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RHEOLOGICAL PROPERTIES OF UZBEKISTAN WHEAT GLUTEN AS A FUNCTION OF DISULFIDE AND H-BOND CONTENTS DURING CONDITIONING

N. V. Korableva* and T. D. Kasymova

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Rheological properties of wheat gluten as a function of the presence of free sulfhydryls and disulfide bonds in addition to their ratio during various conditioning regimes were studied. It was shown that coagulation of the gluten as the temperature increased was accompanied by a decrease in the number of free sulfhydryls and an increase in the number of rheologically active disulfide bonds. Changes occurring in the gliadin and glutenin fractions during various conditioning regimes were seen using IR spectroscopy. It was found that the relative strength of absorption bands due to H-bonds increased as the treatment temperature was raised above 60°C. Significant changes in the protein molecule structure that caused substantial changes in its rheological properties because of thiol–disulfide exchange reactions and H-bond strength occurred during the conditioning.

Keywords: gluten, gliadin, glutenin.

Wheat gluten is an unusually complicated complex of heterogeneous protein components that form a three-dimensional network of linearly cross-linked glutenin subunits and gliadin components through hydrogen, hydrophobic, and disulfide bonds [1]. It is known that the rheological properties of gluten and dough that determine the culinary value of wheat flour are closely related to the presence and quantitative ratio of disulfide and sulfhydryl groups in the wheat grain proteins in addition to the presence in the flour of low-molecular-weight thiols and disulfides [2]. Disulfide bonds fulfill important functions for maintaining the native conformation of the protein molecules [3]. However, their mechanism of action in forming the gluten protein complex with its unique rheological properties is as yet unknown. Some researchers [4–8] have reported a direct dependence of gluten quality and dough physical properties on the content of disulfide bonds in the gluten proteins. Others [9–12] suggested that the gluten quality is determined not by the number of disulfide bonds but the rate of their rupture and reformation in thiol–disulfide exchange reactions. Bogdanov et al. [8] analyzed the number of disulfide bonds and sulhydryl groups in wheat reserve proteins and concluded that gluten proteins of different quality were practically identical with respect to total content of disulfide bonds and that the gluten quality depended on only a certain part of the rheologically active disulfide bonds.

Until now, published data have not allowed an accurate assessment of the distinguishing features of gluten protein fractions (gliadin and glutenin), the type of bonds between its components, and the forms of interactions in the protein complex on which the specific rheological properties of gluten that are responsible for its quality depend.

Our goal was to demonstrate the influence of wheat grain moist-heat treatment (conditioning) on gluten and its fractions as related to the gluten rheological properties and content of free sulfhydryls and disulfide and H-bonds.

Conditioning of wheat grain is an important stage in the processing scheme for producing flour that can advantageously change the gluten rheological properties.

The influence of conditioning in the range 40–80°C on the gluten rheological properties and content of free sulfhydryls and rheologically active disulfide bonds was studied using wheat variety Kroshka that was grown under natural conditions in Uzbekistan for the 2006 and 2008 harvests. The gluten content was determined by rinsing away starch and husks under running water. The rheological properties of the gluten were measured on an IDG-1 instrument; the content of free sulfhydryls in gluten and its fractions, by colorimetry after reaction with 5,5′-dithio-*bis*(2-nitrobenzoic acid).

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax: (99871) 120 64 75, e-mail: nadya1477@yahoo.com. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 360–363, May–June, 2010. Original article submitted June 15, 2009.

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TABLE 1. Change of Gluten Rheological Properties and Quality During Various Conditioning Temperature Regimes

Wheat conditioning temperature, °C	Crude gluten content, %	Dry gluten content, %	IDG reading, units	Quality group
Starting wheat	25.3±0.12	9.98±0.10	110	III (unsatis.)
40	22.16±0.12	9.96±0.87	100	III (unsatis.)
50	22.07±0.14*	9.93±0.02	100	III (unsatis.)
60	21.00±0.15*	9.75±0.10	95	II (satis.)
70	18.00±0.26*	9.38±0.17	90	II (satis.)
80	Not rinsed	–	–	–

n = 5; *p < 0.05.

TABLE 2. Change of Free Sulfhydryl Content, Rheologically Active Disulfide Bonds, and SS/SH Ratio μM/g of Protein During Conditioning

Fraction	Treatment temperature, °C					
	starting wheat	40	50	60	70	80
	Free sulfhydryl groups					
Gluten	8.03±0.07	6.51±0.30	4.45±0.23*	3.75±0.12*	3.06±0.5*	3.08±0.16*
Gliadin	1.21±0.29	1.54±0.11	2.42±0.30*	1.36±0.50*	1.28±0.44	1.07±0.48
Glutenin	7.76±0.38	7.59±0.16	6.83±0.36	6.67±0.02	6.24±0.14	6.11±0.04
	Rheologically active disulfide bonds					
Gluten	13.34±0.06	15.18±0.02*	20.64±0.04*	21.41±0.12*	19.84±0.09*	18.79±0.10*
Gliadin	2.65±0.66	3.86±0.55	7.94±0.38*	9.05±0.41*	13.66±0.16*	21.82±0.62*
Glutenin	14.86±0.12	14.19±0.33	23.50±0.61*	25.51±0.32*	29.74±0.26*	33.54±0.52*
	SS/SH ratio					
Gluten	1.67	2.34	4.65	5.74	6.51	6.13
Gliadin	2.19	2.51	3.28	6.65	10.67	20.39
Glutenin	1.92	1.87	3.44	3.83	4.77	5.49

n = 5; *p < 0.05.

Table 1 lists the rheological properties of gluten and its quality at various conditioning temperatures. Increasing the gluten treatment temperature to 70°C coagulates the gluten, changing the IDK-1 reading from 110 units (quality group III) in starting gluten to 90 units (quality group II) at 70°C. Increasing the wheat treatment temperature further causes partial denaturation of the gluten complex so that the gluten cannot be rinsed out.

Furthermore, increasing the wheat treatment temperature decreases the yield of crude gluten. However, the content of dry gluten remains comparatively constant. This effect may be related to the fact that increasing the treatment temperature reduces the ability of the gluten to be hydrated.

The conditioning process has different effects on the gluten protein fractions. Increasing the treatment temperature from 40 to 60° increases the content of free sulfhydryls in the gliadin fraction. This is accompanied by a reduction of disulfide bonds. However, reduction of disulfide bonds changes the molecular structure by loosening it because all disulfide bonds in gliadin are intramolecular [13, 14]. Increasing the temperature further gradually decreases their amount. The content of rheologically active disulfide bonds in the gliadin fraction increases sharply from 2.65 to 21.82 μM/g of protein as the wheat treatment temperature is raised to 80°C (Table 2). This effect is possibly related to the fact that the temperature increase causes partial denaturation of the gliadin components and their cleavage into components with lower molecular weight, the disulfide bonds in which are much more readily analyzed.

The glutenin fraction contains both intramolecular and intermolecular disulfide bonds [13, 14]. Their reduction would cause decomposition of large molecules into components with lower molecular weights. This would degrade the gluten rheological properties. These data show that carrying out the conditioning process decreases the number of free sulfhydryl groups and increases intermolecular bonds and protein aggregation because of the formation of many disulfide bonds (Table 2), the content of which increases by 2.26 times as the wheat treatment temperature is raised.

The content of free sulfhydryls in gluten is characteristically less than in the total isolated gliadin and glutenin fractions. We assume that this is due to disulfide–sulfhydryl exchange reactions that occur during gluten formation and is related to the formation of new disulfide bonds between gliadin and glutenin molecules in addition to soluble proteins. This is also confirmed by the data in Table 2.

The SS/SH ratio is much greater in strong wheat than in weak wheat according to many observations [2]. Table 2 presents data for the change of the SS/SH ratio resulting from the conditioning process. Increasing the wheat treatment temperature increased this ratio in all instances.

IR spectroscopy studies of lyophilized gliadin samples showed that increasing the treatment temperature to 60°C increased the relative intensity of absorption bands at 2855 cm^{-1} (ν s) and 1650 (σ s) for symmetric stretching vibrations of C–H bonds in methyls and bending vibrations of amino groups. This may indicate that H-bonds are increased in gliadin. Increasing the treatment temperature to 80°C caused absorption bands at 1350 (σ s), 1100 (σ s), and 950 (σ s) for symmetric bending and stretching vibrations of intermolecular hydroxyls to disappear. This indicated a change in the hydration capability of gliadin.

IR spectral analysis of the glutenin fraction showed that increasing the wheat conditioning temperature increased the relative intensity of the absorption band at 1666 cm^{-1} (σ s) and generated a new absorption band at 1410 (σ s) belonging to symmetric bending vibrations of amino groups and C–H bonds, respectively. The main band (1666) also shifted by ~10 cm^{-1} to lower frequency (1653) compared with untreated glutenin. This may also indicate that H-bonds in glutenin strengthened as the conditioning temperature was raised.

Thus, the studies showed that changes in the rheological properties of gluten during wheat grain conditioning are caused by changes in the structure of its protein molecules. Coagulation of gluten as the treatment temperature is increased is due to structural rearrangement of the protein complex as a result of thiol–disulfide exchange and changes in the strength of disulfide and H-bonds.

EXPERIMENTAL

We used wheat grain from the 2008 harvest with 9.69% moisture and 15.2% protein content; 25.3%, gluten; 1.86%, ash. Weighed portions of wheat were moistened gradually to obtain final moisture 16%. The amount of water required for moistening was calculated using the published formula [15]. The grain was treated for 10 min in a moist thermostat at 40, 50, 60, 70, and 80°C. Wheat grain treated in this manner was stored in closed containers at room temperature for 5 h, ground, and used for further analysis. Gluten was separated by the standard method [16] by washing away starch and husks under running water. Rheological properties of gluten were determined on an IDG-1 instrument; the amount of dry gluten, by drying crude gluten as a thin film in a drying cabinet at 105°C to constant mass. They were expressed in percent relative to the mass taken for washing gluten.

Protein fractions were extracted from treated wheat samples by the literature method [17] using successive extraction of reserve wheat proteins by EtOH (70%) and NaOH solution (0.05 N) after extraction of water-soluble fractions by phosphate buffer (0.1 M, pH 7) containing NaCl (1 M).

The resulting gliadin and glutenin protein fractions and gluten samples were lyophilized.

Free sulfhydryls were determined colorimetrically after reaction with 5,5′-dithio-*bis*(2-nitrobenzoic acid) (Elman reagent) by the procedure proposed by Lagrain et al. [10]. Rheologically active disulfide bonds were determined by the method of Weegels et al. [6] as modified by Hayta and Shofield [18].

IR spectra were taken on a Nicolet Magna 560IR IR spectrometer in the range 4000–400 cm^{-1} as mixtures with KBr at sample concentration 1:80.

Statistical processing used the Microsoft Excel computer program and commonly accepted statistical criteria [19]. The results were statistically significant from the control at $p < 0.05$.

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