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THE DISTRIBUTION OF DISULFIDE BONDS IN SOME PROTEIN FRACTIONS OF GLUTEN COMPLEX

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Using wheat varieties of various baking quality, the distribution of S-S bonds in gluten complex molecules were investigated. Therefore the contents of those bonds were determined in the absence and in presence of 7 M urea in whole gluten solubilised in acetic acid, as well as in glutenin and gliadin. In those two separate protein fractions the dynamics of interchain S-S bond reduction was also investigated. In last case the two step reduction was observed; the first reduction step of glutenin ended earlier end on much lower level in that of high quality. It can be concluded, that the distribution, but not the content of S-S bonds in separate protein fractions can be bound with the baking quality of wheat flour.

INTRODUCTION

Flour baking quality is dependent on such factors, as protein fractional composition [5, 8, 12], contents of some particular protein components [9] and the compactness of protein molecules, measured as the specific viscosity of their solutions [1, 13] or the ratio E_{280}/total N [9]. This last property depends on the content, distribution and strenght of covalent bondings, joining together the particular molecules or polypeptide chains, to build up the macromolecule of gluten. Among them the strong disulfide bonds are of significance [2, 7]. According to our earlier works [3, 4], they are not evenly distributed in the gluten molecule and the level of those bonds, which are reducible directly in the absence of urea, is influencing the structural features of proteins, being important for the formation of baking potentialities of flour. Among the S-S bonds reducible in the absence of urea, two types can be distinguished, which are differing by various accessibility for the reducing agent, as their reduction proceeded in two independent steps [4].

In the following experiments the course of S-S bond reduction in gliadin and glutenin isolated from flours of different baking quality, was investigated. The contents of total S-S bonds and those reduced directly in the absence of urea in protein fractions, were also compared. The results of those experiments are discussed in this paper.

MATERIAL AND METHODS

Winter wheat harvested in 1974 was supplied by the Breeding Station Laski. The varieties Saratowskaja 29 and 694/74 exhibited high, but Tetrix and 1347/74 — the low baking quality. For analysis flours of 70% milling were used.

Flours were extracted according to Coates and Simmonds [6] and proteins contained in acetic acid were separated by means of Sephadex G 100 columns 25×600 mm and acetic acid as the eluting agent. In separate fractions or other solutions proteins were determined according to Lowry et al. [11] or in the spectrophotometer VSU-2 at 280 nm. In both cases the standard curves were prepared on freeze dried purified gluten. In pyrophosphate and acetic acid fractions, as well as in separated gliadin and glutenin the total S-S bond contents (in presence of 7 M urea) and those accessible in the absence of urea, after different reduction times were determined, according to own method [3], in which however 2-mercaptoethanol was replaced by dithiothreitol (DTT). The reducing agent was used in the amounts 20 mm^3 DTT per 15 mg of gluten or 40 mm^3 per 10 mg of gliadin or glutenin. The time of total reduction amounted for gluten 24 hours and for gliadin or glutenin — 48 hours. The determinations were carried out after the incubation the protein with the reducing agent at 25°C for the full time or for intermediate ones, when the reduction dynamics was investigated.

RESULTS

The contents of total S-S bonds and those reduced in the absence of urea in pyrophosphate and acetic acid fractions, as well as in separated gliadin and glutenin, are presented in Table 1.

The data demonstrate, that the contents of S-S bonds in pyrophosphate fraction proteins were much higher, than in those of acetic acid fraction, containing gliadin and soluble glutenin. The percent of S-S bonds reduced directly, was much lower in the former fraction (34-53%), than in the latter one and did not show any connection with the baking quality of flour. Similarly, the total amount of S-S bonds in albumins and globulins, amounting 224-253 $\mu aeq \times g^{-1}$ of protein, was not connected with the baking quality. On the other hand proteins soluble in

Tabele 1 Total and intermolecular S-S bond contents in separate protein fractions of investigated flours in μaeq $SH \times g^{-1}$ of protein

Wheat sample	Pyrophosphate			Acetic acid			Glutenin			Gliadin		
	total	intramo-lecular	% of total	total	intramo-lecular	% of total	total	intramo-lecular	% of total	total	intramo-lecular	% of total
1347/74	253	112	44.0	195	103	53.0	113	106	96.0	180	181	100
Tetrix	224	119	53.0	176	109	62.0	122	110	90.0	197	178	90
694/74	241	108	45.0	201	127	63.0	104	89	82.0	169	176	100
Saratowskaja	239	139	58.0	182	132	73.0	134	104	74.0	203	204	100

acetic acid exhibited lower total contents of S-S bonds, amounting 176-201 μaeq x g^{-1}, which seem also not to be bound somehow with the baking quality. But the level, and particularly the percent participation of so called intermolecular S-S bonds (reducible in the absence of urea), as related to the total ones, was demonstrated to be higher in samples of good quality.

If, however, the proteins of acetic acid fraction were separated into gliadin and glutenin, the accessibility of S-S bonds in both subfractions increased significantly, reaching in case of glutenin 80 to 90% and in case of gliadin up to 100% of total ones. In the former case the percent accessibility for the reducing agent in the absence of urea could be oppositely related to the baking quality. Of interest is also the total content of S-S bonds, which is much higher in gliadin, than in glutenin.

In order to demonstrate the reduction of S-S bonds in the absence of urea, proceeding in two steps and to determine the distribution of those bonds reduced in the first or second one, as well as the time and level of the steady state period between those steps, the reduction dynamics were determined. The results of them, separately for glutenin and gliadin, are demonstrated in Table 2. The data indicate, that in all cases investigated, during the reduction of either glutenin or gliadin, after the first reduction step the steady state period was observed, in which the increase of SH groups did not occur. This means, that similarly to the results obtained for whole gluten [4], also in case of glutenin and gliadin, two types of S-S bonds reducible in the absence of urea, exist. Among the selected varieties of high and of low quality the starting time of this steady state occured in glutenin earlier (after 4^{th} or 5^{th} hour of reduction), than in gliadin (after 5^{th}-10^{th} hour), but the differences between the glutenins of high and low quality were, as concerns this feature, insignificant. On the other hand in case of gliadins of lower quality the steady state was initiating after the 5^{th} or 8^{th} hour of reduction, whereas in case of that of higher quality — not earlier than after the 10^{th} or 12^{th} hour. Also the level of S-S bonds reduced in the first step, as related to the total reduction observed in the absence of urea, was different in the various varieties, amounting in glutenins of low quality 60 or 73% and for those of higher quality — about 52%. The reverse relation was observed in case of gliadin, for which the first step of reduction was finished in case of varieties of low quality after the reduction of 52 or 63% of total S-S bonds reducible in those conditions and in case of those of higher quality — after about 66% of total S-S bonds had been reduced. Of interest is also, that the lenght of the steady state period between the first and second reduction steps in glutenin lasted only 3-5 h, whereas in gliadins it was much longer and amounted 7-10 h.

The comparison of data contained in Table 1 and 2 indicates, that the contents of S-S bonds in gliadin, reducible in the absence of urea,

Table 2. The reduction dynamics of intermolecular S-S bonds (accessible in the absence of urea) in glutenin (peak I) and gliadin (peak II), values given in μaeq $SH \times g^{-1}$ of protein

Wheat sample	Peak	Reducing time (hours)													% red. in the I step
		2	3	4	5	6	8	10	12	14	16	24	32	48	
1347/74	I	49.0	—	49.0	64.0	66.5	65.5	68.5	91.0	—	—	109.0	—	—	60.1
	II	44.0	—	61.0	—	60.0	90.0	97.0	92.0	—	99.0	181.0	—	—	52.5
Tetrix	I	54.0	63.5	58.0	80.0	88.0	79.0	80.5	91.0	—	—	110.0	—	—	72.7
	II	44.0	—	52.0	110.0	113.5	98.0	114.0	101.0	—	124.0	180.0	176.0		62.7
694/74	I	25.5	36.0	43.0	—	44.0	46.0	46.5	77.5	—	—	84.0	—	85.5	53.5
	II	12.0	—	25.0	—	—	59.5	97.5	105.0	90,0	115.0	114.0	—	176.0	65.3
Saratowskaja	I	23.0	—	49.0	56.5	54.0	82.0	—	—	—	—	104.5	—	—	52.3
	II	25.0	—	46.5	—	—	119.0	140.0	130.0	135.0	135.0	166.0	202.5	205.5	65.8

was very close or even uniform with the total contents of those bonds, determined in this protein in presence of urea. It indicates, that no (or very little only) so called intramolecular S-S bonds exist in this type of protein. In case of glutenin however the percent of S-S bonds reduced directly were lower and amounted for varieties of lower quality 90 and 94% and for those of higher quality — about 80%. In this case the total level of S-S bonds was much lower, than in case of gliadin. This indicates, that in glutenin about 20% of S-S bonds are of intramolecular character and/or is localised in deeper parts of molecules. This is however, together with the lack of such S-S bonds in gliadin, not comparable with the amounts of S-S bonds of the same accessibility observed in proteins of acetic acid fraction, amounting 30-50% of the total ones.

DISCUSSION

It was revealed earlier [3, 4], that during the reduction of S-S bonds in initial gluten with 2-mercaptoethanol or dithiothreitol proceeded in two steps, divided each other by the stedy state period. This observation has been explained, as the existence of two kinds of S-S bonds in this protein, differing in the accessibility for the reducing agent. The results of Table 2 indicate, that also in glutenin and gliadin similar types of S-S bonds can be distinguished. Of particular interest should be the former protein fraction, because the levels of S-S bonds, being most accessible because of the location in the external layer of molecule, were lower in varieties of high quality (about 52%), whereas in theese of poor quality amounted over 60%. This can suggest, that the distribution of S-S bonds in the complex gluten molecule might influence its compactness [5, 8], though the amount of sampels investigated was rather small. On the other hand, unexpected seems to be the fact, that in varieties of higher quality the easiest reducible S-S bonds were reduced during the shorter time (about 1 hour), than in varieties of lower quality, but possibly it could be due to the smaller amount of them in the former case. The completion time of the first reduction step was also much shorter in gliadins isolated from samples of lower quality, which can indicate for less compact structure of this protein, as compared to those of higher quality. However gliadin represents the protein of rather uncomplicated structure, neverthelles it contains two kinds of S-S bonds among those, reducible in the absence of urea. This is not easy to explain, particularly because of the almost 100% reduction of S-S bonds mentioned, as related to total ones, determined normally in gliadin, amounting 180 $\mu aeq \times 1\ g^{-1}$ of protein (Table 1). In this case the S-S bonds reduced in the first step in the absence of urea, would correspond to intermolecular ones and those reduced in the same conditions in the second step — to intramolecular ones. It can be also possible, that the first step of reduction con-

cerns in part all S-S bonds contained in some lowmolecular gliadin components (e.g. of α- or β-region), which might be easier accessible for the reducing agent, whereas in the second step those intermolecular ones, which are contained in γ or ω-gliadins, which show more compact structures.

Of interest should be also, that the final level of S-S bonds reducible in the absence of urea in glutenin isolated by means of Sephadex G 100 from the acetic acid protein fraction amounted 80-95% of total S-S bonds contained in this protein. This can be hardly understood, as the corresponding values calculated for initial gluten amounted 50-70%. Therefore the most probable explanation should be, that loosening of the molecule structure, causing the increase of S-S bond accessibility occured as the consequence of the protein partition. This does not remain in accord with the behaviour of protein treated in that way, as concerns the accessibility of peptide bonds for peptidase action [10]. It should be mentioned also, that the accessibility of S-S bonds in conditions described, increased to the higher extent in glutenins of lower quality, than in that of higher quality. This confirms our earlier suggestion, concerning the higher mechanical stability of the latter. It should be pointed out at last, that the results obtained in those investigations can not be compared with the data of other authors, as in the last years the significant decrease of interest in the role of S-S bonds in the building up of gluten proteins, can be observed in the literature, which, according to our experience, seems to be incorrect.

CONCLUSIONS

1. In glutenin and gliadin, similarly to the initial gluten, two kinds of S-S bonds reduced in the absence of urea, can be distinguished, which are differing in their accessibility for the reducing agent.
2. In samples of various quality the relationship of this property with the percent content of S-S bonds most easily reducible and the rate of their reduction in glutenin, was observed.
3. The separation of glutenin and gliadin by means of Sephadex G 100 caused in the former the significant increase of the level of S-S bonds reduced in the absence of urea, particularly in samples of lower quality.
4. The reduction rate of S-S bonds in particular fractions and samples of wheat proteins and in various conditions can be due to their distribution in proteins of different baking potentialities.

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LITERATURE

1. Bartoszewicz K., Kączkowski J., Liss W.: Bull. Acad. Polon. Sci. ser. sci. biol., 1972, **20**, 827.
2. Belderok B.: Getreide und Mehl 1967, **17**, 20.
3. Bernacka T., Kączkowski J.: Bull. Acad. Polon. Sci. ser. sci. biol., 1973, **21**, 565.
4. Bernacka-Mieleszko T., Kączkowski J.: Die Nahrung 1975, **19**, 769.
5. Bernacka T., Kączkowski J., Liss W.: Acta Agrobotanica 1977, **30(1)**, 5.
6. Coates J. H., Simmonds D. H.: Cereal Chem., 1961, **38**, 256.
7. Ewart J. A. D.: J. Sci. Food Agric., 1977, **28**, 191.
8. Filutowicz H., Serwatowska J., Kączkowski J.: Acta Soc. Bot. Polon., 1978, **47**, 269.
9. Jankiewicz M., Jankowski S.: II Tagung Intern. Probl. Modern. Getreideverarbeitung und Chemie 1966, 47, 54.
10. Liss W., Kączkowski J.: Acta Aliment. Polon., 1975, **1(25)**, 281.
11. Lowry O. H., Roseborough H. J., Farr A. L., Randall R. J.: J. Biol. Chem., 1951, **193**, 265.
12. Pomeranz Y.: J. Sci. Food Agric. 1965, **16**, 586.
13. Shorina O. C., Vakar A. B., Kretovich V. L.: Prikł. Biochim. Mikrobioł. 1966, **2**, 1.

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ROZMIESZCZENIE WIĄZAŃ S-S W NIEKTÓRYCH BIAŁKACH KOMPLEKSU GLUTENU PSZENNEGO

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Streszczenie

Stosując próby odmian pszenicy ozimej o zróżnicowanej wartości technologicznej badano rozmieszczenie wiązań dwusiarczkowych S-S w glutenie i jego frakcjach. W tym celu oznaczano zawartość wiązań S-S w nieobecności 7 M mocznika (tzw. powierzchniowych lub międzyłańcuchowych oraz z jego dodatkiem (wewnątrzłańcuchowych) w glutenie rozpuszczonym w kwasie octowym, a także w gluteninie i gliadynie. Wyniki tych oznaczeń, podane w tab. 1 wskazują na nierównomierne rozmieszczenie tych typów wiązań w glutenie i gluteninie różnej jakości. W celu wykazania nierównomierności rozmieszczenia wiązań S-S międzyłańcuchowych o różnej dostępności dla czynnika redukującego zbadano w gluteninie i gliadynie dynamikę redukcji tych wiązań, której wyniki przedstawiono w tab. 2. Podobnie jak przy dynamice redukcji międzyłańcuchowych wiązań S-S w glutenie wyjściowym, również w jego frakcjach wykazano dwustopniowość procesu, przy czym etapy te były różnej długości z występującą na różnym poziomie redukcji przerwą. Pierwszy etap w przypadku gluteniny wyższej jakości kończył się wcześniej i na niższym poziomie w porównaniu z pozostałymi próbami. Szybkość redukcji mierzona na początku I etapu była zarówno w gluteninie, jak i w gliadynie 2-krotnie wyższa w przypadku prób niższej jakości, niż u pozostałych. Wnioskuje się, że rozmieszczenie, a nie zawartość wiązań S-S w poszczególnych frakcjach białek ma wpływ na wartość wypiekową pszenicy.