underlying concept of this gluten-processing method is presented in Fig. 8.

EWART's recent work [16, 17] confirms the importance of the distribution of disulphide bonds. The theory of rheologically active SH-groups (BLOKSMA [18], JONES [19]) also stresses the rheological importance of the sites of the S—S-bonds.

3.3.2. *The role of hydrogen bonds*

The gluten contain a great number of side chains forming hydrogen bonds (Table 2). This fact supports the assumption that the hydrogen bonds

Table 2

Functional groups in gluten proteins
(HOLME 1966, POMERANZ 1968)
(mmol/100 g protein)

Group	Amino acids	Gliadin	Glutenin
Acidic	glutamic acid aspartic acid	27	36
Basic	lysine arginine histidine tryptophane	39	52
Amide	glutamine asparagine	309	266
Sulphhydril and disulphide	cysteine cystine	12	12
Total ionic	(acid + basic)	66	87
Total polar	(hydroxy + amide)	381	365
Total nonpolar		390	301

also contribute to the rheological properties of gluten. Many experimental facts confirm the possible role of non-convalent bonds. Most insoluble glutens can be dispersed in strong urea solutions or in other hydrogen-bond-disrupting agents. Hydrogen bonding was also shown to be responsible for aggregation and disaggregation phenomena of proteins during separation by gel chromatography (JANKIEWICZ and POMERANZ [20]). The contribution of hydrogen bonds and reactive sulphur-containing groups of proteins to the rheological properties of dough were studied by JANKIEWICZ and POMERANZ [20] by adding urea and N-ethylmaleic imide. VAKAR et al. [21] reported that freshly washed gluten becomes stronger and more elastic after dipping in to D_2O. These facts also indicate that hydrogen bonds play an important role in the gluten structure. The role of amido groups was studied by HOLME and BRIGGS [6] and BECKWITH et al. [4]. In our laboratory we investigated chemically modified glutens.

The influence of desamidation. A great number of amidated carboxy groups are present in gluten proteins. In view of this number, high in comparison with other polar groups, the role of amido groups in the formation of secondary bonds might be important. We therefore determined penetration indexes of glutens desamidated to various degrees, and measured the viscosities of desamidated gluten dissolved in acetic acid and in 8 mol/dm^3 urea solutions. Results are listed in Tables 3, 4 and 5.

The data demonstrate that, compared to control samples, desamidated glutens are of softer consistence, i.e. their rheological properties are inferior.

Table 3

Rheological properties of desamidated glutens

Sample No.	Difference in penetration ΔP(%) Compared to control sample					
	degree of desamidation (%)					
	10	30	50	70	90	100
1	15	24	31	39	39	42
2	12	23	35	46	47	50
3	17	29	39	51	55	57
4	10	21	31	35	34	39
5	8	17	29	37	42	42
6	7	23	29	35	37	39
7	13	21	34	45	49	53
8	15	21	29	42	47	48
9	11	19	33	42	47	48
10	14	23	32	44	43	43

Table 4

Instrinsic viscosity of desamidated gluten solutions in acetic acid

Ser. No.	[η] dl/g degree of desamidation						Acetic acid concentration
	10	30	50	70	90	100	
1	0.425	0.445	0.462	0.480	0.505	0.510	0.05 mol/dm³
	0.430	0.435	0.440	0.437	0.435	0.420	1.0 mol/dm³
2	0.480	0.485	0.492	0.507	0.580	0.510	0.05 mol/dm³
	0.475	0.480	0.481	0.475	0.470	0.469	1.0 mol/dm³
3	0.460	0.475	0.490	0.502	0.500	0.499	0.05 mol/dm³
	0.462	0.467	0.470	0.465	0.459	0.458	1.0 mol/dm³
4	0.502	0.520	0.530	0.536	0.535	0.537	0.05 mol/dm³
	0.490	0.501	0.506	0.508	0.506	0.501	1.0 mol/dm³
5	0.397	0.412	0.431	0.432	0.430	0.429	0.05 mol/dm³
	0.400	0.405	0.405	0.402	0.400	0.390	1.0 mol/dm³

Table 5

Intrinsic viscosity of the solutions of desamidated gluten in 8 mol/dm³ urea

Ser. No.	[η] dl/g degree of desamidation					
	10	30	50	70	90	100
1	0.480	0.475	0.475	0.478	0.468	0.450
2	0.550	0.552	0.545	0.547	0.530	0.527
3	0.501	0.497	0.502	0.504	0.486	0.479
4	0.560	0.550	0.552	0.540	0.531	0.535
5	0.480	0.482	0.480	0.470	0.447	0.450

The differences, in terms of relative per cent penetration increase with the degree of desamidation, substantially at the start, and then tending towards a limit value.

As far as solubilities are concerned, desamidated gluten is more difficult to dissolve in strong acid media (below pH 3), but is easly dissolved — in contrast to controls — in pH 8. phosphate buffer. Considering the substantial increase of free carboxyl groups and the acid character of the protein formed, this fact seems to be understandable.

Based on the results of viscosity measurements it can be stated that desamidation primarily affects the viscosities of solutions in acetic acid. According to the data in Table 4, this modification leads to an increase in

intrinsic viscosity, demonstrating that a change had occurred in the conformation of the molecules, which results in higher asymmetry of the structure. For an explanation, one might suggest that the removal of the amido groups involves the elimination of secondary, e.g. hydrogen bonds, resulting in a looser structure. Presumably, due to the dissociation of carboxyl groups liberated within a molecule, repulsive forces will be operative between groups with identical charge. This assumption is supported by the experimental finding that increase in viscosity is substantially less in 1.0 mol/dm^3 acetic acid at higher pH and lower dissociation, or that on these conditions no increase of viscosity is found in some cases. Probably on the one hand, electrostatic repulsion is weaker, and on the other hand, new hydrogen bonds are formed between groups at sterically favourable sites.

Similar conclusions can be drawn from viscosity data relating to glutens and to desamidated glutens dissolved in 8 mol/dm^3 urea. A comparison of viscosity data of solutions in 0.05 mol/dm^3 acetic acid and in 8 mol/dm^3 urea reveals that as compared to the acetic acid solution the increase in viscosity of not desamidated control samples is significantly higher than that of partially desamidated samples. The difference can be explained on the basis that in amidated glutens there are substantially more hydrogen bonds present, and these are disrupted by the urea added and thus the conformations are altered.

At lower (pH/1 mol/dm^3 acetic acid) desamidated gluten samples also show viscosity data widely differing from those of solutions with urea. In some cases these values are very nearly the same as those for the control samples. This finding supports the idea of new hydrogen bonds being formed as mentioned in the discussion of the data shown in Table 4.

Effect of esterification. Esterification is one of the possibilities to transform the free carboxyl groups. Partial conversion of amido groups into esters is also feasible. In our experiments the rheological properties of glutens esterified with methanol or ethanol were studied together with the viscosity of the solutions prepared from the derivatives with 0.05 mol/dm^3 acetic acid. Results are listed in Tables 6 and 7.

The data in Table 6 show that with increasing degrees of esterification the rheological properties of gluten deteriorate, relaxation time becomes significantly shorter. In the first stage corresponding to the esterification of the free carboxyl groups present, no essential change occurs; this suggests that the role of free carboxyl groups in the formation of secondary bonds is insignificant.

The viscosity data indicating a substantial decrease in intrinsic viscosity implicate more compact and less asymmetric molecules, possibly due to the fact that alkylated protein is highly hydrophobic, and hence its hydration degree will be lower.

Table 6

Re axation of gluten esterified with methanol

Ser. No.	Relaxation time, sec.						
	extent of methylation, mmol/g						
	0	0.30	0.50	1.0	1.5	2.0	3.0
1	82	79	60	54	48	45	46
2	45	44	36	30	28	25	26
3	73	70	60	52	45	40	41
4	55	56	51	41	35	32	34
5	69	65	54	48	40	36	30

Table 7

Intrinsic viscosity of solutions of gluten esterified with methanol

Ser. No.	[η] dl/g						
	extent of methylation, mmol/g						
	0	0.3	0.5	1.0	1.5	2.0	3.0
1	0.442	0.439	0.384	0.350	0.321	0.295	0.280
2	0.495	0.480	0.401	0.362	0.318	0.288	0.275
3	0.480	0.469	0.360	0.331	0.297	0.291	0.302
4	0.530	0.529	0.460	0.431	0.390	0.321	0.305
5	0.420	0.415	0.340	0.291	0.270	0.258	0.261

Effect of acylation. In order to study the rheological properties of acylated gluten, the penetration values of the hydrated gluten derivatives and the viscosities of their solutions in 0.1 mol/dm^3 acetic acid were measured. Results are summarized in Tables 8 and 9.

The data reveal that the rheological properties of acylated gluten are very much inferior to those of native gluten. The decrease of cohesivity suggests that primary amino groups play a substantial role in the formation of intermolecular non-covalent bonds.

Experimental results show that no major alteration in viscosity takes place; after an initial small decrease the viscosity values remain practically constant. Thus it can be concluded that no important change of molecular conformation occurs, or that primary amino groups participate principally in the formation of intermolecular bonds.

Table 8

Penetration values of acylated gluten

Ser. No.	Penetration, 0.1 mm per cent of acylation			
	0	40	80	100
1	72	142	152	160
2	58	108	112	135
3	85	120	135	142
4	70	105	121	143
5	94	143	162	180
6	45	92	108	125
7	48	89	102	117
8	73	104	130	135

Table 9

Intrinsic viscosity of the solutions of acylated gluten

Ser. No.	$[\eta]^{Y}$ dl/g per cent of acylation			
	0	40	80	100
1	0.425	0.396	0.392	0.388
2	0.480	0.428	0.420	0.422
3	0.460	0.432	0.417	0.418
4	0.502	0.477	0.472	0.465
5	0.397	0.368	0.362	0.365
6	0.485	0.444	0.427	0.430
7	0.510	0.482	0.469	0.461
8	0.447	0.417	0.405	0.396

3.3.3. *Effect of hydrophobic bonds*

Gluten proteins contain several amino acids with hydrophobic side chains (alanine, leucine, phenylalanine, isoleucine, valine, proline). Moreover, taking into consideration that the hydrophobic parts of longer polar side chains (e.g. in the case of lysine and glutamic acid) may also interact, there can be no doubt about the potential possibility of the formation of hydrophobic bonds.

Dough and gluten formation proceed in aqueous media. Owing to the fact that an interaction of the non-polar groups with water is "unfavourable"

from the thermodynamic viewpoint, the thermodynamic tendency points towards a linkage of the non-polar groups with each other (with a consequent weakening of the interaction between these groups and water). This problem is dealt with in the review by KAUZMAN [22]. In general, the formation of hydrophobic bonds is an endothermic process, i.e. the change in thermodynamic potential is negative since the effect of the change in entropy ($T\Delta S$) exceeds that of the change in enthalpy (ΔH). Up to a certain temperature limit, the strength of hydophobic bonds increases with increasing temperature, so that hydrophobic bonds are of particular importance from the viewpoint of thermal stability of proteins.

The solubility of gliadin in non-polar solvents, and the influence of the latter on solubility are also indicative of the possible importance of the role of hydrophobic bonds. All this shows convincingly that a study of the hydrophobic bonds is unavoidably necessary for the understanding of factors which influence the structure and rheological properties of gluten proteins.

Up to now wheat protein research paid little attention to this problem. Only the observation might perhaps be mentioned that the rheological properties of doughs are changed already by small quantities of certain aliphatic hydrocarbons (MUELLER et al. [22], PONTE et al. [24—26]).

In the present work indirect methods were used. We studied the effect of compounds able to react with the hydrophobic groups of the gluten complex, and can, through these groups, interfere with the hydrophobic bonds existing earlier.

Effect of hydrocarbons on the rheological properties of gluten

These tests were carried out as follows. Dehydrated gluten was contacted with water containing a known amount of hydrocarbons. After hydration and swelling, the hydrated mass was subjected to mechanical working until a coherent, homogeneous material was obtained. The excess solution was removed, and the relaxation test was performed as described earlier. The experimental results are presented in Table 10.

These data show that the rheological properties of gluten are affected unfavourably by the presence of higher aliphatic hydrocarbons. When tested organoleptically, gluten becomes less stretchable and crumbly. In the case of pentane and hexane, an increase in the relaxation time and in the force necessary to cause a deformation of identical degree can be observed, particularly with glutens of poorer quality.

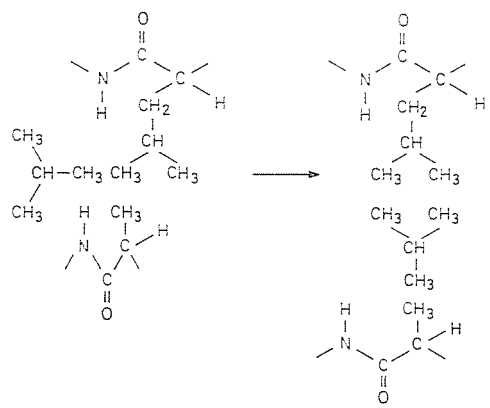
The pronounced changes which can be detected even at the relatively low concentrations used, definitely indicate an interaction between the hydrocarbons and the proteins of gluten. As concerns the character of this interaction, on the basis of thermodynamical considerations one can assume that

Table 10

Effect of hydrocarbons on the rheological properties of gluten

No.	Relaxation time (sec)					
	Control	Pentane	Hexane	Heptane	Octane	Undecane
	0.03 mol/100 g gluten					
1	102	106	101	94	82	65
2	95	95	96	82	76	72
3	88	91	82	71	66	50
4	73	72	70	72	68	53
5	69	65	63	58	55	48
6	62	65	60	55	54	45
7	58	60	62	59	51	45
8	53	48	49	44	40	37
9	43	44	40	38	37	35
10	38	45	40	38	40	41

a linkage is formed between the hydrocarbons and the hydrophobic side chains of proteins. In the case of pentane and hexane, a weaker bond is formed, extending or rather protecting those hydrophobic nuclei, which — in the course of hydration, osmotic uptake and swelling, and peptization — prevent the aggregates from unlimited swelling and disintegration. When higher hydrocarbons are added, the interaction may become stronger due to the higher affinity, so that existing interactions between side chains may cease, i.e. existing hydrophobic bonds may be ruptured, and replaced by bonds between the added hydrocarbon and the side chains. This situation is analogous to that assumed for the rupture of the hydrogen bonds by urea. The two analogous processes are illustrated by the following scheme.



Effect of fatty acids on the rheological properties of gluten

The procedure was the same as in the preceding series of experiments; some of the fatty acids were dissolved, others were emulsified in the aeqeous medium.

The rheological properties of the glutens treated under these conditions are summarized in Table 11.

The data in the table show an interesting pattern. The changes in rheological properties differ, depeding on the acid and the gluten. In the case of formic and acetic acid, the peptizing effect predominates, which brings about rapid deterioration of the rheological properties. Acids with increasing numbers of carbon atom up to and including valeric acid, cause an increase in relaxation

Table 11

Effect of fatty acids on the rheological properties of gluten

No.	Relaxation time (sec)								
	Acid added: 0.01 mol/dm³ solution (emulsion)								
	Control	Formic	Acetic	Propionic	Butyric	Valeric	Palmitic	Oleic	Stearic
1	102	39	40	56	72	85	80	b	75
2	95	47	30	50	68	80	82	b	71
3	88	39	28	52	59	69	79	b	80
4	73	48	a	41	48	53	63	b	65
5	69	41	a	44	50	60	62	b	58
6	62	42	a	48	47	50	52	b	47
7	58	29	a	32	39	40	52	b	49
8	52	a	a	30	30	42	48	b	41
9	43	a	a	27	30	40	42	46	30
10	38	a	a	25	28	32	32	57	28

a = not measurable (sticky, spreading mass)

b = not measurable (crumbing, disintegrating mass)

time, while palmitic and stearic acid lead to a slight deterioration of the rheological properties.

To explain the observed changes it may be assumed that, similarly to pentane and hexande, fatty acids with 3—5 carbon atoms bring about hydrophobization. On the other hand, the decreasing relaxation time observed with higher fatty acids indicates that the interaction of more strongly hydrophobic compounds with proteins may result in the rupture of existing hydrophobic bonds.

Unsaturated oleic acid does not fit at all into the series. Oleic acid causes a change similar in character to a very high degree of thermal denaturation.

Presumably this is caused by the interaction of oleic acid with a preferred side chain.

Effect of hydrocarbons on the formation of gluten in the presence of urea

In earlier works concerned with the role of hydrogen bonds, the effect of the addition of increasing amounts of urea on the rehydration of dry gluten and on gluten formation has been studied. These experiments were now repeated with the difference that various hydrocarbons were added to the urea solution (0.03 mol per 1 g of gluten). The experimental results obtained under these conditions indicate the absence of gluten formation or a considerable decrease in the amount of gluten formed already at a lower urea concentration. The results are plotted in Figs 9 and 10.

The observed behaviour can be explained by assuming that a combined addition of urea and hydrocarbon hinders not only the formation of intermolecular hydrogen bonds but also the formation of the corresponding hydrophobic bonds, as a result of the interaction between hydrocarbon and nonpolar side chains of the protein.

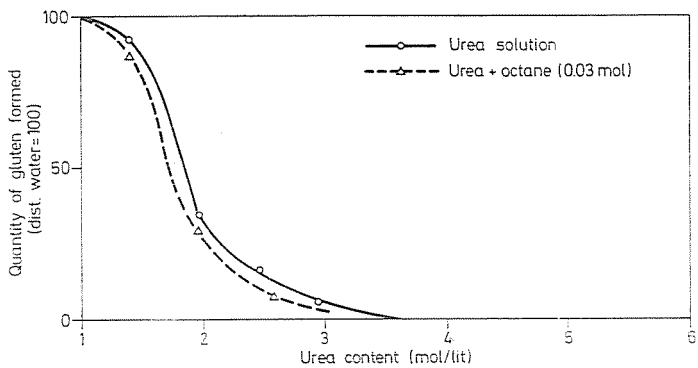


Fig. 9. Effect of octane on the formation of gluten in the presence of urea

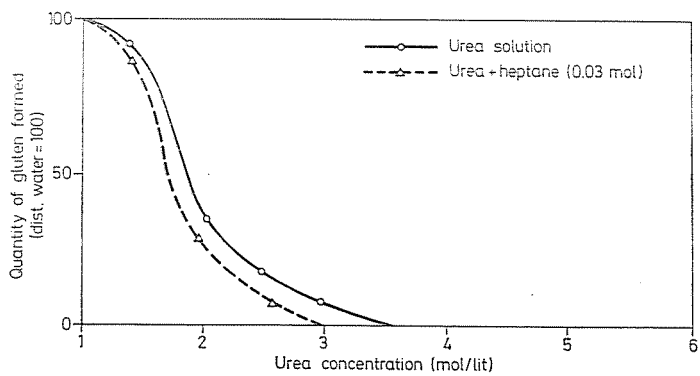


Fig. 10. Effect of heptane on the formation of gluten in the presence of urea

Summary

The general statement is that two main groups of factors influence the rheological properties of the gluten

— the quality and quantity of the protein fractions in the gluten complex (amino acid composition, molecular mass and conformation etc.)
— the interactions between the different protein components of the gluten complex (disulphide bonds, hydrogen bonds, hydrophobic interactions etc.)

In the framework of the research conducted in our laboratory the influence of the following factors was investigated:

— amino acid composition
— ratio of low and high molecular mass fractions
— interactions between protein components.

We found that the cystine content, the degree of amidation and the content of amino acids with hydrophobic chains have a significant effect on the rheological properties.

The lower molecular mass fractions determined by gel chromatography, gel electrophoresis or peptization were found to impair the rheological quality of the gluten complex.

The quantity and distribution of disulphide bonds play a very important role in the determination of the rheological properties. The distribution of the disulphide bonds can be changed by reduction and reoxidation of the gluten complex.

The investigation of chemically modified glutens showed that in forming intermolecular hydrogen bonds, amido and primary amino groups play the main role.

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