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Effects of Wheat Gluten Hydrolysate and Its Ultrafiltration Fractions on Dough Properties and Bread Quality

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Summary

Two fractions (50-K and permeate) from a proteolytic hydrolysate (degree of hydrolysis, DH=3.8 %) of wheat gluten were separated using ultrafiltration (UF) membrane with molecular mass cut-off of 50 kDa. The effects of the wheat gluten hydrolysate (WGH) and its UF fractions on the mixing behaviour and viscoelastic properties of wheat dough were presented. The WGH and its UF fractions modified the mixing properties of dough. The addition of these fractions improved the viscoelastic characteristics of wheat dough. A significant ($p<0.05$) effect of 50-K fraction on these characteristics of wheat dough was observed. After adding these fractions, the bread was considered acceptable by the sensory panel. Also, 50-K fraction resulted in significant ($p<0.05$) increase in the crumb firmness, while the bread made with wheat flour with WGH and permeate (P) fraction showed softer crumbs compared to that of wheat flour. Moreover, these fractions had anti-staling properties for bread during storage. Hence, the wheat gluten hydrolysate and its UF fractions are the products with promising potential in the baking products.

Key words: gluten hydrolysate, ultrafiltration fractions, dough properties, bread quality evaluation, texture profile analysis

Introduction

Wheat gluten, a by-product of the wheat starch industry, is a typical water-insoluble protein. Wheat gluten belongs to wheat storage proteins including glutenins and gliadins. Gliadins are polymorphic polypeptides with a molecular mass (M_r) between 30 000 and 80 000, whereas glutenins are multi-chained polypeptides and vary in M_r from about 80 000 to several million (*1,2*). In the food industry, wheat gluten is mainly used as an additive for improvement of baking quality of flour. In wheat dough making, gluten protein molecules become hydrated and interact to form a three-dimensional structure, determining the rheological properties of dough (*3,4*).

Much research has been focused on enhancing wheat gluten functional properties to extend its utilization. Enzymatic hydrolysis has been proved to be able to improve the solubility and develop the emulsifying and foaming properties of wheat gluten (*5,6*). Molecular mass distribution of the peptides in the protein hydrolysate is one of the most important factors in producing the protein hydrolysates with desired functional properties to be used as functional materials (*7*). The peptide chain length in the protein hydrolysates has no significant effect on the functional properties; any specific functional properties need optimal degree of hydrolysis (*8*). Large molecular mass peptides are presumed to be associated with the improvement of the functionality of the hydrolysates. Drago and Gonzalez (*9*) showed that enzymatic

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hydrolysis of wheat gluten led to the fact that most of the key hydrolysate functional properties differ from those of the intact protein, they depend to a great extent on the molecular size or the degree of hydrolysis (DH). The extent of hydrolysis will depend on the final use of the hydrolysate. It is necessary to keep a balance between molecular size and flexibility. In the previous study, we found that the functional properties of the peptides obtained by enzymatic hydrolysis of gluten protein and membrane ultrafiltration were significantly different (*10*). Although other modified proteins have been widely used in the food industry, no literature on the effect of the modified wheat gluten on the food quality is available.

The aim of the present study is to investigate the effects of wheat gluten hydrolysate (WGH) and its ultrafiltration (UF) fractions on the dough properties (mixing and viscoelastic properties). The mixing and viscoelastic properties of wheat dough were determined using Farinograph and Alveograph. Change in bread quality of wheat flour with these fractions was evaluated. Moreover, potential use of the WGH and its fractions in breadmaking was also tested.

Materials and Methods

Materials and reagents

A commercial blend of wheat flour was provided by Haixiang Flour (Zhengzhou, China). Commercial wheat gluten with 71.5 % (wet mass) of crude protein and 6.8 % of moisture was provided by Lianhua Co., Ltd. (Zhoukou, China). The commercial enzyme (Protamex™, 105 U/g) was kindly provided by Novozymes, A/S (Beijing, China). Protamex™ (EC 3.4.21.62/3.4.24.28) is a *Bacillus* protease complex developed for the hydrolysis of food proteins and fulfills the purity demands for food-grade enzymes set by the Joint FAO/WHO Expert Committee on Food Additives (JEFTA) and the Food Chemical Codex (FCC). Optimal working conditions for Protamex™ are reported to be pH=5.5–7.5 at temperature of 35–60 °C (*11*).

Preparation of wheat gluten hydrolysate and its ultrafiltration fractions

Preparation of wheat gluten hydrolysate and its UF fractions was carried out according to our previous method (*10*). The hydrolysate without membrane filtration was called WGH. A fraction of 8 % (by mass per volume) of aqueous dispersion of wheat gluten was incubated in a water bath at 48 °C for 10 min. When the gluten dispersion reached 48 °C, protease was added, at enzyme to substrate ratio of 2000 U/g. Proteolysis was performed at pH=6.8. At the end of the incubation period the enzyme was inactivated by heating for 10 min at 100 °C. The resulting hydrolysate was then rapidly cooled to about 25 °C in an ice bath and consecutively filtered through membrane filter with 50-kDa mass cut-off (A/G Technology Co., model UFP-5-C, Needham, MA, USA). Finally, the retentate (50–K fraction) and permeate (P fraction) were freeze-dried and stored at –20 °C.

Determination of the degree of hydrolysis

The degree of hydrolysis of WGH was measured by the *o*-phthaldialdehyde (OPA) (*12*). The gluten hydrolysate powder was solubilized at 1.25 mg/mL, in 12.5 mM Na borate buffer, pH=8.5, and 2 % (by mass per volume) sodium dodecyl sulphate (SDS). A volume of 50 μL of this solution was mixed with 1 mL of reagent composed as follows: 50 mL of 0.1 M Na borate buffer, pH=9.3, 1.25 mL of 20 % (by mass per volume) SDS solution, 100 mg of N,N-dimethyl-2-mercaptoethylammonium chloride (DMMAC), and 40 mg of OPA dissolved in 1 mL of methanol. The mixture was allowed to stand for 2 min before measuring the absorbance at 340 nm. The number of amino groups was determined with reference to the L-leucine standard curve (between 0.5 and 5 mM). The increase in amino groups between native gluten and hydrolysate was attributed to proteolysis and DH was calculated by the following equation:

$$DH=[(\alpha-n_i)/n_T]\cdot 100/\% \qquad /1/$$

where n_T is the total number of amino groups in native gluten after total hydrolysis with 6 M HCl for 24 h, n_i is the number of amino groups in native gluten and α is the number of free amino groups measured in the gluten hydrolysate. DH was the mean of four determinations.

Evaluation of dough quality properties

Farinograph absorption, dough development time and stability time were determined according to the methods of the American Association of Cereal Chemists (AACC) (*13*). Alveograph parameters were also measured by standard methods of the American Association of Cereal Chemists (*14*). The content of WGH and its fractions in wheat flour was 1 % (by mass).

Baking procedure

Baking performance was carried out according to our previous method with some modifications. The dough formulation comprised (in g): wheat flour 100, compressed yeast 1.6, sodium chloride 1.5 and shortening 3.0. The addition of water depended on a Farinograph test using the 500-BU (Brabender units) line. The resulting dough was allowed to rest for 15 min in a cabinet at 30 °C and 70 % relative humidity (RH). The bulk dough was then sheeted by a roller having two rolls of 50·12.6 cm^2. The dough was divided into pieces of 80 g, hand-moulded, proofed at 30 °C and 96 % RH up to its maximum volume, and then baked for 18 min at 200 °C. Bread loaf specific volume was determined by rapeseed displacement. Bread was stored at 20 °C and 70 % RH for different time periods.

Bread quality evaluation

Bread quality was evaluated by mass, volume (determined by rapeseed displacement in a loaf volume meter), specific volume, moisture content, acceptance and crumb texture.

Bread firmness measurements were made with texture profile analysis (TPA) model of Texture Analyzer (TA-XT2i, Stable Micro Systems, England) according to our previous method (*15*). Before measurement, bread

was cooled at ambient temperature for 2 hours. On the test days, bread slices (15-mm thickness) were compressed using a 50-mm diameter aluminium plunger with a 5-kg load cell. The rates of pretest, test and posttest were 3.0, 1.7 and 1.7 mm/s, respectively. The compression curves of the breadcrumb (distance *vs*. force) were plotted, and the force readings (in Newton) at 25 % compression were taken as a measure of bread firmness in accordance with the AACC method 74-09 (*16*). Six slices were analyzed from each loaf. The parameters recorded were hardness, chewiness, cohesiveness, springiness and resilience.

Statistical analysis

All the tests were done in triplicate and data were averaged. Standard deviation was also calculated. Duncan's multiple-range test (*17*) was used to evaluate significantly different ($p<0.01$) means for each sample.

Results and Discussion

Degree of gluten hydrolysis

The degree of gluten hydrolysis in the study was (3.8±0.2) %. After membrane ultrafiltration (UF), the WGH was separated into two fractions, 50-K and P. The yield of the UF fractions was 56.7 and 43.3 %, respectively.

Influence of wheat gluten hydrolysate and its ultrafiltration fractions on dough mixing properties

The addition of WGH and its UF fractions caused differences in the dough mixing behaviour measured by the Farinograph. Table 1 presents the main parameters recorded in the Farinograph. Water absorption (WA) or percentage of water is the water content required to yield dough consistency of 500 BU. Dough development time (DDT) is time to reach maximum consistency in minutes. Stability is time during which dough consistency remains at 500 BU, and mixing tolerance index (MTI) is the difference in consistency between the highest peak and that 5 min later (in BU).

WGH and its UF fractions mainly modified the water absorption. Great increase was achieved by the addition of these fractions and the extent of the increase depended on the structure and molecular mass distribution of the added fractions. The highest water absorption was found with the addition of 50-K fraction, followed by WGH and P fraction. Dough development time and stability value are indicators of the flour strength, with higher values suggesting stronger dough. The gluten hydrolysate led to the decrease in dough development time, while 50-K fraction resulted in significant ($p<0.05$) increase. These fractions did not modify the stability, with the exception of WGH, which decreased it. The addition of 50-K fraction resulted in the significant ($p<0.05$) decrease of MTI, compared to the other two fractions. The Farinograph result shows that the addition of WGH and its UF fractions confers different mixing properties to the dough, probably due to their characteristics and molecular mass distribution. Enzymatic hydrolysis of the proteins results in three fundamental modifications: (*i*) an increase of the polar group number, which increases the product hydrophilicity, (*ii*) a decrease of the molecular mass chains, and (*iii*) changes in molecular conformation (*18*). Enzymatic hydrolysis modified the structure of wheat gluten. After hydrolysis, secondary linkages in wheat gluten proteins were destroyed. They weakened the cohesion and adhesion strength of gluten network. These modifications resulted in the changes in the rheological properties of dough.

Change in the viscoelastic characteristics of wheat dough

The effects of added WGH and its UF fractions on the Alveograph characteristics of wheat flour dough are shown in Table 2. These characteristics include the deformation energy (*W*), tenacity or resistance to extension

Table 1. Farinograph analysis of wheat dough containing WGH and its UF fractions

	WA/%	DDT/min	Stability/min	MTI/BU
Wheat dough	60.8±2.4ac	4.5±0.2b	5.5±0.4a	90±4.6bc
+1 % WGH	62.3±1.8c	3.9±0.1ac	4.8±0.3ab	93±5.1b
+1 % 50-K	65.5±2.7d	6.8±0.3de	5.5±0.3ac	82±4.9a
+1 % P	61.3±2.1ab	4.6±0.1bc	5.4±0.2ac	92±5.0bc

*WA, water absorption; DDT, dough development time; MTI, mixing tolerance index. The values in the table are the means of triplicates. Means within a column followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test

Table 2. Effect of WGH and its UF fractions on the Alveograph characteristics of wheat dough

	Wheat dough	+1 % WGH	+1 % 50-K	+1 % P
P/mm H_2O	53±3.4c	59±2.1bc	69±3.7a	56±2.5cd
L/mm	123±5.6a	121±4.3a	106±2.9b	128±5.2ac
$W/10^{-4}$ J	136±5.1bc	145±5.8c	183±6.4a	141±3.7bc
P/L	0.5	0.46	0.65	0.43
Proteolytic degradation/%	16.7±0.8a	5.4±0.2b	10.3±0.5c	3.2±0.1b

The values in the table are the means of triplicates. Means within a row followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test

(*P*), dough extensibility (*L*) and curve configuration ratio (*P/L*) of dough. The proteolytic degradation was determined by measurement of the Alveograph parameters after 3 h of incubation of dough pieces (*19*). The *P* value (dough resistance to deformation or tenacity) is an indicator of the ability of dough to retain gas. The *P* values increased with the addition of WGH and its UF fractions compared to the control. The highest effect was exhibited by 50-K fraction (69 mm H_2O), followed by WGH, while P fraction (56 mm H_2O) had the least influence. This is probably due to interactions between the added fractions and the wheat proteins.

L (the extensibility of dough) was generally known to be as a predictor of the processing characteristics of the dough. The gluten hydrolysate and P fraction did not modify the *L* values of the dough. *L* value was significantly ($p<0.05$) reduced by adding 50-K fraction (106 mm) compared with the wheat flour dough sample (123 mm). The resulting effect on *P* and *L* became evident in the *P/L* value, which provides information about the elastic resistance and extensibility balance of flour dough. Hence, the addition of 50-K fraction led to the highest *P/L* ratio (0.65 *vs.* 0.5 in the wheat flour dough sample). The influence on *W* (the deformation energy) depended on the fractions considered. *W* values were increased by WGH and its UF fractions. The 50-K fraction produced the highest *W* value (183 *vs.* 136 in the wheat flour dough sample). Moreover, it is important to note that the addition of these fractions promoted a marked decrease of the proteolytic degradation, and therefore the addition of these fractions led to a great improvement of wheat protein behaviour, allowing long proofing times.

Change in bread quality evaluation

Table 3 summarizes the differences in bread quality. The loaf volume of wheat flour bread with WGH increased, compared to the wheat flour bread ($p<0.05$). Additionally, the loaf volume of wheat flour bread with added WGH was higher than that of wheat flour with UF fractions. This is different from the reports of Batey (*20*), who found that gluten hydrolysate did not improve the bread loaf volume significantly. In terms of specific bread volume, complement of P fraction resulted in significant ($p<0.05$) decrease. The breads from wheat flour containing WGH and P fraction had higher moisture content compared to the bread from wheat flour, as observed in Table 3. The bread with 50-K fraction was the exception to this trend, since its moisture content was lower than of the bread with wheat flour.

According to the TPA shown in Table 3, addition of 50-K fraction increased significantly ($p<0.05$) the hardness of bread, while the addition of WGH and P fraction gave softer crumbs. The same trends were observed with the chewiness of the bread ($p<0.05$). Other parameters from the TPA did not show obvious changes. The crumb softness effect produced by WGH and P fraction is noteworthy in this study.

After adding WGH and its UF fractions, the loaves of bread were judged by the consumer panelists as acceptable, scoring >5 for each specific sensory characteristic and overall acceptability (Table 3).

Change in bread firmness during storage

Fig. 1 shows the change (increase) in bread crumb firmness during storage at 25 °C. The addition of gluten hydrolysate and its UF fractions led to slow increase in the firmness of bread during storage. The lowest firmness was observed in bread in the presence of 1 % WGH after storage for 3 days. Although the initial firmness of the bread containing 1 % 50-K fraction was higher than that of the bread made of wheat flour, its final firmness was far lower than the control. Thus, slower increase in

Table 3. Effect of WGH and its UF fractions on the wheat bread evaluation

	Bread with flour	+1 % WGH	+1 % 50-K	+1 % P
Loaf volume/mL	630±27c	645±31a	618±24bc	621±27c
Specific volume/(mL/g)	4.3±0.2ac	4.5±0.1a	4.1±0.2ab	3.8±0.1d
Moisture content/%	35.7±1.2a	37.8±1.5ac	32.4±1.0ab	36.5±1.1a
Sensory analysis				
Grain	5.9±0.3ac	6.4±0.2ab	5.6±0.5ac	5.9±0.1a
Crumb smoothness	6.1±0.5c	6.3±0.3ac	6±0.2bc	6±0.4c
Aroma	6.6±0.4a	7.3±0.4c	6.9±0.2ab	6.7±0.3a
Flavour	6.8±0.6c	7.5±0.4b	7±0.3ac	6.9±0.5c
Overall acceptability	6.3±0.3ab	6.9±0.5ac	6.1±0.1ab	6.5±0.6a
TPA parameters				
Hardness	253.6±34.5a	198.7±27.1ac	272.1±36.9b	208.4±25.7a
Chewiness	198.4±20.1c	136.2±12.3cd	201.5±15c	142.7±15.8b
Cohesiveness	0.756	0.758	0.754	0.755
Springiness	0.961	0.965	0.972	0.968
Resilience	0.502	0.511	0.498	0.504

[a]Nine point hedonic scale ratings: 9=extremely like and 1=extremely dislike. The values of loaf volume, specific volume and TPA parameters in the table are the means of triplicates. Sensory analysis was performed according to the section of Materials and Methods. Means within a row followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test

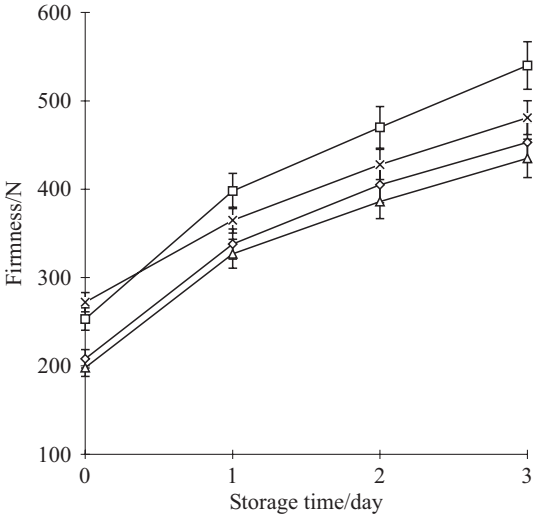

Fig. 1. Change in bread crumb firmness during storage at 25 °C and 70 % RH. The values in this figure are means of triplicates □ control; △ gluten hydrolysate; × 50-K; ◊ permeate

bread crumb firmness after the addition of 1 % WGH and its UF fractions indicates that these fractions have anti-staling properties in bread during storage.

Conclusion

From the overall results, it could be concluded that the addition of 1 % of wheat gluten hydrolysate (WGH) and 1 % of its UF fractions to wheat flour modified the mixing properties of the dough. The addition of these fractions improved the viscoelastic characteristics of wheat dough. 50-K fraction had significant ($p<0.05$) effect on these characteristics.

After adding these fractions, the bread was considered acceptable by the sensory panel. 50-K fraction resulted in significant ($p<0.05$) increase in the crumb firmness of bread, while the addition of WGH and P fraction decreased the hardness of bread crumbs compared to that of wheat flour. Moreover, these fractions showed anti-staling properties during storage. Hence, the WGH and its UF fractions have a promising potential in the baking products.

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References

1. P.I. Payne, M.A. Nightingale, A.F. Krattiger, L.M. Holt, The relationship between HMW glutenin subunit composition and the breadmaking quality of British-grown wheat varieties, *J. Sci. Food Agric. 40* (1987) 51–65.
2. P.R. Shewry, N.G. Halford, A.S. Tatham, High molecular subunits of wheat glutenin, *J. Cereal Sci. 15* (1992) 105–120.
3. W. Li, B.J. Dobraszczyk, J.D. Schofield, Stress relaxation behaviour of dough, gluten protein and gluten fractions, *Cereal Chem. 80* (2003) 333–338.
4. A.A. Tsiami, A. Bot, W.G.M. Agterof, Rheology of mixture of glutenin subfractions, *J. Cereal Sci. 26* (1997) 1–9.
5. A. Kato, K. Shimokawa, K. Kobayashi, Improvement of the functional properties of insoluble gluten by pronase digestion followed by dextran conjugation, *J. Agric. Food Chem. 39* (1991) 1053–1056.
6. E. Linares, C. Larre, M.M. Le, Y. Popineau, Emulsifying and foaming properties of gluten hydrolysates with an increasing degree of hydrolysis: Role of soluble and insoluble fractions, *Cereal Chem. 77* (2000) 414–420.
7. W.D. Deeslie, M. Cheryan, Fractionation of soy protein hydrolysates using ultrafiltration memebranes, *J. Food Sci. 57* (1991) 411–413.
8. J. Alder-Nissen, H.S. Olsen: The Influence of Peptide Chain Length on Taste and Functional Properties of Enzymatically Modified Soy Protein. In: *Functionality and Protein Structure*, A. Pour-El (Ed.), American Chemical Society, Washington, DC, USA (1993) p. 125.
9. S.R. Drago, R.J. Gonzalez, Foaming properties of enzymatically hydrolysed wheat gluten, *Innov. Food Sci. Emerg. Technol. 1* (2001) 269–273.
10. J.S. Wang, M.M. Zhao, X.Q. Yang, Y.M. Jiang, Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration, *J. Cereal Sci. 44* (2006) 93–100.
11. Protamex™ Product Sheet, B7 16d-GB, Novo Nordisk A/S, Bagsvaerd, Denmark (1998).
12. H. Frisher, H. Meisel, E. Schlimme, OPA method modified by use of N,N-dimethyl-2-mercaptoethylammonium chloride as thiol components, *Fresenius Z. Anal. Chem. 330* (1988) 631–633.
13. Official Methods of Analysis, American Association of Cereal Chemists (AACC), St Paul, MN, USA (2000) Method 54–21.
14. Official Methods of Analysis, American Association of Cereal Chemists (AACC), St Paul, MN, USA (2000) Method 54–30A.
15. J.S. Wang, M.M. Zhao, X.Q. Yang, Y.M. Jiang, Improvement of emulsifying properties of wheat gluten hydrolysate/λ-carrageenan conjugates, *Food Technol. Biotechnol. 44* (2006) 25–32.
16. Official Methods of Analysis, American Association of Cereal Chemists (AACC), St. Paul, MN, USA (1996) Method 74–09.
17. R.G.D. Steel, J. Torrie, D. Dickey: *Principles and Procedures of Statistics*: *A Biometrical Approach*, McGraw-Hill, New York, USA (1980).
18. R.D. Phillips, L.R. Beuchat: Enzyme Modification of Proteins. In: *Protein Functionality in Foods*, J.P. Cherry (Ed.), ACS Symposium Series No. 147, Washington, DC, USA (1981) pp. 275–298.
19. A. Colas, Tests of rheological properties used for determination of proteolytic activity, *Annales de Technologie Agricole*, *23* (1974) 241–247 (in French).
20. I.L. Batey, Enzymatic solubilization of wheat gluten, *J. Appl. Biochem. 7* (1985) 423–429.