Food Sci. Biotechnol. Vol. 15, No. 2, pp. 173 ~ 176 (2006)

Food Science and Biotechnology

Effect of Enzymatically Hydrolyzed Vital Wheat Gluten on Dough Mixing and the Baking Properties of Wheat Flour Frozen Dough

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Abstract The effect of enzymatically hydrolyzed vital wheat gluten (EHG) on dough mixing and the baking quality of wheat flour frozen dough was examined. Three different proteases, pepsin, trypsin, and chymotrypsin, were tested individually, sequentially paired, or in combination of all three enzymes. Addition of 1% EHG produced no observable effect on the mixing properties of wheat flour dough. However, addition of 2.5% pepsin-hydrolyzed gluten decreased the mixing tolerance of the wheat flour, and 1% trypsin-hydrolyzed gluten increased the loaf volume of both frozen and non-frozen dough. This finding suggests that trypsin-hydrolyzed vital wheat gluten may serve as a baking additive in replacement for $KBrO_3$ to improve frozen dough quality.

Key words: pepsin, trypsin, chymotrypsin, vital wheat gluten, wheat flour, frozen dough, mixing property

Introduction

It is known that the addition of gluten increases dough elasticity and loaf volume, therefore low protein flour supplemented with vital wheat gluten replaced with higher protein flour in the production of frozen dough (1, 2). Addition of vital wheat gluten also reduced the proofing time of non-frozen dough by strengthening the dough and improving its gas-sealing property. So far, however, vital wheat gluten could not reduce the proofing time of frozen dough due to loss of yeast activity (3).

Wheat gluten is now becoming available with improved solubility and enhanced foaming and emulsifying properties (4-15), and its application is growing increasingly diverse. Chemical gluten modification utilizes primarily mild acids or bases to replace uncharged hydrogen-bonding groups with charged hydrophilic groups (6-9). In biological modification, proteolytic enzymes have been used to increase gluten solubility and dispersibility (10-13). Use of gluten peptide in baking has rarely been reported. Asp *et al.* (16) found that addition of enzymatically modified gluten reduced dough-mixing times without affecting the loaf volume and Crowley *et al.* (17) used glutamine-containing peptides as baking ingredients to supplement for glutamine deficiency at catabolic stress.

The here-present work evaluates the effect of enzymatically hydrolyzed vital wheat gluten (EHG) on the mixing properties of wheat flour dough and the baking quality of wheat flour frozen dough. Proteases have substrate specificity and catalyze the hydrolysis of specific chemical bonds (18). Therefore, this study was designed to assess the effects of enzyme hydrolyzed glutens on dough mixed with wheat flour. Three proteases, pepsin, trypsin, and chymotrypsin were applied individually or in combination to produce various EHG versions and to test their impact on the baking quality of frozen dough.

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Received April 18, 2005; accepted November 24, 2005

Materials and Methods

Materials Straight grade wheat flour containing 12.17% protein (N × 5.74) and 13.80% moisture, and vital wheat gluten, containing 7.33% moisture and 83.54% protein, were purchased from Samyang Co. (Seoul, Korea) and Amylum Co. (Amylum France, Mesnil-Saint-Nicaise, France), respectively. Instant yeast, white sugar, salt, and shortening were obtained from Saf (Saf levure, Marcq, France), Samyang, Hae-pyo (Hae-pyo, Seoul, Korea), and Alfs shortening (Heinz Sam-lip Oil, Seoul, Korea), respectively. Pepsin (EC 3.4.23.1, 2500 units at pH 2.0, 37°C), trypsin (EC 3.4.21.4, 1000-2000 BAEE units at pH 7.6, 25°C), chymotrypsin (EC 3.4.21.1, 40-60 units at pH 7.8, 25°C), and $KBrO_3$ were obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA).

Hydrolysis of gluten by proteases A fixed ratio of enzyme protein to vital wheat gluten was used for each enzyme based on the enzyme's relative efficiency in solubilizing gluten established in preliminary tests (data not shown). For every 40 g of gluten, 300 mg of chymotrypsin, 300 mg of trypsin, or 400 mg of pepsin were added. Figure 1 depicts how 40 g of vital wheat gluten were dispersed and stirred in 300 mL of distilled water for 5 min. The slurry was then adjusted to the optimum pH (2 N NaOH or HCl) and temperature for each enzyme (pepsin: pH 2.0/45°C, trypsin pH 7.6/30°C, and chymotrypsin pH 8.0/30°C) and continuously mixed for 1 hr (pepsin and chymotrypsin) or 3 hr (trypsin). The enzymatic reaction was stopped by boiling the slurry at 100°C for 15 min and allowing it to cool to room temperature. After adjusting to pH 6.6 the slurry was centrifuged at 2500×g for 20 min (20PR-52D; Hitachi, Japan). The pellet was resuspended in 300 mL of distilled water and centrifuged at 2500×g for 20 min. The final pellets (GP: pepsin hydrolysate, GT: trypsin hydrolysate, GC: chymotrypsin hydrolysate) were lyophilized and passed through a 60-mesh sieve. To examine the effects of acidity, alkalinity, and elevated temperature on enzyme hydrolysis, controls were prepared using the hydrolysis

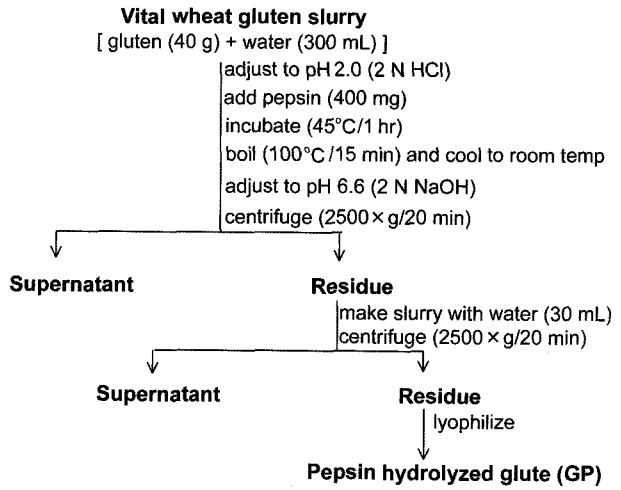

Fig. 1. Flow chart of pepsin hydrolysis of wheat vital gluten.

protocol described above in absence of the enzymes (GNP: GP without pepsin, GNT: GT without trypsin, GNC: GC without chymotrypsin).

Sequential hydrolysis of gluten with all the three enzymes is depicted in Fig. 2. After 3 hr of trypsin hydrolysis, the enzyme was heat-inactivated by boiling at 100°C for 15 min and allowing it to cool to room temperature. The slurry was then adjusted to pH 8.0, hydrolyzed with chymotrypsin at 30°C for 1 hr, heat-inactivated, and again allowed to cool to room temperature. The third hydrolysis step proceeded similarly (pepsin, pH 2.0, 45°C for 1 hr, heat inactivation). Finally, the mixture was centrifuged (2500×g, 20 min) and re-suspended in 300 mL of water. After an additional centrifugation, the final pellet (GPTC) was lyophilized and passed through a 60-mesh sieve. Sequential paired hydrolysis with pepsin/trypsin (GPT), trypsin/chymotrypsin (GTC), and chymotrypsin/pepsin (GCP) was done analogously.

Mixogram study Mixing properties were examined according to AACC procedure 54-40 (19) in a 10-g mixograph (National Mfg. Co., Lincoln NE, USA). $KBrO_3$ was dissolved in formula water and added to the flour during mixing. Powdered EHG was mixed with the flour on a dry-weight basis.

Baking test Breads were prepared according to modified AACC 10-10B (19) procedures. The concentration of the control bread ingredients was calculated in reference to wheat flour weight (baker's %) as shown in Table 1. The amounts of EHG and $KBrO_3$ added were 1% and 30 ppm of flour weight, respectively. Powdered EHG was mixed with flour, while $KBrO_3$ was dissolved in the formula water and added back to the flour. The dough was mixed for 9.5 min in a dough mixer (Hobalt A120; Hobart, Troy, MI, USA) at ambient temperature and divided into 130 g of piece. Fermentation was done in a proofer (Softmill; Dae-Hung, Seoul, Korea) for 1 hr at 30°C and 85±5% relative humidity. The fermented dough was immediately baked in an electrical deck oven (Softmill; Dae Hung) with four independently operating decks at 200°C (top) and 190°C (bottom) for 18 min.

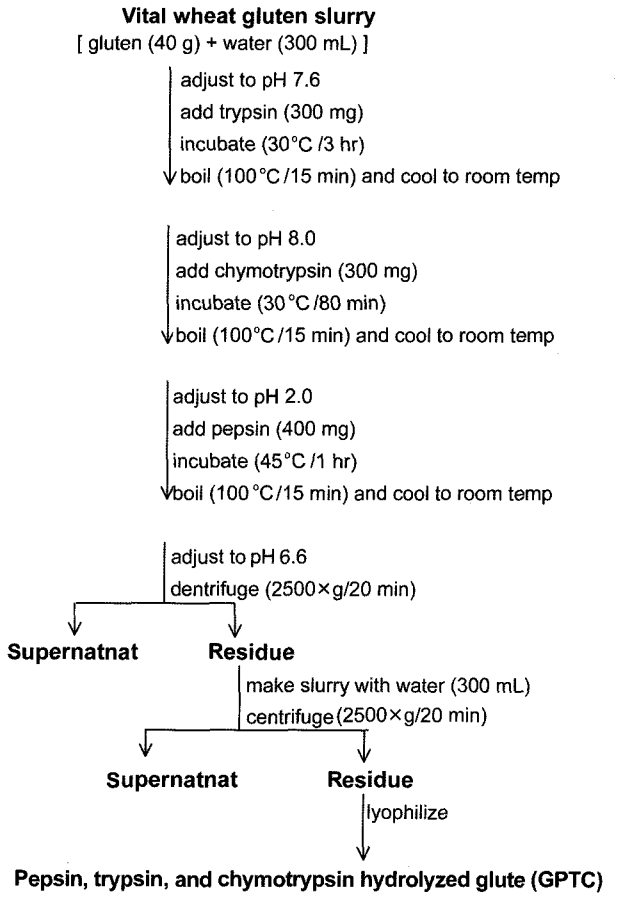

Fig. 2. Flow chart of sequential hydrolysis of vital wheat gluten with trypsin, chymotrypsin, and pepsin.

To examine the effects of EHG on frozen dough, mixed dough (28±2°C) was rapidly hand-balled 20 times, wrapped with polyethylene film (Kim's wrap, Chung-Jung Food, Seoul, Korea), and immediately freezed (PDF 9014; Il Sin, Seoul, Korea) at -70°C for 1 hr. The dough was then stored in a -20°C air freezer (SR-62EA; Samsung, Seoul, Korea) for 1 week. The two-step freezing method was adopted from Kim and Koh (20) to ensure minimal ice crystal formation. Prior to the experiments, the dough was transferred into a proofer (85±5% relative humidity) to defrost at 4°C for 16 hr and ferment at 30°C for 1.5 hr after which it was immediately baked as described above. Loaf volume and weight were determined at room temperature 1 hr after baking according to the AACC 10-10B (18)

Table 1. Formula of wheat flour bread

Ingredient	Baker's	Batch
Flour	100%	300 g
Water	65%	195 g
Yeast	1.77%	5.3 g
Sugar	6%	18 g
Salt	1.5%	4.5 g
Shortening	3%	9 g
Peptide	1%	3 g

procedure. Loaf volume was determined by the rapeseed displacement test (21). Specific volume (SV) was calculated as the ratio of bread volume to bread weight. All experiments were done five times, each producing three different loaves. All statistical analyses were done with SAS software version 6.11 (22). Differences among samples were analyzed with GLM (General Linear Model) and multiple comparisons employed Duncan's multiple range test ($p<0.05$).

Results and Discussion

We investigated the effect of EHG on dough mixing by adding EHG 1 or 2.5% to wheat flour in a mixograph. Figure 3 and 4 demonstrate that low EHG addition had no effect on the mixture, while addition of 2.5% gluten hydrolyzed by pepsin (GP, GPT, GCP, GPTC) decreased optimum mixing times and mixing tolerance. Among the pepsin hydrolyzed glutens, GP, hydrolyzed with pepsin alone, produced the most observable changes of mixing properties, whereas the sequentially hydrolyzed glutens, GPT, GCP, and GPTC, produced less effect than GP. The addition of GNP, GNT, and GNC increased, rather than decreased, the mixing tolerance and optimum mixing time. These results indicated that the effectiveness of EHG on the mixing properties resulted from enzyme hydrolysis of gluten rather than the acid, base or heating treatments of the gluten.

The effect of EHG on the loaf volume was assessed by substituting 1% of the wheat flour mixture with EHG. As shown in Table 2, the addition of GT significantly increased the loaf volume (SV=4.78) of the non-frozen dough breads, compared to the control (SV=4.56), and the dough containing $KBrO_3$ (SV=4.60). Addition of GPT and GPTC also significantly ($p<0.05$) increased loaf volume of non-frozen dough (SV=4.77 and SV=4.66, respectively). For frozen dough, the addition of the trypsin hydrolyzed glutens, GT, GPT, GTC, and GPTC, significantly ($p<0.05$) improved loaf volume to 3.57-3.50 compared to the control frozen dough bread (SV=3.46). Especially, GT and GTC containing dough were improved to equal loaf volume of the $KBrO_3$ containing dough (SV=3.57). These results indicate the potential of GT and GTC as baking additives and as replacement for $KBrO_3$ to improve frozen dough quality. Although $KBrO_3$ is an essential additive to increase frozen dough bread quality, a replacement has been required due to safety problems (23-26).

Overall, it is concluded that among EHG, the addition of pepsin hydrolyzed glutens produced the most obvious influence on the mixing characteristics of wheat flour, while the addition of trypsin hydrolyzed glutens was the most effective in improving the loaf volume of both non-frozen and frozen dough breads. The addition of gluten to flours would normally be expected to increase, rather than decrease, the mixing requirements. However, the action of proteolytic enzymes on gluten yields a product in which the viscoelastic and rheological properties of gluten have

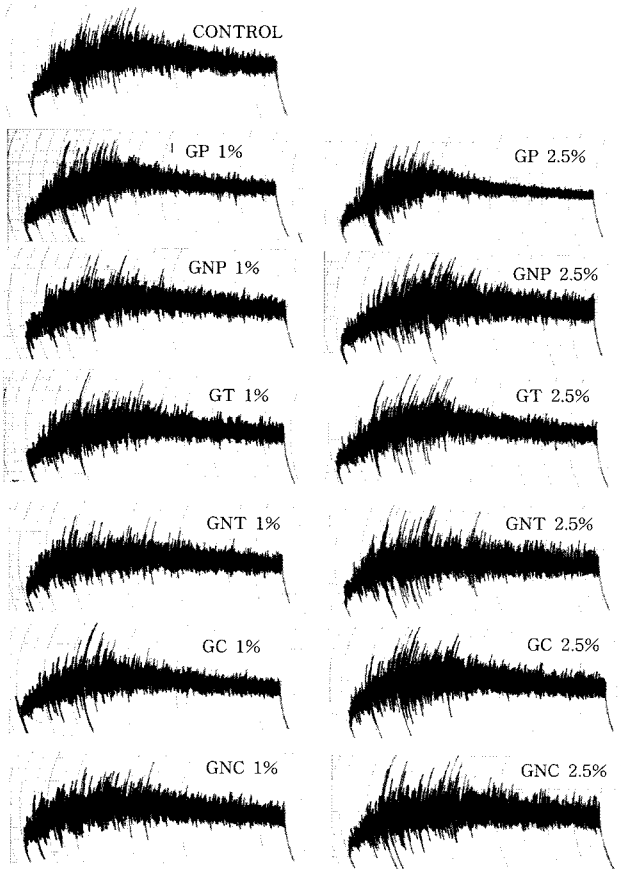

Fig. 3. Mixograms of wheat flour doughs with protease-hydrolyzed gluten. GP: pepsin-hydrolyzed gluten; GT: trypsin-hydrolyzed gluten; GC: chymotrypsin-hydrolyzed gluten; GNP: same as GP without pepsin; GNT: same as GT without trypsin; GNC: same as GC without chymotrypsin.

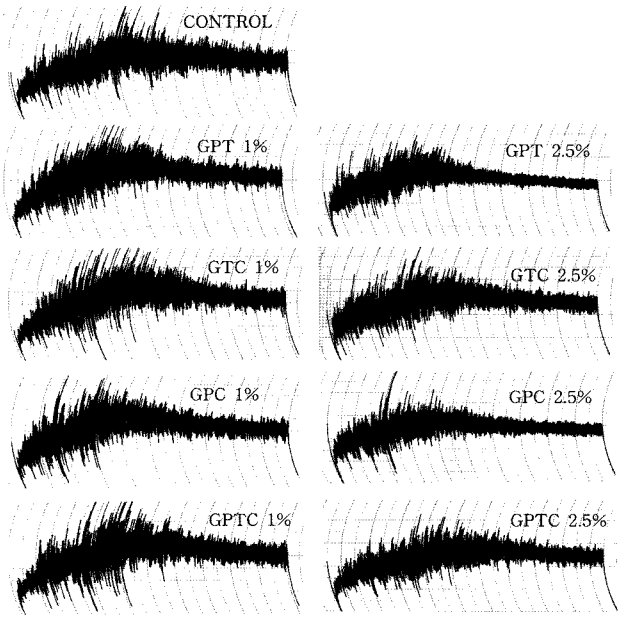

Fig. 4. Mixograms of wheat flour dough with addition of gluten hydrolyzed sequentially with two or three different proteases. GPT: sequential gluten hydrolysate with pepsin and trypsin; GTC: sequential gluten hydrolysate with trypsin and chymotrypsin; GPTC: sequential gluten hydrolysate with pepsin, trypsin and chymotrypsin.

Table 2. Specific loaf volume of breads with added peptides

	Non-frozen dough	Frozen dough
Control	$4.56^{bc2)}(0.45)^{1)}$	$3.46^{b}(0.54)$
$KBrO_3$	$4.60^{abc}(0.38)$	$3.57^{a}(0.64)$
GP	$4.59^{bc}(0.43)$	$3.37^{bc}(0.42)$
GT	$4.78^{a}(0.33)$	$3.57^{a}(0.58)$
GC	$4.63^{ab}(0.36)$	$3.48^{b}(0.42)$
GNP	$4.46^{c}(0.40)$	$3.37^{bc}(0.39)$
GNT	$4.62^{ab}(0.33)$	$3.26^{c}(0.52)$
GNC	$4.54^{bc}(0.33)$	$3.30^{bc}(0.34)$
GPT	$4.77^{a}(0.58)$	$3.51^{ab}(1.16)$
GTC	$4.69^{ab}(0.25)$	$3.57^{a}(1.05)$
GCP	$4.74^{a}(0.64)$	$3.45^{b}(0.61)$
GPTC	$4.66^{ab}(0.10)$	$3.50^{ab}(0.75)$

[1)]The values in the parenthesis are the standard deviation of 5 times of experiments.
[2)]Different letters at the columns indicate the significant difference at $p<0.05$.

been completely changed. Hydrolysis of some of the peptide bonds in gluten produces a mixture of polypeptides of lower, but still significantly large, molecular weight. Lower molecular weight, soluble peptide has greater influence on the mixing properties than the large molecular residue and addition of this hydrolyzed product has a marked effect on the mixing properties. Trypsin possesses very narrow substrate specificity compared to pepsin and chymotrypsin, and hence produces comparatively large molecular hydrolysate. Therefore, trypsin hydrolyzed gluten must have less influence on the mixing properties than the pepsin and chymotrypsin hydrolyzed glutens. However, this effect on dough improvement varied considerably with mixing properties. With its comparatively large molecular hydrolysates, GT was more effective in increasing loaf volume. The effect of the alkalase hydrolyzed gluten (16) was in agreement with that of the pepsin hydrolyzed gluten which reduced mixing time and tolerance, but which did not significantly affect loaf volume.

Acknowledgments

This work was supported by grant number 2000-1-22000-008-2 from the Basic Research Program of the Korea Science and Engineering Foundation.

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