Rheology, Microstructure, and Baking Characteristics of Frozen Dough Containing *Rhizopus chinensis* Lipase and Transglutaminase

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ABSTRACT

Cereal Chem. 88(6):596–601

The beneficial effects of a new recombinant lipase (*Rhizopus chinensis* lipase [RCL]) and transglutaminase (TG) were investigated on frozen dough systems and their breadmaking quality. Rheological properties and microstructure of doughs were measured using a dynamic rheometer, rheofermentometer F3, and scanning electron microscopy (SEM). Measurements of viscoelastic properties showed that both G' and G'' of dough containing RCL and TG were greater than those of the control after 35 days of frozen storage. The SEM micrographs showed that dough containing RCL and TG had the most starch granules embedded in or attached to the gluten network, and the gluten seemed more powerful and resilient than for the control dough after 35 days of frozen storage. Results of the gas production and dough development tests indicated that RCL and TG improved the rheofermentative characteristics of frozen dough. RCL and TG could improve water-holding capacity and significantly increase the glycerol content of the control dough. Image analyses showed that bread crumbs containing RCL and TG had a more open network and uniform crumb structure, which resulted in higher specific volume. This combination also yielded a product with higher sensory scores for test breads.

The frozen dough market has grown in recent years because of consumer demand for convenient, high-quality baked products. Frozen dough is thawed, proofed, and baked to serve to customers as oven-fresh bread and offers a number of advantages compared with the conventional method. Customers enjoy a freshly baked loaf of bread, and the food service industry saves on labor and equipment costs while facilitating transportation (Wang et al 2006; Kim et al 2008). However, a major shortcoming of frozen dough is that its breadmaking quality deteriorates substantially as time in frozen storage increases (Wolt and D'Appolonia 1984a, 1984b; Inoue and Bushuk 1991; Huang et al 2008; Asghar et al 2011). Two factors have been identified as possible reasons for the observed deterioration: 1) decreased gassing power resulting from a decline in yeast activity (Ribotta et al 2003) and 2) gradual loss of dough strength (Inoue and Bushuk 1991).

Additives are used in bakeries to facilitate processing, to compensate for variations in raw materials, to guarantee constant quality, and to preserve freshness and food properties. A variety of additives and ingredients have been used to modify dough behavior during freezing (Ribotta et al 2004; Huang et al 2008; Kim et al 2008; Huang et al 2011). Transglutaminase (TG) (EC 2.3.2.13) catalyzes protein cross-linking through the formation of inter- or intramolecular ε (γ-glutamyl) lysine isopeptidic bonds. These bonds cause the homologous and heterologous polymerization of proteins (Motoki and Seguro 1998). The formation of cross-linked polypeptide chains by the action of TG may lead to the secondary formation of disulphide bonds, thereby decreasing the free thiol group content (Gujral and Rosell 2004). Therefore, TG may improve dough elasticity and crumb strength. TG is often used in weak gluten systems, such as damaged flour, rice flour bread, and gluten-free bread because TG may transform weak gluten into strong gluten through its effects on rheological behavior (Gujral and Rosell 2004; Caballero et al 2005; Moore et al 2006). There is some evidence that TG in frozen dough polymerizes proteins, enhances the properties of dough, and stabilizes the dough structure, all of which may help reduce problems in the production of frozen-dough bread that result, at least in part, from the weakening of dough strength (Hozova et al 2002; Huang et al 2008).

Lipase is a carboxylesterase that catalyzes the hydrolysis of acylglycerol, liberating free glycerol. One of the properties that glycerol could confer is increased freeze tolerance of yeast cells (Myers and Attfield 1999; Huang et al 2011). Some surfactant-like lipids, such as phospholipids and galactolipids, have also been shown to be products of lipase that can form lipid-protein complexes through the interaction of lipophilic portions with hydrophobic regions of proteins (Martín et al 2006) and improve water-holding capacity (Lilbaek et al 2006), which might influence the rheological properties of bread dough (Jascanu and Stefoane 2005). Lipase made via conventional methods has been associated with very high production costs that made it impractical to implement into the mainstream baking industry. A new recombinant lipase (*Rhizopus chinensis* lipase [RCL]) from *R. chinensis* CCTCC M201021 (He et al 2008; Yu et al 2009) could reduce production costs significantly and make the use of lipase a possibility for the baking industry (Zhang et al 2010).

The combined effects of RCL and TG at different concentrations on frozen dough bread were studied in our preliminary experiments through response surface methodology (*data not shown*), and the optimum amount was obtained for each enzyme. The objectives of this study were to investigate the reasons for the abilities of RCL and TG at the optimum amounts to improve frozen dough systems utilizing a dynamic rheometer, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and rheofermentometer F3. Further related properties of thiol groups and the glycerol content were chemically analyzed. The quality of the final product made from frozen dough was evaluated by making bread and by analysis of crumb structure parameters with ImageJ software (http://rsb.info.nih.gov/ij/). Finally, a test panel evaluated the bread samples.

MATERIALS AND METHODS

Materials

Commercial bread flour (brand name Jiafeng) was obtained from East Ocean Oils and Grains Industries (ADM joint venture, Zhangjiagang, China). The bread-flour moisture, ash, and protein content were 12.8, 0.58, and 13.5% (14% mb), respectively, as

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http://dx.doi.org/10.1094/CCHEM-07-11-0082

analyzed by AACC International Approved Methods 44-15.02A, 08-01.01, and 46-12.01, respectively (2010). Shortening (Fortune, Zhangjiagang, China), instant dry yeast (Swallow, Shanghai, China), sugar, and salt were obtained from a local market in Wuxi, China. TG from microbial fermentation was obtained from Yiming Fine Chemicals (Taixing, China), with an activity of 20 U/g. RCL was prepared in the key laboratory of the Industrial Biotechnology department, which is part of the Ministry of Education of Jiangnan University (Wuxi, China) by submerged cultures from genetically modified *R. chinensis* CCTCC M201021 with an activity of 13,800 U/g.

Dough Preparation and Frozen Dough

A "no time" dough procedure was used with flour, water (60%, flour weight basis [fwb]), instant dry yeast (2% fwb), salt (1% fwb), shortening (8% fwb), and sugar (8% fwb) to produce the fresh dough samples. Experimental dough was similarly prepared containing RCL and TG at 700 and 100 U/kg (fwb), respectively.

Dough was mixed in a Bud mixer (Shanghai Bud Food Co., Shanghai, China) until the gluten was optimally developed. For the baking test, the dough was divided into 120 g pieces, rounded, and molded. For the rheofermenter gas production and dough development test, 150 g dough pieces were prepared. The molded dough was placed in an aluminum pan and wrapped in a polyethylene sheet. After mixing, the dough was frozen within 10 min to limit the activity of the yeast, and the room temperature was maintained at 16°C. Dough was frozen at –38°C for 3 hr and then stored at –18°C in a blast freezer for 7, 21, and 35 days (BD-100LT, Qingdao Haier, Qingdao, China).

After frozen storage, the dough was thawed at 25°C and 55% rh for 1 hr and fermented at 38°C and 80% rh for 1.5 hr in a proof cabinet and then baked for 25 min in a deck oven (SM-603T, Sinmag Machinery, Wuxi, China) set at 170°C top and 210°C bottom.

Dynamic Rheology Measurements

An AR 1000 controlled-stress rheometer (TA Instrument, New Castle, DE) with parallel-plate geometry (40 mm diameter) as described by Caballero et al (2005) was used to study the dynamic oscillatory rheological behavior of frozen dough. The dough was prepared as described previously but without instant dry yeast. Frozen dough was thawed for 1 hr at 25°C and placed between parallel plates, the gap was adjusted to 1 mm, and the excess dough was removed. To prevent drying at the edges, mineral oil was used during testing. Before measurements were taken, the dough was allowed to rest for 5 min. Tests were performed at 30°C. Measurements were made in the linear viscoelastic region at a target strain of 1%. Oscillatory tests with a frequency sweep of 0.1–10 Hz were conducted, and the dynamic rheological properties of samples were assessed by the storage modulus, G' (elastic modulus), and the loss modulus, G'' (viscous modulus). Measurements were performed in triplicate.

Quantification of Free Thiol Groups

Changes in free thiol groups were determined with Ellman's reagent according to the method of Gujral and Rosell (2004). Tris-glycine (Tris-Gly) buffer consisted of 8.5m*M* Tris, 9.2m*M* glycine, and 0.3m*M* ethylenediaminetetraacetic acid, pH 8.0. The GuHCl/Tris-Gly solution contained 5*M* guanidine hydrochloride. Ellman's reagent contained 4 mg of 5,5' dithiobis-2-nitrobenzoic acid in 1 mL of Tris-Gly buffer (pH 8.0) and was prepared daily.

Each sample (200 mg) was taken from a frozen dough piece that had been thawed. The sample was suspended in 1 mL of GuHCl/Tris-Gly solution, vortexed for 10 min, and centrifuged at 16,000 × *g* for 5 min. GuHCl/Tris-Gly solution (1.5 mL) and Ellman's reagent (0.5 mL) were added to 300 µL of the clear supernatant, and the absorbance was read at 412 nm. The results were calculated against a cysteine standard curve. The values recorded were the mean of three replicates.

SEM

The microstructures of fresh dough, dough after 35 days of frozen storage without additives (control), and dough with RCL and TG in combination were observed with a QUANTA-200 SEM (FEI, Eindhoven, The Netherlands). Dry yeast was not included in the formula to prevent structural changes caused by yeast activity. Frozen dough samples stored for 35 days were thawed for 1 hr at 25°C. Samples were fixed with 2.5% (v/v) glutaraldehyde, rinsed with 100m*M* phosphate buffer, fixed with 1% (v/v) osmium tetroxide, and rinsed with 100m*M* phosphate buffer. Samples were then dehydrated sequentially with 30, 50, 70, 90, and 100% (v/v) ethanol and ethanol displaced with isoamyl acetate. Samples were dried at the critical point (CPD-005, Bal-Tec, Macclesfield, UK), followed by sputter-coating with gold in a metalizer (SCD-005, Bal-Tec). Micrographs were taken at two magnifications (600× and 1,200×) at 10 kV.

Determination of Freezable Water Content

Each sample (about 10–11 mg) was taken from frozen dough pieces that had been stored at –18°C for 7, 21, or 35 days and thawed for 1 hr at 25°C. Samples were placed in hermetically sealed aluminum pans and analyzed with a Pyris 1 DSC (Perkin-Elmer, Waltham, MA). An empty pan was used for reference. Samples were heated at 5°C/min from –40 to 20°C. Analyses were performed at least in duplicate. The enthalpy changes associated with thermal transition were obtained by integrating the area of each pertinent endothermic melting peak around 0°C corresponding to ice melting. The enthalpy of this transition (ΔH) was used to evaluate the freezable water content.

Determination of Glycerol Content

Samples (10 g) were taken from frozen dough piece that had been thawed and fermented. The sample was thoroughly mixed with 90 mL of distilled water in a test tube, and 10 mL of the solution was removed for quantification of glycerol. Glycerol was assayed by a modified titration method (Mostafa 2001), in which glycerol was oxidized by sodium periodate to form formic acid, and then sodium hydroxide was used to titrate the formic acid that had been produced. The recorded values were the mean of three replicates and are expressed in gram of glycerol content per gram of dough (g/g of dough).

Gas Production and Dough Development Tests

A rheofermentometer F3 (Chopin, Villeneuve-La-Garenne, France) was used to measure the volume (mL) of CO_2 production (V_{CO_2}), the gas retention rate (R, %), and two parameters of dough development—the maximum height of dough (H_m [mm]), and maximum height of gaseous release (H'_m [mm])—following the method described by Czuchajowska and Pomeranz (1993) with minor modifications. The amount of dough was adjusted to 150 g, and before testing, frozen dough was thawed for 1 hr at 25°C. The test was conducted at 30°C for 3 hr with a 2,000 g cylindrical weight and performed in duplicate.

Crumb Structure Analysis

Crumb structure analysis was determined by the method of Ozkoc et al (2009) with some modifications. Loaves were mechanically sliced into 10 mm thick slices. Three or four central loaf slices were positioned at the center of a flatbed scanner (Panasonic KX-MB228CN, Fukuoka, Japan) scanned individually at a resolution of 300 dpi, and analyzed with ImageJ software (Datta et al 2007; Ozkoc et al 2009). This software compares the contrast between the two phases (pores and solids) in the image. The scanned color image was first cropped to a field of view of 3 × 3 cm and converted to grayscale. Given bars of known lengths, pixel values were converted into distance units. The grayscale images were thresholded with the Otsu algorithm through the ImageJ software. Pore area as a fraction of total area

(AF), the number of pores per square centimeter (cell density [CD]), and the uniformity of pores were analyzed.

Measurements of Bread Specific Volume and Sensory Evaluation

Bread loaf volume was measured 1 hr after baking following a seed-displacement method, and specific volume (mL/g) was calculated from the loaf weight (g) and volume (mL). The results were the mean of three replicates.

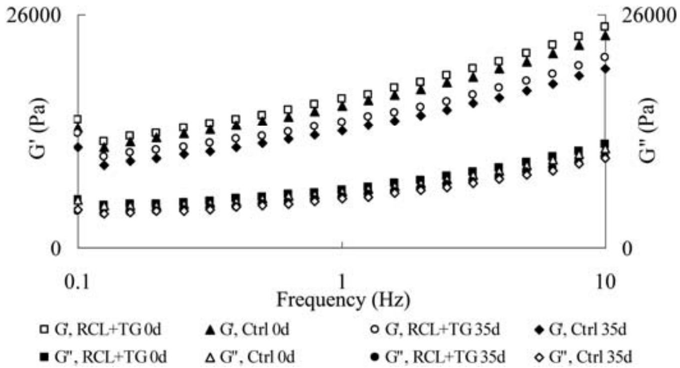

Fig. 1. Effects of *Rhizopus chinensis* lipase (RCL) and transglutaminase (TG) on storage modulus (G') and loss modulus (G'') of fresh dough and dough after 35 days of frozen storage.

TABLE I
Effects of *Rhizopus chinensis* Lipase (RCL) and Transglutaminase (TG) on Free Thiol Content and Specific Volume of Dough Samples That Were Frozen for Different Lengths of Time[a]

Frozen Storage Time (Day)	Sample	Free Thiol Content (mmol/g of dough)	Specific Volume (cm^3/g)
0	Control	0.63 ± 0.006a	7.74 ± 0.04ef
	RCL + TG	0.60 ± 0.005a	7.83 ± 0.05f
7	Control	0.98 ± 0.004d	7.28 ± 0.06d
	RCL + TG	0.71 ± 0.004b	7.64 ± 0.03e
21	Control	1.13 ± 0.004e	6.98 ± 0.05b
	RCL + TG	0.81 ± 0.004c	7.63 ± 0.03e
35	Control	1.21 ± 0.005f	6.71 ± 0.04a
	RCL + TG	0.85 ± 0.002c	7.04 ± 0.03c

[a] Mean (n = 3) ± standard deviation. Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

Sensory evaluation was carried out on the bread samples within 3–5 hr of baking. The samples served were sliced (10 mm thick) and evaluated by 31 trained panelists of master's degree and Ph.D. candidates from the College of Food Science and Technology, Jiangnan University. The panel was composed of 12 females and 19 males ranging from 18 to 43 years of age and hailing from multiple countries. Sensory attributes of bread were measured blindly with a nine-point hedonic scale indicating the degree of liking, with 9 being like extremely, 5 being neither like nor dislike, and 1 being dislike extremely. The attributes tested were appearance, color, flavor, mouth feel, and overall acceptability.

Statistical Analysis

A general linear model (analysis of variance) and pairwise comparison with Duncan's method were performed with SPSS analytical software.

RESULTS AND DISCUSSION

Dynamic Rheology

The viscoelastic properties of control dough without enzymes and with RCL and TG were studied by dynamic oscillatory measurements. Figure 1 shows frequency sweep results of fresh and frozen doughs with G' and G'' as a function of frequency. A decrease in G' and G'' after 35 days of frozen storage was observed; G' was higher than G'' at any given frequency, indicating that the dough was more elastic than viscous and that frozen storage had a negative effect on the viscoelastic properties of the doughs.

After 35 days of frozen storage, both G' and G'' of dough containing RCL and TG were greater than those of the control, and the free thiol group content was found to be decreased 29.75% compared with the control dough (Table I). This result indicates that the interactive effects of RCL and TG could modify the viscoelastic behavior of the frozen dough. Similar improvements were also observed by Gujral and Rosell (2004) and Huang et al (2008), who reported that TG increased the viscoelasticity of dough and, thus, the dough structure was strengthened because of the cross-linking action. Furthermore, it has been postulated that lipase modifies the interaction between the flour lipids and gluten (Martín et al 2006) and promotes the oxygenation of free thiol groups, thereby facilitating formation of disulfide bonds (Jascanu and Stefoane 2005).

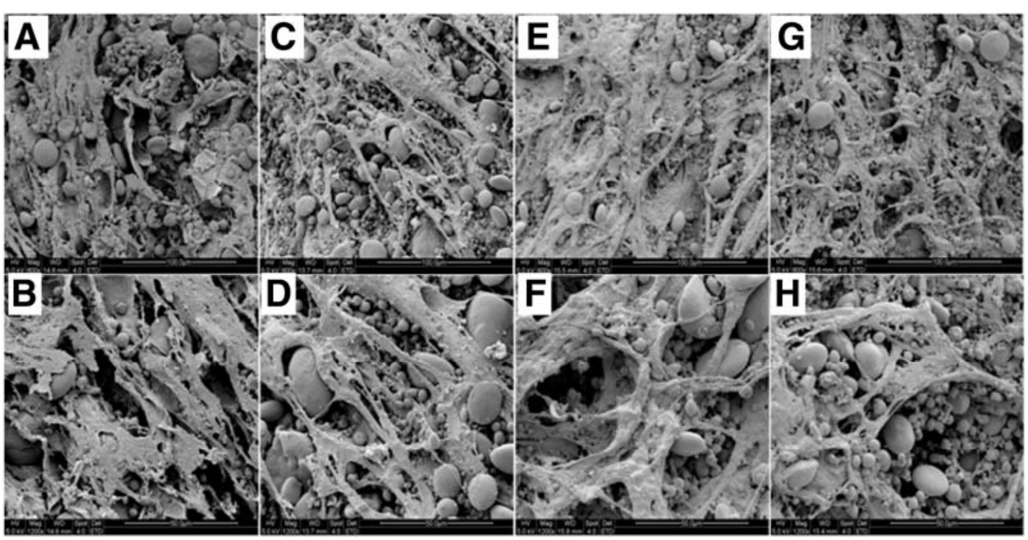

Fig. 2. Scanning electron micrographs. **A** and **B,** fresh dough samples without enzymes; **C** and **D,** fresh dough samples with *Rhizopus chinensis* lipase (RCL) and transglutaminase (TG); **E** and **F,** dough samples after 35 days of frozen storage without enzymes; and **G** and **H,** dough samples after 35 days of frozen storage with RCL and TG. Micrographs A, C, E, and G were magnified 600× and B, D, F, and H were magnified 1200×.

Frozen Dough Microstructure

In this research, SEM was used to visualize the ultra structure of doughs prepared without yeast. During and after 35 days of frozen storage, not many well-developed gluten structures were observed in doughs without RCL and TG addition; instead, more starch granules were evident, especially at 1,200× magnification (Figure 2). Typical micrographs showed that most starch granules were embedded in a well-developed gluten matrix (i.e., one that formed a continuous film), but the control dough showed a few loose starch granules, and the gluten strands appeared less continuous, more disrupted, and thinner than those of dough containing RCL and TG (Figure 2). Dough containing RCL and TG had the most starch granules embedded in or attached to the gluten network, and the gluten seemed stronger and more resilient than that of the control dough after 35 days of frozen storage. Ribotta et al (2004) also found a damaged gluten network with porous and ruptured structures in dough stored for 60 days at –18°C. Huang et al (2008) found that treatment of frozen dough with TG could repair the weakened gluten structure caused by frozen storage.

Freezable Water Content

The effects of RCL and TG on the amount of freezable water in dough samples that had been frozen for different lengths of time are presented as DSC thermograms (–30 to 10°C temperature range) in Figure 3. An increase of ΔH with increasing storage time at –18°C was observed, and ΔH of dough containing RCL and TG, fresh and frozen for 35 days, was 25.31 and 30.73 J/g, respectively, which represented an increase of 21.42% over a period of 35 days of frozen storage. By contrast, the ΔH of dough without additives, fresh and frozen for 35 days, was 28.25 and 42.32 J/g, respectively, which represented an increase of 49.81% after 35 days of frozen storage. These numbers indicate that RCL and TG improved the water-holding capacity of dough during frozen storage and decreased the freezable water content. According to Lilbaek et al (2006), lipase produced some surfactant-like lipids (e.g., phospholipids and galactolipids), which improved water-holding capacity of dough; moreover, as reported by Gerrard et al (1998), TG also improved the water absorption of dough.

Glycerol Content

The effects of RCL and TG on glycerol content of doughs before and after proofing following 7, 21, and 35 days of frozen storage are shown in Figure 4. The glycerol content decreased with increasing storage time and changed dramatically during the first seven days of frozen storage. After 35 days of frozen storage, the glycerol content of dough containing RCL and TG was 0.0391 and 0.0428 g/g of dough before and after proofing, representing decreases of 5.3 and 3.8% when compared with the corresponding fresh dough. Glycerol contents of dough samples without additives were 0.0365 and 0.0389 g/g of dough before and after proofing, respectively; the glycerol content of these samples decreased by 7.1 and 4.4% compared with the fresh dough control. These results indicate that RCL and TG significantly increased glycerol content of control dough and that there was more available glycerol existing in the frozen dough containing RCL and TG compared with the control dough. Myers and Attfield (1999) studied the effects of glycerol on the leavening capacity of frozen dough and indicated that at least 2% (fwb) glycerol content could result in substantial effects on frozen dough leavening. Exogenous lipase could catalyze polar and nonpolar lipids to produce glycerol, but endogenous wheat lipase was inactive on the nonpolar lipids (Castello et al 1998). This finding suggests that dough containing RCL could have a higher glycerol content compared with that of the control. According to Myers and Attfield (1999), glycerol helps yeast quickly adapt to the osmotically stressed dough, which reduces the lag of yeast and thereby increases yeast viability. It follows that dough containing RCL and TG has better yeast fermentation capacity during frozen storage.

Rheological and Fermentation Properties

The effects of RCL and TG on $V\text{co}_2$ generated during 3 hr of fermentation, R, and two parameters of dough development, H_m and H'_m, as determined with the rheofermentometer, are shown in

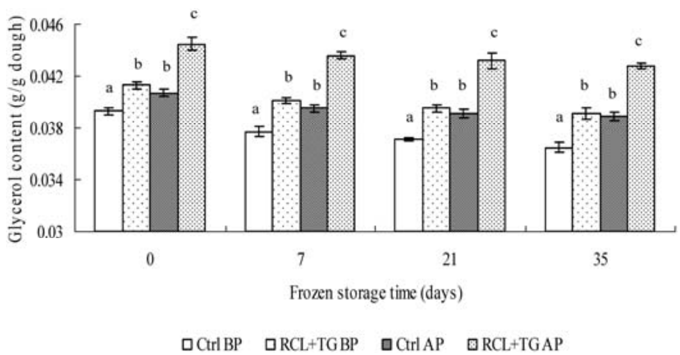

Fig. 4. Effects of *Rhizopus chinensis* lipase (RCL) and transglutaminase (TG) on glycerol content in dough samples that were frozen for different lengths of time. BP and AP refer to dough before and after proofing. Different letters above the columns indicate statistically significant values at the same storage time ($P < 0.05$).

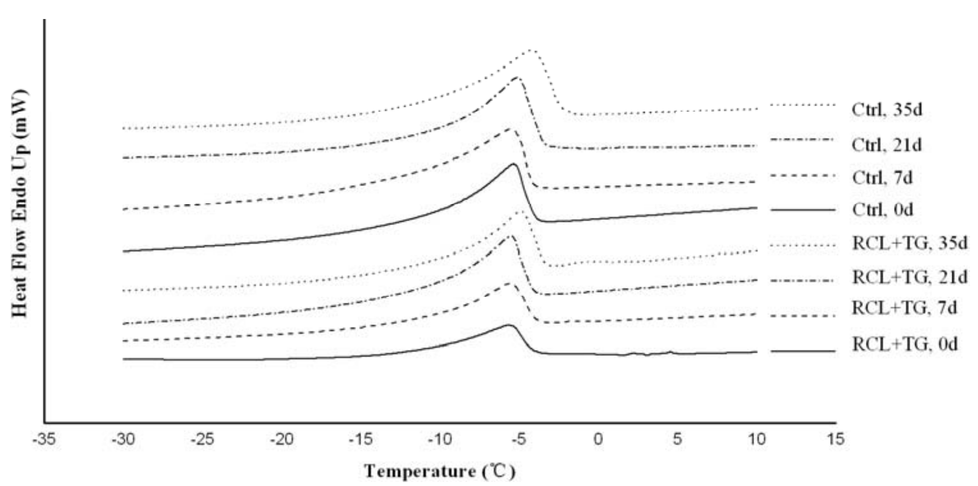

Fig. 3. Effects of *Rhizopus chinensis* lipase (RCL) and transglutaminase (TG) on the amount of freezable water in dough samples that were frozen for different lengths of time.

Table II. Generally speaking, H_m, H'_m, and Vco_2 decreased dramatically during the first seven days of frozen storage and changed more gradually with prolonged storage. When RCL and TG were added, H_m and H'_m increased significantly compared with the control at each time point following frozen storage, and the Vco_2 of dough containing RCL and TG increased by 18.13, 20.56, 20.82, and 19.14% after 0, 7, 21, and 35 days, respectively, compared with the control dough, which had been stored at –18°C. The combination of RCL and TG also increased the *R*, which, in turn, increased the dough stability during fermentation. These results indicated that RCL and TG improved the fermentative characteristics of frozen dough and significantly increased the yeast gas-production rate.

Baking Characteristics of Bread

CD, AF, and the uniformity of cells were analyzed as the main factors of crumb cell structure (Figure 5). A better crumb cell structure was described as a more uniform crumb with a higher AF (Ozkoc et al 2009). As shown in Figure 5, CD and AF of crumbs without enzymes decreased with increasing storage time at –18°C; by contrast, CD and AF of crumbs with RCL and TG increased, and the AF of crumbs containing RCL and TG was larger than that of the control. These results might be attributable to alterations in dough rheological property changes (Martín et al 2006). As a result of the duration of frozen storage and other changes (e.g., temperature fluctuations), gas cells ruptured and stability decreased, resulting in decreased CD and AF of control dough as observed in Figure 5. TG increased the viscoelasticity of the dough and enhanced the gluten structure that had been weakened or ruptured during frozen storage (Gujral and Rosell 2004; Huang et al 2008), and lipase modified the interaction between the flour lipids and the gluten (Martín et al 2006). These changes led to the formation of an interfacial membrane between air and bulk that was more difficult to disrupt and, therefore, provided better protection against bubble collapse (Bos and van Vliet 2001). Stojceska and Ainsworth (2008) reported similar findings: lipase significantly improved crumb porosity and made the air cells more evenly distributed by liberating monoglycerides and surfactants from lipids (Azizi et al 2003).

This study provides additional information about a more open network and uniform crumb structure of dough containing RCL and TG compared with the control dough after 35 days of frozen storage. Additionally, the final product had a 6% higher specific volume for the loaf than did the control dough following 35 days of frozen storage (Table I).

Sensory Evaluation

Thirty-one trained panelists performed sensory analysis with a nine-point hedonic scale of control samples and ones containing RCL and TG following 35 days of frozen storage. The scores for the bread containing RCL and TG ranged from 7.24 to 7.67, whereas those of the control bread ranged from 5.67 to 6.71. Statistical analysis showed that all of the factors except for the flavor were significantly different ($P < 0.05$) between the control and bread containing RCL and TG. As noted by some of the panelists, adding enzymes extensively improved the bread's mouth feel.

CONCLUSIONS

Overall, RCL and TG could improve the characteristics of frozen dough. Results from dynamic rheology measurements showed that both G' and G'' of dough containing RCL and TG were greater than those of the control after 35 days of frozen storage. The SEM micrographs showed that dough containing RCL and TG had the most starch granules embedded in or attached to the gluten network, and the gluten seemed stronger and more resilient than for the control dough after 35 days of frozen storage. RCL and TG improved the water-holding capacity of dough during frozen storage, thereby decreasing the amount of freezable water. RCL and TG could significantly increase available glycerol content of dough

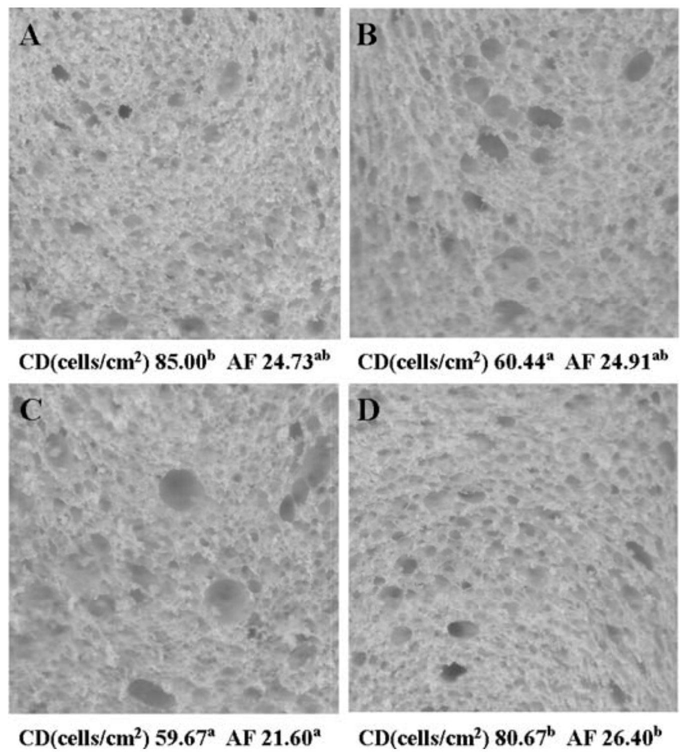

Fig. 5. Image analysis of crumb structure. **A,** fresh dough without enzymes; **B,** fresh dough with *Rhizopus chinensis* lipase (RCL) and transglutaminase (TG); **C,** after 35 days of frozen storage without enzymes; and **D,** after 35 days of frozen storage with RCL and TG. CD = cell density; AF = area fraction. The results are the average of three repetitions. Values followed by different superscripted letters indicate statistically significant values at the same storage time ($P < 0.05$).

TABLE II
Effects of *Rhizopus chinensis* Lipase (RCL) and Transglutaminase (TG) on Rheofermentometer Parameters of Dough Samples That Were Frozen for Different Lengths of Time[a]

Frozen Storage Time (Day)	Sample	H_m (mm)	H'_m (mm)	Vco_2 (mL)	R (%)
0	Control	68.20 ± 1.39e	46.00 ± 0.92b	993.00 ± 5.20c	98.00 ± 0.36a
	RCL + TG	68.30 ± 0.78e	51.70 ± 1.35d	1,173.00 ± 2.65f	99.40 ± 0.26bc
7	Control	51.50 ± 0.82c	43.70 ± 1.13a	895.00 ± 4.59b	99.80 ± 0.06e
	RCL + TG	65.40 ± 2.04e	49.90 ± 1.85cd	1,079.00 ± 4.00e	99.80 ± 0.10e
21	Control	44.00 ± 3.47b	43.60 ± 0.82a	831.00 ± 5.57a	99.60 ± 0.06e
	RCL + TG	55.90 ± 1.21d	48.90 ± 0.79c	1,004.00 ± 4.58d	99.90 ± 0.06e
35	Control	32.00 ± 2.95a	43.50 ± 1.06a	836.00 ± 1.00a	99.30 ± 0.20b
	RCL + TG	55.90 ± 0.89d	49.20 ± 0.26c	996.00 ± 2.65c	99.80 ± 0.06e

[a] Mean ($n = 2$) ± standard deviation. Means in the same column followed by the same letter are not significantly different ($P > 0.05$). H_m = maximum height of dough; H'_m = maximum height of gaseous release; Vco_2 = volume of CO_2 production; and R = gas retention rate.

and sustain a high glycerol content after 35 days of freezing when followed by proofing. The results of the gas production and dough development tests indicated that RCL and TG could improve the rheofermentative characteristics of frozen dough. Bread crumbs containing RCL and TG had a more open network and a more uniform crumb structure, which resulted in higher specific volume. Sensory evaluation found that this combination also yielded a product with higher sensory scores for test breads.

ACKNOWLEDGMENTS

We are grateful for financial support from the National Natural Science Foundation of China (31071595 and 20576046), the National Agricultural Science and Technology Achievement Transfer Fund Project of China (2009GB23600520), and earmarked funding for the Modern Agro-industry Technology Research System (No. nycytx-14) from the Ministry of Agriculture of China. We also thank the Key Laboratory of Industrial Biotechnology of the Ministry of Education, Jiangnan University, Wuxi, China, for providing the RCL.

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[Received July 4, 2011. Accepted October 9, 2011.]