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Some methodical aspects in investigations on wheat gluten

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Abstract
3 M urea has been shown to cause considerable, and only partially reversible conformational changes of gluten molecules. Homogenization has proved to act mechanically, breaking down some molecular bonds.
No structural changes could be observed during freeze drying gluten, as well as after brief heating of its acetic acid extracts.

INTRODUCTION

One of the basic problems concerning wheat flour is to find the relationship between its baking quality and the structural differences of gluten protein contained in it. The experiments on the structure of gluten are very labourious, because of its complex structure, as well as of rather small resistance to chemical and mechanical factors. Therefore the results obtained in various laboratories sometimes differ significantly and depend largely on the applied methods of isolation and purification of gluten.

Because of the lability of gluten, it is practically impossible to fully eliminate structural changes of protein in any operation concerning its dispersion, isolation and separation into constituents. Therefore the main task in more precise investigations is to apply operations involving as small structural changes of protein as possible. This work was carried out in order to obtain information on the influence of methods commonly used in more detailed investigations of gluten structure on maintaining its initial conformation. The experiments deal with methods of dispersion, using 3 M urea as a dispersing agent, the influence of freeze drying and that of heating the dispersion in order to inactivate proteolytic enzymes.

MATERIAL AND METHODS

As experimental material 8 samples of wheat were used, the glutens of which could be classified as very strong ('Bezenczugska' and 'Saratowska'), strong ('Bezosta' and 'Manitoba'), medium ('Eros' and 'Mironowska') and weak ('Dańkowska 40' and Polish mixed). This classification was based on farinographic measurements of dough (Bernacka, Kączkowski, Liss 1971), laboratory bakings and some rheological indexes (flow rate, elasticity and swelling ability).

The influence of the manner of dispersing the flour and gluten on structural changes of protein was investigated by viscosity measurements. Gluten was dispersed by means of homogenization or shaking. Some dried gluten samples were homogenized at 2—3000 rpm during 15 min. using the MSE homogenizer, others — dispersed by shaking twice, in a laboratory shaker, one hour each time, with 24 hours of break. In both cases 12% sodium salicylate was used as a dispersing agent. After centrifuging the slurry at 7000 rpm the supernatant was subjected to the viscosity determination directly, as well as in the presence of urea or sodium sulphite of final concentrations 7 M and 0.01 M respectively (Kączkowski et al. 1968; Shorina, Wakar, Kretowich 1966). The results were used for calculations of characteristic viscosity $[\eta]$, axial relation and specific hydrodynamic volume of protein molecules.

The efficiency of extraction of freeze dried gluten by shaking in 0.05 M acetic acid was tested by comparing a dispersion of the sample with one portion of the agent, with that in which successively two volumes of dispersing agent were used. In the latter case the slurry after first shaking was centrifuged as above and the solid was shaken again using the second portion of acetic acid and centrifuged, both supernatants being joined together.

The influence of freeze drying on structural changes in gluten has also been tested. Gluten was washed out from flour during 90 min. under tap water and freeze dried in Vickers apparatus at a vacuum of 0.25—0.5 mm Hg during 5 hours at the temperature of material —10—+30°C and that of chamber +3—+30°C. Gluten was then dispersed in 0.02 M acetic acid by two one hour shakings with a 24 hour break using one portion of dispersing agent. Flour was first extracted 3 times using pyrophosphate buffer of pH 7.0 (to remove albumins and globulins) and then using acetic acid as above. In case of dispersing the freeze dried gluten albumins and globulins were considered to be removed during washing out.

As the measure of protein denaturation the increase of negative optical rotation coefficient of gluten dispersion was adopted. The optical rotation was determined using a Hilger M 511 polarimeter and the hydro-

gen lamp (438 nm), according to the procedure described previously (Dałek, Liss, Kączkowski 1970). This measure was also used for experiments concerning the influence of 3 M urea on the structure of protein. In investigations mentioned protein solutions were measured directly, as well as in presence of 3 M and 7 M urea. Measurements of samples, in which 3 M urea after treatment had been removed using dialysis against acetic acid were also conducted.

The influence of 5 min heating on the activity of native proteolytic enzymes of gluten was measured as the increase of non-protein Nitrogen in protein extract after 7 days of storage. As experimental material the 0.02 M acetic acid extracts of flour were used, from which albumins and globulins had been removed by 3 times pyrophosphate extraction. One part of the acetic acid extract was heated at the temperature 100° during 5 min and then immediately cooled, whereas the other one remained unheated. The samples of both extracts were stored at 0—2° as well as at 20°C during 7 days, and the increases of non-protein N were determined using the method of Reifer and Tarnowska (1950), after precipitation the proteins by means of trichloracetic acid of final concentration 6%.

The influence of heating the gluten protein extracted by acetic acid in a boiling water bath on its structure was also tested using polarimetric measurements. In this case a sodium lamp (589 nm) and the polarimeter Hilger M 413 were used.

RESULTS

Methods of dispersing gluten

The comparison of dispersing gluten by homogenization and shaking was carried out by means of the determination of hydrodynamic properties of dispersed protein molecules. These measurements should demonstrate the influence of the mechanical factor (homogenization) on the maintenance of hydrogen and disulphide bonds. The values derived from viscosity measurements of gluten originating from 3 sorts of wheat, are given in Table 1.

The results given in Table 1 show, that in all samples dispersed by homogenization, the higher hydrodynamic indexes of direct solutions, as well as more significant changes of them under the action of 7 M urea, can be observed. This indicates considerable loosening of gluten structure, as the effect of rupture of some molecular bonds by homogenization. The increase of characteristic viscosities of homogenized samples (without additions), as compared with those dispersed by shaking amount to 62%

Table 1

Characteristic viscosities [η], viscosity coefficients — ν and axial relations — f in gluten dispersed by means of shaking and homogenization

Sort of wheat Varieties		Shaking				Homogenization			
		[η]	ν	f	z	[η]	ν	f	z
Bezosta	1	0,262	23,4	14,4	100	0,425	37,9	19,8	100
	2	0,410	36,6	19,4	157	1,000	89,4	33,2	235
	3	0,115	10,3	8,4	44	0,175	15,6	11,0	41
Eros	1	0,260	23,2	14,2	100	0,330	29,5	17,0	100
	2	0,450	40,1	20,4	173	0,850	75,8	30,2	258
	3	0,115	19,8	12,8	85	0,180	16,2	11,4	55
Dańkowska 40	1	0,215	19,2	12,7	100	0,410	36,6	19,4	100
	2	0,450	40,1	20,4	209	0,720	64,3	27,3	176
	3	0,185	16,5	11,5	86	0,210	18,8	12,4	51

1 — direct solution, 2 — solution in 7 M urea, 3 — solution in 0,01 M sodium sulphite, z — % change of [η] under the influence of factor added, as compared with the direct solution.

for strong gluten ('Bezosta') and 91% for the weak one ('Dańkowska'). When 7 M urea was added these values amounted to 144 and 60% respectively. These values can suggest that more significant loosening of gluten structure under the action of a mechanical factor took place in case of the weak one — than in the strong one. Gluten of medium quality ('Eros') showed the smallest increase of [η] under homogenization — 27%, which can suggest — that other factors bound with mechanical rupture of some molecular bonds may also play a part in dispersing the gluten. The greater tractability of weak gluten, as concerns the loosening of its structure is shown also in case of samples dispersed by shaking. The percent increases of [η] in presence of 7 M urea, as compared with those without additives were the highest for the weakest gluten samples (156.5% for strong gluten, 173% for medium and 209.3% for weak one). After the homogenization of weak gluten ('Dańkowska') the influence of 7 M urea was much smaller than that observed in both strong and medium ones. This can be interpreted as the consequence of the mechanical rupture in homogenization of more hydrogen bonds in case of weak gluten than in stronger ones.

The efficiency of the extraction in 0.05 M acetic acid of freeze dried gluten

The results of protein extraction in the first and second portion of dispersing agent in case of two samples of freeze dried gluten are presented in Table 2.

Table 2

The efficiency of protein extraction in % of protein content in dried gluten

Sort of wheat Varieties	1	2	3	Increase of two-step extraction as compared with the first-step
Mironowska	55.4	17.7	73.1	32
Rokicka	55.9	22.7	78.6	41

1 — Extractability by shaking using one portion of solvent; 2 — Protein % in the second portion of solvent; 3 — Total extraction in %.

The increase of efficiency of two-step extraction, as compared with one-step extraction, amounting to 30 and 40% indicates, that the two-step extraction should be useful when higher efficiency is needed.

The influence of freeze drying

The specific rotations of gluten protein solutions extracted from flour or freeze dried gluten using 0.02 M acetic acid, measured directly, as well as in presence of 7 M urea are presented in Table 3.

Table 3

Specific rotations in angular degree of protein solutions extracted from flour and freeze dried gluten using 0.02 M acetic acid

Sort of wheat Varieties	Direct solution		With 7 M urea		Increase of specif. rotation		
	flour	gluten	flour	gluten	In 7 M urea % increase flour	glut.	freeze drying % increase
Saratowska	−113.7	−113.5	−159.3	−154.5	40	36	0
Polish mixed	−121.6	−130.4	−170.3	−178.3	40	37	7
Manitoba	−117.6	−126.6	−150.3	−169.0	29	33	7

The results show that in case of Saratowska wheat, containing very strong gluten, no changes of specific rotation under the influence of freeze drying measured directly, as well as in presence of urea, can be observed. Also in the case of samples, containing weaker gluten (Manitoba and Polish mixed) the increase of specific rotation of freeze dried gluten extract, as compared with that extracted from flour does not exceed

7%. The increase of specific rotation under the influence of urea in all samples of wheat and in both sorts of extracts was of the same order of value amounting to 30—40%. These results suggest that freeze drying of strong gluten does not cause any change of protein structure. In the case of weaker ones this procedure involves only small (if any) structural changes.

The influence of 3 M urea

In order to test the structural changes of gluten under the action of 3 M (and 7 M) urea which is recommended by some authors (Jankiewicz, Pomeranz 1965; Meredith, Wren 1966), as well as the possibility of the reversion of these changes after the removal the agent by means of dialysis, experiments were carried out, the results of which are given in Table 4.

Table 4

The influence of urea on specific rotations in angular degree of gluten protein solutions in 0.02 M acetic acid

	Saratowska		Bezenczugska	
	I sample	II sample	I sample	II sample
Direct solution	−95.7	−99.2	−84.6	−96.7
with 3 M urea	−108.7	−109.5	−135.2	−126.0
with 7 M urea	−140.0	−144.6	−153.8	−170.6
after removal 3 M urea using dialysis	−120.5	−111.1	−114.2	−120.8

The changes of specific rotations of protein dispersion in presence of 3 M urea, as compared with the initial dispersion indicate conformational changes — possibly the breakdown of some hydrogen bonds. Much deeper changes of this type were observed, when urea concentration was 7 M, which is considered sufficient to break all accesible hydrogen bonds, and thus fully unfold polypeptide chains. When 3 M urea had been dialysed off, the value of specific rotation did not return to that of initial dispersion. This means that after the removal of modifying agent, the broken hydrogen bonds did not form again, or formed to a limited degree only. Therefore the modifying action of 3 urea is irreversible and this dispersing agent should not be recommended.

The influence of heating

In order to test the stability of native proteolytic enzymes in gluten extracted from flour, experiments were carried out on heating the extracts in a boiling water bath and the maintenance of proteolytic activity

after this procedure. The increases of non-protein nitrogen in heated and nonheated extracts of some flours are presented in Table 5.

The results obtained show that 5 min heating in a boiling water bath is not sufficient for complete inactivation of proteolytic enzymes in gluten. The increases of non-protein N in heated samples are much smaller

Table 5

The effect of heating of gluten dispersions in acetic acid on the activity of proteolytic enzymes, measured as an increase of nonprotein N in mg/g of flour

Sort of wheat Varieties	Initial non-protein N	Treating the sample	Temp. 0—2°C		Temp. 20°C	
			1	2	1	2
Bezenczugska	3.62	5 min heating	3 86	7	4.05	12
		unheated	4.35	20	7.81	116
Saratowska	4.13	5 min heating	5.28	28	5.23	26
		unheated	7.58	83	9.43	128
Polish mixed	4.24	5 min heating	4.60	10	5.49	29
		unheated	8.62	102	13.60	221

1 — nonprotein N in mg/g of flour; 2 — % increase of nonprotein N, as compared with its initial content.

than in unheated ones, but the activities observed indicate has remaining proteolytic activity to be 10—30% of the initial one.

Initial proteolytic activities observed in particular samples of wheat flour were not equal and the increases of non-protein N in unheated, as well as in heated ones were also different. It is possible that these data may depend on the strongh of gluten, because in the case of Polish mixed the activities were almost everywhere higher, than those of very strong gluten ('Saratowska' and 'Bezenczugska' wheats). Also the stability of proteolytic enzymes during the heating, as well as their behaviour in temperature range 0—20°C, seem to be unequal. Therefore, it can be suggested that either in different gluten samples various proteolytic activities may occur, or in glutens of various qualities different accessibility of peptide bonds for proteolytic enzymes can be observed.

In order to test the influence of heating on structural changes in proteins, specific rotations of gluten dispersions extracted from flour and freeze dried gluten before and after heating, were measured. The results are given in Table 6.

In Table 6 only very small (if any) changes of optical rotations after heating, as compared with nonheated dispersions, can be observed. It means that this operation does not involve structural changes in gluten and therefore can be applied in procedures where acetic acid is used as a dispersing agent.

Table 6

The influence of heating of gluten solutions on its specific rotation, in angular degree

Sort of wheat Varieties	Before heating		After heating	
	flour	gluten	flour	gluten
Saratowska	−113.7	−113.5	−117.7	−115.4
Manitoba	—	−126.6	—	−134.1
Polish mixed	−121.8	—	−119.2	—

DISCUSSION AND CONCLUSIONS

The results obtained in this work give new information concerning the behaviour of gluten proteins during some procedures carried out in order to isolate and purify it. The problem not solved until now was the method of dispersing gluten. In Wakar's laboratory sodium salicylate (Mc Calla 1935) is applied because of rather good efficiency and the inhibition the proteolytic enzymes. We found acetic acid to be a more convenient agent, if further separation and purification have to be carried out, though a less efficient one. Some authors apply homogenization at 2—3000 rmp and/or the addition of 3 M urea in order to increase the efficiency of dispersion. Meredith and Wren (1966), for example, using 3 M urea in 0.1 M acetic acid obtained 95% of dispersion of wheat flour proteins. Jankiewicz and Pomeranz (1965) found that the effect of this factor on structural changes of gluten is almost fully reversible. On the other hand our results suggest that the structural modification of polypeptide chains under the action of 3 M urea is significant and irreversible. Therefore the application of this dispersing factor in investigations on gluten structure should not be recommended.

The dispersion of gluten protein using homogenization is commonly used. Wakar suggested that because of drastic mechanical forces this method may involve significant structural changes in gluten, which is very sensitive to mechanical factors. Our results confirmed fully this suggestion showing that homogenization involved considerable changes of hydrodynamic properties of gluten molecules, as compared with the dispersions obtained by shaking. Therefore dispersing gluten using homogenization should not be recommended.

To increase the efficiency of protein dispersion, the two step acetic acid extraction of flour or freeze dried gluten, using two successive portions of solvent, was found to be useful.

In some cases washed out and freeze dried gluten is the more convenient material for investigations, as compared with flour extract. Re-

sults presented in this work did not indicate any significant change of gluten structure during such a mild operation and therefore this procedure can be applied also in more detailed structural research.

In investigations in which a longer period of storage of the protein dispersions in acetic acid is needed, the activity of native proteolytic enzymes has to be taken into consideration. Therefore their inactivation using 3—5 min heating in a boiling water bath is commonly carried out. Our investigations showed that even 5 min heating does not fully inactivate proteolytic enzymes, and thus 6—7 min heating should be necessary. On the other hand Wakar (1969) suggested that this operation should involve some structural changes in gluten. Our experiments on 3 min heating however did not demonstrate any change of specific rotation, and therefore it can be suggested that also somewhat longer heating would not cause significant structural changes in gluten.

The methodical investigations discussed above were carried out using glutens of various technological quality. Comparing the results the observation may be made, that weak gluten is more sensitive than the strong type to external factors which can modify protein structure.

SUMMARY

The influence of some methodical factors, used for gluten isolation and purification, on its conformational changes, was investigated. The experiments concerned the application of 3 M urea as a dispersing agent, the manner of dispersing (homogenization or shaking), the influence of freeze drying, as well as that of heating in order to inactivate proteolytic enzymes. As a measure of the denaturating action of factors investigated, the determinations of viscosity, and thus hydrodynamic properties of sodium salicylate solutions, as well as those of optical rotations of acetic acid extracts, were applied.

3 M urea has been shown to cause considerable, and only partially reversible conformational changes of gluten molecules, and the homogenization acted mechanically, breaking down some molecular bonds. Therefore these factors should not be applied in more detailed investigations concerning gluten structure. On the other hand no structural changes during freeze drying gluten, as well as short heating of its acetic acid extracts could be observed. But 5 min heating was shown to inactivate the proteolytic activity only in 70—90%. The results shown also, that weak gluten is less resistant to chemical, as well as mechanical factors than stronger ones.

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Niektóre aspekty metodyczne badań nad glutenem pszennym

Streszczenie

Przebadano wpływ szeregu czynników metodycznych, stosowanych przy izolowaniu i oczyszczaniu glutenu pszennego, na zmiany w jego konformacji cząsteczkowej. Doświadczenia dotyczyły stosowania 3 M mocznika, jako czynnika dysperg ującego, sposobu rozpraszania (przez homogenizację, lub wstrząsanie), wpływu liofilizacji oraz ogrzewania wyciągu w celu zniszczenia enzymów proteolitycznych. Jako miarę wpływu denaturującego badanych czynników stosowano pomiary lepkości, a więc własności hydrodynamicznych roztworów w salicylanie sodowym oraz skręcalności optycznej wyciągów w kwasie octowym.

Wykazano, że 3 M mocznik powoduje znaczne i tylko częściowo odwracalne zmiany konformacji cząsteczek glutenu, a homogenizacja działa mechanicznie na porozrywanie wiązań cząsteczkowych; czynniki te nie powinny więc być stosowane w bardziej szczegółowych badaniach nad strukturą glutenu. Nie stwierdzono natomiast wpływu liofilizacji ani kilkuminutowego ogrzewania na konformację glutenu, jednakże 5-minutowe ogrzewanie inaktywowało enzymy proteolityczne tylko w 70—90%. Uzyskane wyniki świadczą również, że gluten słaby jest mniej odporny na czynniki chemiczne, jak i mechaniczne, niż gluten mocniejszy.

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