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Effects of Milk Proteins and Gums on Quality of Bread Made from Frozen Dough following Freeze-Thaw Cycles

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Abstract The quality of frozen bread dough made with the milk proteins casein (C), whey (W), and the gums sodium alginate (A) and κ-carrageenan (K), was investigated to develop methods to suppress the deterioration of the frozen dough quality. The control had a lower dough volume than dough with additives during freeze-thaw cycles. In bread stored at 5°C, the moisture content of bread prepared with whey plus sodium alginate (WA) decreased less than that of the control. The control also had a lower specific loaf volume than breads made with added milk proteins and gums. The hardness of the control bread and bread made with casein plus sodium alginate (CA) and whey plus κ-carrageenan (WK) increased during freeze-thaw cycles, although that of the control increased more than the others. There was no significant difference in sensory preference among breads with and without milk proteins and gums. Addition of CA and WA improved the baking quality by reducing the deterioration of frozen dough and retarding the staling of bread.

Keywords: milk proteins, gums, frozen dough, freeze-thaw cycles

Introduction

Frozen dough is widely used in the bread-making industry to reduce labor costs, while still supplying fresh bread to consumers. It is generally believed that the use of frozen dough will increase in the future (1), however the quality of bread baked from frozen dough deteriorates as a result of the dough's storage at sub-zero temperatures (2). The loss of quality in frozen dough may result from two primary factors: deterioration of dough strength and yeast viability (3).

Another major problem is the loss of quality that results from mishandling of the frozen dough during transportation and storage (4). Temperature changes, from the initial freezing temperature to the storage temperature, as well as the lowest temperature reached, all affect yeast viability (5) and dough quality. For example, ice crystal formation in the frozen dough is attributed to the physical disruption of the gluten network and affects dough strength as well as gas retention properties of the dough, consequently reducing the final bread quality (3). Ice crystallization and recrystallization are closely related to water movement in the dough during freezing, frozen storage, and transportation of the frozen dough to bakery outlets.

Additives have been used in bread formulations to overcome the aforementioned problems. For example, vital gluten improved both loaf volume and breadcrumb structure, while decreasing the fermentation time of the dough (2). In another study, Aibara *et al.* (1) reported that the specific loaf volume was improved by adding shortening to frozen dough. Inoue *et al.* (6) reported that three components of shortening systems (type of oil, type of surface-active agent added to the oil, and type and composition of the emulsion system) affected the maintenance of baking and the rheological properties of dough subjected to extended frozen storage. In milled rice bread, adding gums, fats, and glutens also improved baking quality (7, 30). Oxidizing agents, such as vitamin C and emulsifiers, such as diacetyltartaric acid (DATEM), have also been used to overcome the disadvantages of frozen dough. In addition, sugar esters have been used to protect the gluten network of frozen dough during frozen storage (8).

Hydrocolloids have been used in food products to improve moisture retention, control water mobility, and maintain overall product quality during storage (2). Ribotta *et al.* (2) and Sahlstrom *et al.* (9) suggested that the addition of mono- and diacetyltartaric acid and guar gum to dough yielded the best bread loaf volume after freezing the dough. Furthermore, Kyung and Lee (8) reported that hydro-colloids such as xanthan gum, guar gum, and carrageenan extend the shelf-life of frozen dough. Carrageenan is often used as a stabilizer in ice cream and other frozen products (2).

Studies involving milk proteins such as casein and whey have also been reported. With regard to fermentation and bread baking, the functionalities of whey protein and casein were reported by Erdogdu-Arnoczky *et al.* (10), while the rheological properties, including viscosity, elasticity, and texture, of dairy products were reported by Velez-Ruiz and Babosa Canovas (11). Zhu and Damoaran (12) reported the relationship between conformational changes in denatured whey protein isolates and their foaming properties. Furthermore, sodium caseinate and whey protein improved the baking performance of frozen dough, decreased proof time, increased specific volume, and improved texture (13).

While many reports indicate that the inclusion of some gums and milk proteins will retard the deterioration of frozen dough (8, 13-15), there have been few studies on the complete effects of adding mixtures of milk proteins

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and gums to frozen dough.

Milk proteins, such as casein and whey protein, aid gluten activity, and reduce the size of ice crystals formed in the dough, while gums such as carrageenan and sodium alginate prevent the growth of ice crystals (14). Therefore, the addition of milk proteins and gums was expected to control the deterioration of frozen dough quality. Also, when gums are added to proteins the emulsion capacity and stability both increase. These properties were expected to improve the stability of the dough and retard the staling of the bread.

The purpose of this study was to investigate the effects of mixtures of milk proteins and gums on the quality of frozen dough and bread made from frozen dough following storage and freeze-thaw cycles. The effects of various mixtures of milk proteins and gums on the gluten network in frozen dough were evaluated through micro-structural observation and measurement of frozen dough volume. Specific loaf volume, color, and sensory characteristics of baked bread were also examined. In addition, the moisture content and hardness of bread stored at 5°C for 4 days were measured to investigate the effects of these additives on the staling of bread.

Materials and Methods

Materials Strong flour (Korea-Dongah Flour Mills, Seoul, Korea) with a moisture content of 14% was used to prepare the dough, along with instant dry yeast (Marcq, en-Barœul, France), yeast food (Samlip Co., Siheung, Korea), shortening (LotteSamkang Co., Seoul, Korea), sugar (Samyang Co., Ulsan, Korea), salt (Hanju, Muan, Korea), skim milk powder (Seoul Milk Co., Seoul, Korea), and ascorbic acid (Hebei Welcome Pharmaceutical Co., Shjiazhuang, Hebei, China). Casein (Matsutani Inc., Itami, Japan), whey protein (Davidco Foods International, Inc., USA), κ-carrageenan (Korea carrageen. Co. Ltd., Sunchon, Korea), and sodium alginate (MSC Co., Yangsan, Korea) were used as additives, using the following mixtures of proteins and gums: casein-κ-carrageenan (0.6% each), casein-sodium alginate (0.6% each), whey-κ-carrageenan (0.6% each), and whey-sodium alginate (0.6% each). The appropriate amounts of milk proteins and gums added were determined by preparative experiments.

Methods ***dough preparation***: The various bread dough formulations are given in Table 1. Dough mixtures were prepared using a no-time straight-dough procedure (AACC Approved Method 10-10A). The dough was then mixed in a spiral mixer (PM-250 S.S; Shihung, Gyeonggi, Korea) with stepwise mixing: 2 min low-speed mixing at 100 rpm, 2 min high-speed mixing at 190 rpm, 2 min low-speed mixing at 100 rpm after the addition of margarine and 5 min high-speed mixing at 190 rpm. Dough was divided into 430 g pieces and molded into loaves. The molded dough was wrapped in polyethylene bags before freezing at -40°C.

Storage conditions ***frozen storage***: The molded dough was frozen at -40°C for 3 hr and then moved to a freezer at -20°C for 8 weeks of storage. Frozen dough samples were stored at -20°C for 1 day or 2, 4, 6, or 8 weeks.

Table 1. Formulation for bread with added milk proteins and gums (% flour basis)

Ingredients	Control	CK	CA	WK	WA
Flour	100	100	100	100	100
Water	63	63	63	63	63
Instant dry yeast	2.5	2.5	2.5	2.5	2.5
Yeast food	0.5	0.5	0.5	0.5	0.5
Sugar	6	6	6	6	6
Salt	2	2	2	2	2
Skim milk powder	3	3	3	3	3
Shortening	4	4	4	4	4
Ascorbic acid	40 ppm	40 ppm	40 ppm	40 ppm	40 ppm
Casein	-	0.6	0.6	-	-
Whey protein	-	-	-	0.6	0.6
Carrageenan	-	0.6	-	0.6	-
Sodium alginate	-	-	0.6	-	0.6

Freeze-thaw cycles: The dough samples were frozen at -40°C and then stored at -20°C for 1 day. They were subsequently subjected to 1, 2, or 3 freeze-thaw cycles as follows: Frozen dough was held at -2°C for 15 hr and then re-frozen at -20°C for 3 days. This thawing and refreezing treatment was repeated two more times. The procedure for the freeze-thaw cycles for frozen dough is shown in Fig. 1.

Baking procedure: Frozen dough stored at -20°C was thawed at 5°C for 15 hr, re-rounded, shaped, and proofed for 50 min (35°C, 85% RH). The proofed dough mixtures were baked in the oven at 160 and 180°C for 40 min. The overall baking procedure for the frozen dough is shown in Fig. 2.

Dough volume: Ten g of dough was placed in a graduated cylinder (100 mL) and changes in the height of dough before and after proofing at 35°C, 85% RH for 50 min were measured. The resulting change in height was expressed as dough volume.

Microscopic observation: The cryofracturing technique was used to prepare the frozen dough for scanning electron microscopy. First, the dough was frozen with liquid

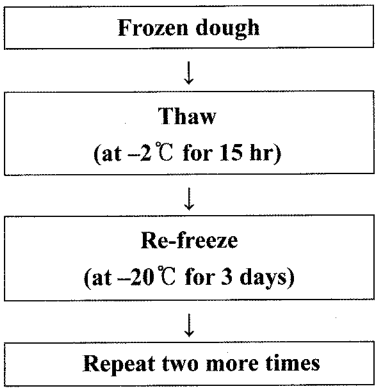

Fig. 1. Freeze-thaw cycles for frozen dough.

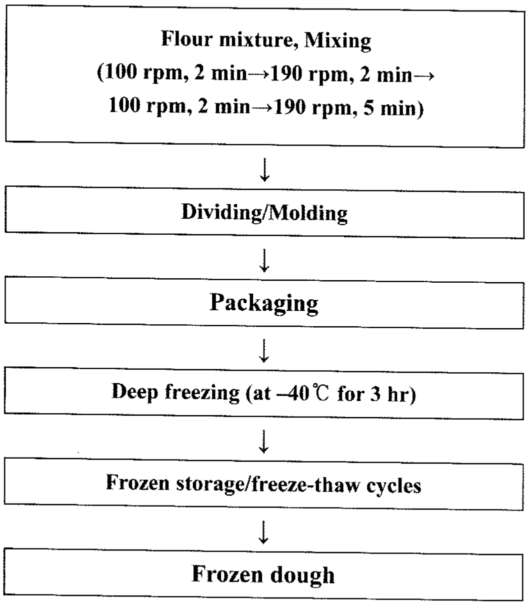

Fig. 2. Dough preparation procedure by the straight-dough method.

nitrogen. The frozen dough was then fractured to expose the interior surfaces and freeze-dried in a freeze dryer. The dough samples were mounted on specimen stubs and sputter coated with gold (15). Samples were observed with a scanning electron microscope (Hitachi S-2400, Tokyo, Japan) at an accelerating voltage of 15 kV.

***Specific loaf volume of bread*:** After baking and cooling (60 min), the bread loaf volume was measured by the rapeseed displacement method, and the ratio of volume to mass (specific volume) was then calculated (16).

***Bread crumb color*:** The color in the interior of the bread was measured against standards using a chromameter (CM-3500d; Minolta Co., Tokyo, Japan). The results were expressed as L (lightness), a (redness), and b (yellowness) values. The color measurements were done in duplicate.

***Textural analysis*:** Textural changes in the breads were analyzed instrumentally using a TA-XT2 texturometer (Model TA-XT2; Stable Micro Systems, Godalming, England) equipped with 2.0-cm diameter plunger. 1.3 cm crumb slices were 50% compressed. The hardness of just-baked bread and bread aged at 5°C for 4 days was measured and compared.

***Moisture content of bread*:** The moisture content of bread was determined according to the AACC (44-15A) method. Decrease in moisture content was measured by comparing the moisture content of just-baked breads and those aged at 5°C for 4 days.

***Sensory evaluation*:** Sensory evaluation was conducted using a 5-point hedonic scale with a score of 1 representing ‘dislike very much’ and a score of 5 representing ‘like very much’. The sliced breads were served in random order to 10 trained panelists. The evaluated parameters were crust color, break-shred, crumb color, grain, aroma, texture, taste, and overall acceptability.

Statistical analysis All experiments and measurements were performed in duplicate, and the results were expressed as mean±standard deviation. The data obtained were statistically analyzed using the SAS program (v 8.0). Means were compared using Duncan’s multiple range test, and results were considered significant at a level of $p<0.05$.

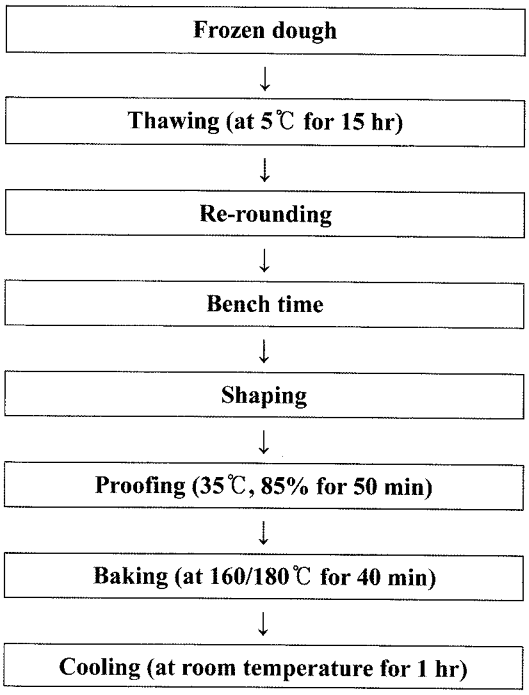

Fig. 3. Procedure for making bread from frozen dough.

Results and Discussion

Characteristics of frozen dough ***Dough volume*:** Dough volume was measured to investigate the effect of milk proteins and gums on the quality of dough subjected to one to three freeze-thaw cycles. The results are shown in Table 2. A gradual decrease in dough volume occurred in frozen dough with each successive freeze-thaw cycle, up to three cycles. The observed decrease in dough volume might be due to ice crystals that contribute to the weaken-

Table 2. Dough volume of frozen dough with milk proteins and gums subjected to one to three freeze-thaw cycles (Unit:cc)

Samples[1]	Freeze-thaw cycles[2]		
	1	2	3
Control	^{x}22.83±1.44^{a}	^{y}21.20±0.62^{a}	^{z}18.57±0.29^{b}
CK	^{x}22.33±0.63^{a}	^{y}21.17±1.77^{ab}	^{yz}19.43±0.50^{b}
CA	^{x}22.42±1.90^{a}	^{y}21.11±0.38^{ab}	^{yz}19.73±0.95^{b}
WK	^{xy}22.00±1.00^{a}	^{yz}21.07±0.38^{ab}	^{y}20.26±0.67^{b}
WA	^{x}23.44±0.49^{a}	^{x}22.77±0.50^{ab}	^{x}21.63±1.07^{b}

[1] CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100 %.

[2] Values are mean±standard deviation. ^{x,y,z}: values with different superscripts within columns are significantly different by Duncan’s multiple range test at $p<0.05$. ^{a,b,c}: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

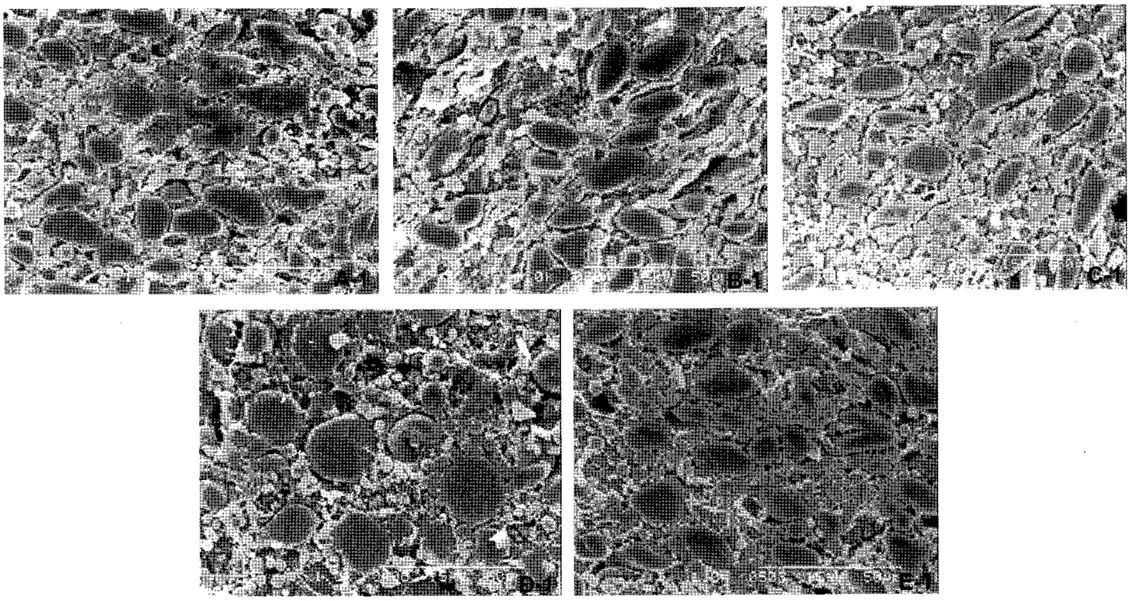

Fig. 4. Scanning electron micrographs of frozen dough subjected to 1 freeze-thaw cycle. A-1, Control; B-1, Casein-Carrageenan; C-1, Casein-Sodium alginate; D-1, Whey-Carrageenan; E-1, Whey-Sodium alginate.

ing of the three-dimensional protein network responsible for gas retention in the dough (4). These results are in agreement with a previous report that frozen dough displayed a significant decrease in extensigram maximum resistance after several freeze-thaw cycles (17). The loss of dough volume seemed to be caused by weakening of the dough during the freeze-thaw cycles.

After one freeze-thaw cycle, not all of the differences among the various dough mixtures were significant. After two freeze-thaw cycles, differences in dough volumes between dough mixtures with and without milk proteins and gums were observed. After three freeze-thaw cycles, the dough mixtures with milk proteins and gums had larger dough volumes than the control dough. These effects seemed to be due to the properties of milk proteins and gums that prevent ice crystal growth and protect the dough. In particular, whey and sodium alginate (WA) were found to be better than the other milk proteins and gums at improving dough volume. Among all the dough mixtures, dough with WA had the highest volume after two or three freeze-thaw cycles, 22.77 and 21.63 cc, respectively. Whey protein might confer a protective effect on the gluten network in frozen dough (13), while sodium alginate provides freeze-thaw stability and reduces the size of the ice crystals formed (14). As explained above, disulfide bonds formed by the denaturation of whey protein during fermentation also seem to affect the gluten network foam (18) and the overall stability of the dough (19).

Dough structure: The ultrastructure of gluten and starch in the frozen dough was observed by SEM following each freeze-thaw cycle (one to three cycles). The dough structure was increasingly altered with each freeze-thaw cycle. According to Berglund *et al.* (4), dough subjected to either one or three freeze-thaw cycles exhibited considerable damage to the gluten network, including separation of starch granules from the gluten network, as well as alterations in the protein network. These changes in frozen dough could be caused by the formation and growth of ice crystals size during freezing and thawing as a result of temperature changes during storage.

Figure 4 shows the dough structure after one freeze-thaw cycle. After one freeze-thaw cycle, there were no structural differences between dough with and without milk proteins and gums. Dough mixtures subjected to one freeze-thaw cycle had relatively coherent structures, with starch embedded in the gluten matrix, when compared to the structure of dough subjected to two or three freeze-thaw cycles.

Dough mixtures subjected to two or three freeze-thaw cycles are shown in Fig. 5 and 6. Each dough exhibited greater alteration with increasing numbers of freeze-thaw cycles as starch granules separated more from the gluten sheet. As explained above, conformational changes in the dough were caused by the formation and growth of ice crystals as a result of freeze-thaw cycles, and these changes were similar to those reported by Inoue *et al.* (20). In the control dough, the starch granules separated more from the gluten matrix than in dough containing milk proteins and gums. These results were in agreement with another study where the addition of gums gave the dough a thicker gluten strand and a more stable structure (15). Gums such as carrageenan and sodium alginate may protect the gluten structure against ice crystals (14). Alginate especially increased the freeze-thaw stability of the dough (14) and might improve dough stability when added in conjunction with proteins (21).

Characteristics of bread made from frozen dough ***Moisture content of bread crumbs***: The moisture content of bread made from dough subjected to one to three freeze-thaw cycles was measured just after baking and after 4 days of storage at 5°C. Table 3 shows the moisture content of just-baked bread and bread aged at 5°C for 4 days. In just-baked bread, there was no significant reduction in moisture content with increasing numbers of freeze-thaw cycles. These results were similar to those of bread made

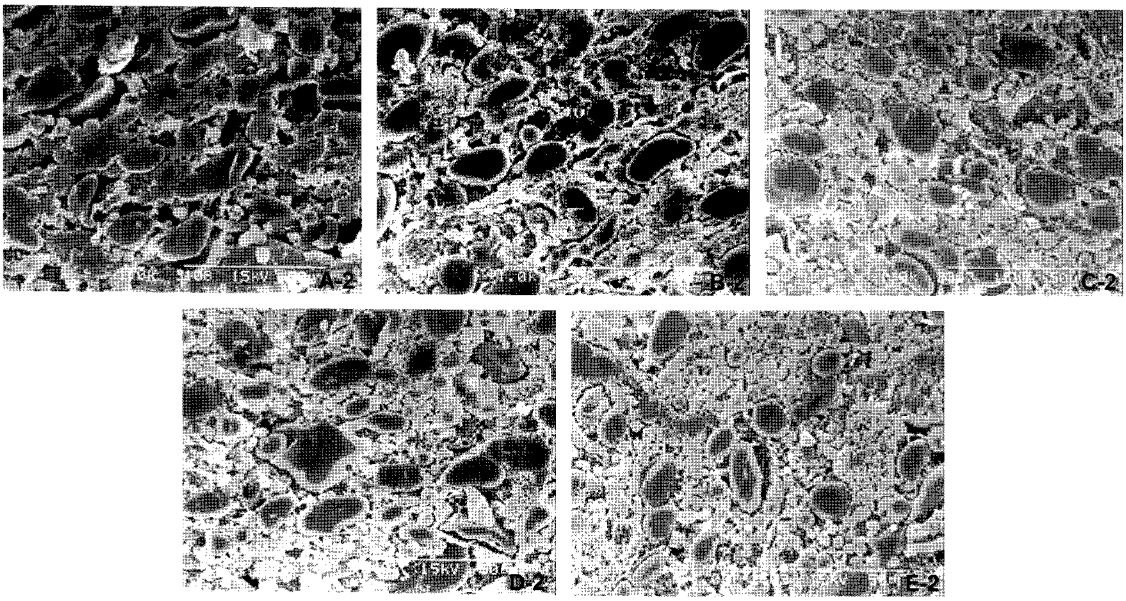

Fig. 5. Scanning electron micrographs of frozen dough subjected to 2 freeze-thaw cycles. A-2, Control; B-2, Casein-Carrageenan; C-2, Casein-Sodium alginate; D-2, Whey-Carrageenan; E-2, Whey-Sodium alginate.

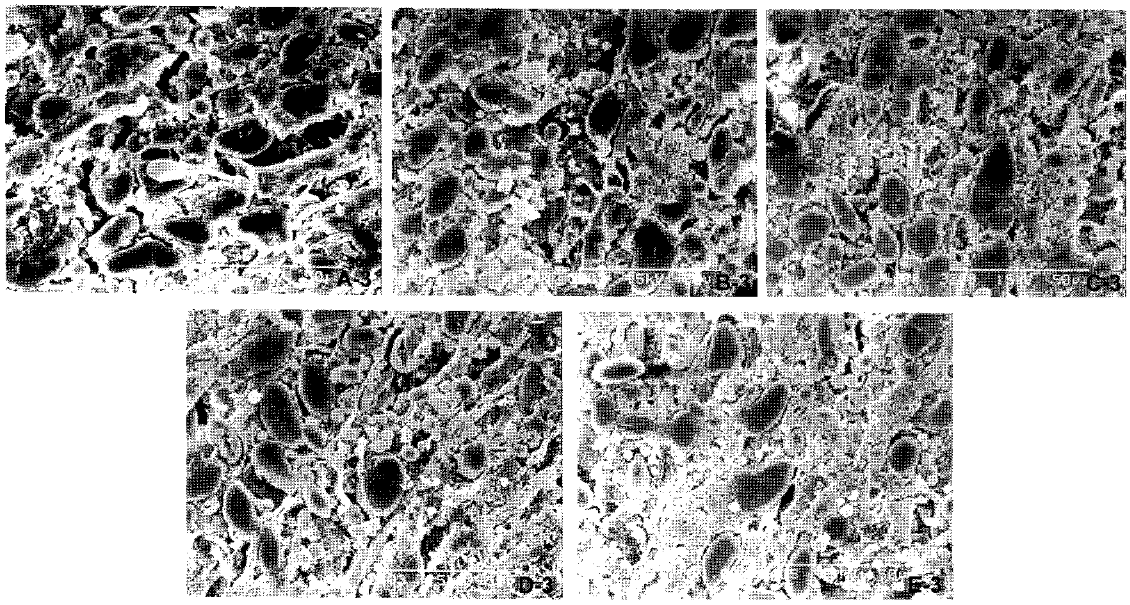

Fig. 6. Scanning electron micrographs of frozen dough subjected to 3 freeze-thaw cycles. A-3, Control; B-3, Casein-Carrageenan; C-3, Casein-Sodium alginate; D-3, Whey-Carrageenan; E-3, Whey-Sodium alginate.

from frozen dough that had been stored for 8 weeks. From these results, the effects of milk proteins and gums on the moisture retention of the baked bread could not be confirmed.

After 4 days of storage, the moisture content of all breads decreased. These decreases resulted from a continuous migration of moisture from crumb to crust during storage (22). The decrease of moisture content was not significantly different between bread with and without milk proteins and gums after one freeze-thaw cycle. In breads made from dough subjected to two or three freeze-thaw cycles, the moisture content of the control bread decreased more than that of bread with milk proteins and gums. These effects could be due to the water binding properties of gums (14) and milk proteins (10). Furthermore, improvements in dough stability with added milk proteins and gums (23) seemed to positively affect the moisture retention of bread during storage.

***Specific loaf volume*:** The specific loaf volume was measured in breads made with dough subjected to one to three freeze-thaw cycles (Table 4). The specific loaf volume was closely associated with the dough volume described above, that is, bread made from frozen dough with a high dough volume had a higher specific loaf volume than bread made from dough with a lower dough volume.

Table 3. Moisture content of just-baked bread and bread aged at 5°C for 4 days. Bread was made from frozen dough subjected to up to three freeze-thaw cycles (Unit: %)

Samples[1)]	Freeze-thaw cycles[2)]					
	1		2		3	
	0 day	4 day	0 day	4 day	0 day	4 day
Control	$^{x}43.04\pm0.32^{a}$	$^{x}40.75\pm0.33^{b}$	$^{x}43.16\pm0.3^{A}$	$^{x}39.54\pm0.53^{B}$	$^{x}43.15\pm0.22^{c}$	$^{x}39.65\pm0.87^{d}$
CK	$^{x}43.18\pm0.44^{a}$	$^{x}41.00\pm0.52^{b}$	$^{x}43.27\pm0.26^{A}$	$^{x}40.23\pm0.34^{B}$	$^{x}43.07\pm0.55^{c}$	$^{x}40.48\pm0.65^{d}$
CA	$^{x}42.96\pm0.57^{a}$	$^{x}40.87\pm1.44^{b}$	$^{x}42.95\pm0.08^{A}$	$^{x}40.57\pm0.76^{B}$	$^{x}43.00\pm0.31^{c}$	$^{x}40.10\pm0.69^{d}$
WK	$^{x}42.88\pm0.22^{a}$	$^{x}40.52\pm0.99^{b}$	$^{x}42.96\pm0.37^{A}$	$^{x}39.95\pm0.68^{B}$	$^{x}42.93\pm0.29^{c}$	$^{x}39.69\pm0.52^{d}$
WA	$^{x}43.14\pm0.21^{a}$	$^{x}40.81\pm0.37^{b}$	$^{x}43.28\pm0.43^{A}$	$^{x}40.14\pm0.88^{B}$	$^{x}42.93\pm0.02^{c}$	$^{x}39.37\pm0.84^{d}$

1)CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100%.
2)Values are mean±standard deviation. $^{a,b:A,B:c,d}$: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

Table 4. Specific loaf volume of breads made from frozen dough with milk proteins and gums subjected to up to three freeze-thaw cycles (Unit: cc/g)

Samples[1)]	Freeze-thaw cycles[2)]		
	1	2	3
Control	$^{z}4.28\pm0.04^{a}$	$^{y}4.09\pm0.15^{ab}$	$^{z}3.72\pm0.15^{c}$
CK	$^{y}4.39\pm0.09^{a}$	$^{y}4.08\pm0.18^{b}$	$^{y}4.01\pm0.09^{b}$
CA	$^{xy}4.47\pm0.08^{a}$	$^{y}4.05\pm0.12^{b}$	$^{y}3.97\pm0.06^{bc}$
WK	$^{y}4.38\pm0.12^{a}$	$^{y}3.92\pm0.12^{b}$	$^{z}3.82\pm0.08^{bc}$
WA	$^{x}4.55\pm0.13^{a}$	$^{x}4.35\pm0.14^{ab}$	$^{x}4.31\pm0.09^{b}$

1)CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100%.
2)Values are mean±standard deviation. ^{x,y,z}: values with different superscripts within columns are significantly different by Duncan's multiple range test at $p<0.05$. ^{a,b,c}: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

Table 5. Color of bread crumbs made from frozen dough with milk proteins and gums subjected to up to three freeze-thaw cycles

Value	Samples[1)]	Freeze-thaw cycles[2)]		
		1	2	3
L	Control	$^{x}77.49\pm1.17^{a}$	$^{y}77.12\pm1.52^{a}$	$^{x}77.95\pm0.77^{a}$
	CK	$^{x}77.12\pm0.95^{a}$	$^{xy}78.10\pm0.31^{a}$	$^{x}77.45\pm0.90^{a}$
	CA	$^{x}76.98\pm0.87^{a}$	$^{xy}77.82\pm0.85^{a}$	$^{x}78.03\pm1.02^{a}$
	WK	$^{x}76.84\pm0.62^{b}$	$^{x}78.72\pm0.62^{a}$	$^{x}78.60\pm1.18^{a}$
	WA	$^{x}77.18\pm0.66^{b}$	$^{xy}78.24\pm0.36^{a}$	$^{x}78.07\pm0.19^{a}$
a	Control	$^{y}\mathrm{z}2.12\pm0.75^{a}$	$^{y}2.24\pm0.70^{a}$	$^{y}2.33\pm0.81^{a}$
	CK	$^{x}2.60\pm0.92^{a}$	$^{x}2.40\pm0.74^{a}$	$^{x}2.58\pm0.31^{a}$
	CA	$^{x}2.57\pm0.91^{a}$	$^{y}2.28\pm0.83^{a}$	$^{x}2.49\pm1.01^{a}$
	WK	$^{y}2.20\pm0.50^{a}$	$^{x}2.38\pm0.73^{a}$	$^{x}2.51\pm0.71^{a}$
	WA	$^{y}\mathrm{z}2.15\pm0.54^{a}$	$^{z}2.11\pm0.41^{a}$	$^{y}2.40\pm0.84^{a}$
b	Control	$^{x}17.65\pm1.18^{a}$	$^{x}17.95\pm1.00^{a}$	$^{x}17.92\pm1.34^{a}$
	CK	$^{x}18.93\pm0.65^{a}$	$^{x}18.54\pm1.29^{a}$	$^{x}18.70\pm0.16^{a}$
	CA	$^{x}18.35\pm1.77^{a}$	$^{x}17.85\pm1.44^{a}$	$^{x}18.43\pm1.75^{a}$
	WK	$^{x}17.73\pm1.26^{a}$	$^{x}18.02\pm1.79^{a}$	$^{x}17.95\pm1.01^{a}$
	WA	$^{x}17.57\pm1.47^{a}$	$^{x}17.27\pm0.60^{a}$	$^{x}18.46\pm1.57^{a}$

1)CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100%.
2)Values are mean±standard deviation. ^{x,y,z}: values with different superscripts within columns are significantly different by Duncan's multiple range test at $p<0.05$. ^{a,b,c}: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

The specific loaf volume decreased as the number of freeze-thaw cycles increased. These results are in agreement with those of Inoue *et al.* (20) and Hsu *et al.* (23). They reported that with increasing number of freeze-thaw cycles, final proofing time increased and loaf volume decreased. Decreased loaf volume may be caused by disruptions in dough structure and reductions in gas retention (16).

Among breads subjected to one freeze-thaw cycle, the control bread had the lowest specific loaf volume, 4.28 cc/g. In addition, the control bread generally had a lower specific loaf volume than breads with milk proteins and gums after two or three freeze-thaw cycles. This was also observed in bread made from dough stored at -20°C for 8 weeks. These results are in agreement with reports by other investigators. Lee *et al.* (15) and Rogers *et al.* (24) reported that gums improved the loaf volume of bread made from frozen dough and served as cryoprotectants. According to Kenny *et al.* (13), sodium caseinate improved the baking properties of frozen dough and increased the specific volume. The addition of milk proteins and gums is thought to improve the gas retention of dough by protecting the gluten network from disruptions by ice crystals (14, 15). As explained above, gums also increased the extensity of dough by increasing the amount of non-gelatinized starch during fermentation and baking, and these properties seemed to improve the loaf volume of the bread.

In particular, bread with WA exhibited significant improvement in specific loaf volume. These results seemed to be due to the added alginate which increased the freeze-thaw stability of the dough (14), and by milk proteins together with gums, increasing the dough stability (21). Furthermore, whey proteins have disulfide bonds that could support the gluten network foam during the initial baking stages in the oven (18).

***Color*:** Crumb color (L, a, and b values) in bread made from frozen dough subjected to one to three freeze-thaw cycles is shown in Table 5 The L value of breads with WK (whey plus κ-carrageenan) and WA were 76.8 and 77.2, respectively, after one freeze-thaw cycle, and were 78.7 and 78.2, respectively, after two freeze-thaw cycles. Thus, the L values of bread with WK and WA milk proteins increased through two freeze-thaw cycles after which L values did not change. In terms of the L value, the control bread was not significantly different from breads with milk proteins and gums, even after three freeze-thaw cycles. After one freeze-thaw cycle, the L values of milk proteins were lower than that of the control, indicating a dark crumb color (25). The L values of all breads were in the range of 77.0 to 79.0.

The number of freeze-thaw cycles did not affect the a value of bread crumbs. After one freeze-thaw cycle, the a values of breads with CK and CA were higher than those of breads with WK and WA, as well as the control bread. Bread with WA had the lowest a value, 2.1, but breads with other milk proteins and gums generally had higher a values than the control, indicating a more reddish tint in the bread crumbs. In breads made from frozen dough subjected to three freeze-thaw cycles, the a value of the bread with WA was still lower than that of breads with other milk proteins and gums, and was similar to the a value of control bread.

The b values of bread crumbs were not affected by the addition of milk protein and gums or the number of freeze-thaw cycles. The b values in all breads ranged from 17.3 to 18.9.

When comparing the color values of bread, the milk proteins and gums did not have any significant effects on bread color. However, adding whey to bread reduced both the a and b values of the resulting bread crumbs. As might be expected, the addition of whey positively affected consumers who preferred white bread to yellow bread (26).

***Texture (hardness)*:** The hardness of just-baked bread and bread aged at 5°C for 4 days was measured to compare the staling of bread made with and without milk proteins and gums (Table 6).The range of hardness was 0.21 to 0.26 for all just-baked breads. There were no significant differences between them, regardless of the additives used or the number of freeze-thaw cycles. In a similar study, Ribotta *et al.* (2) reported that just-baked bread with guar gum had a similar firmness to bread made without guar gum.

The hardness of bread increased during storage at 5°C for 4 days. This is related to the loss of moisture content in the bread and the retrogradation of starch (22) during storage. During the 4-day storage period, the hardness of the control bread increased more than that of bread prepared with milk proteins and gums. These results are in agreement with those of other investigators. For example, Davidous *et al.* (27) and Rosell *et al.* (28) suggested that hydrocolloids like guar gum, alginate, and xanthan gum can be used as anti-staling agents, probably as a result of moisture retention and the emulsification of milk proteins and gums (15). In addition, improved emulsification stability caused by the addition of milk proteins and gums could also contribute to improvements in the texture of the bread (12).

After one freeze-thaw cycle, the hardness of breads did not change, regardless of the presence and types of milk protein and gum. The breads with WK and WA had a lower hardness than the control bread or breads with CK and CA after two freeze-thaw cycles. This result is consistent with those of Zadow (29) who reported that breads with added whey protein were perceived by panelists to retain their freshness longer than the control. These results suggest that whey protein affects the hardness of the bread. Therefore, it seems likely that the addition of whey protein attenuates the increase in hardness of the bread that normally occurs during storage. After three freeze-thaw cycles, there were no significant differences in the hardness of breads with and without milk proteins and gums. Therefore, milk proteins and gums, especially WA, could be used as anti-staling agents in wheat breads.

***Sensory evaluation*:** The results of the sensory tests of bread made with dough subjected to one to three freeze-thaw cycles are summarized in Table 8 to 10. In all attributes of the sensory test, there were no differences in sensory preference between breads with and without milk proteins and gums that had been subjected to up to three freeze-thaw cycles. Furthermore, there were no significant differences in sensory color between the control bread and bread with milk proteins and gums. These results were

Table 6. Hardness of just-baked breads and breads aged at 5°C for 4 days. Breads were made from frozen dough with milk proteins and gums subjected to up to three freeze-thaw cycles **(Unit: kg_f)**

Samples[1]	Freeze-thaw cycles[2]					
	1		2		3	
	0 day	4 day	0 day	4 day	0 day	4 day
Control	$^{x}0.21\pm0.01^{a}$	$^{x}0.54\pm0.03^{b}$	$^{x}0.26\pm0.04^{A}$	$^{xy}0.66\pm0.07^{B}$	$^{x}0.24\pm0.01^{c}$	$^{x}0.68\pm0.02^{d}$
CK	$^{x}0.22\pm0.02^{a}$	$^{x}0.55\pm0.06^{b}$	$^{x}0.23\pm0.01^{A}$	$^{xy}0.62\pm0.03^{B}$	$^{x}0.25\pm0.01^{c}$	$^{x}0.63\pm0.06^{d}$
CA	$^{x}0.23\pm0.03^{a}$	$^{x}0.58\pm0.04^{b}$	$^{x}0.26\pm0.00^{A}$	$^{x}0.69\pm0.01^{B}$	$^{x}0.25\pm0.00^{c}$	$^{x}0.65\pm0.01^{d}$
WK	$^{x}0.23\pm0.03^{a}$	$^{x}0.55\pm0.04^{b}$	$^{x}0.26\pm0.02^{A}$	$^{y}0.61\pm0.02^{B}$	$^{x}0.25\pm0.01^{c}$	$^{x}0.66\pm0.03^{d}$
WA	$^{x}0.23\pm0.03^{a}$	$^{x}0.54\pm0.05^{b}$	$^{x}0.23\pm0.02^{A}$	$^{y}0.60\pm0.00^{B}$	$^{x}0.24\pm0.01^{c}$	$^{x}0.64\pm0.02^{d}$

[1] CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100%.

[2] Values are mean±standard deviation. $^{a,b;\ A,B;\ c,d}$: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

Table 7. Sensory evaluation of breads made from frozen dough with milk proteins and gums subjected to one freeze-thaw cycle

Characteristic	Samples[1,2]				
	Control	CK	CA	WK	WA
Crust color	4.29±0.13^{a}	4.18±0.60^{a}	4.18±0.60^{a}	4.13±0.18^{a}	3.90±0.14^{a}
Break-shred	3.67±0.33^{a}	3.55±0.63^{a}	3.55±0.63^{a}	3.97±0.04^{a}	3.80±0.15^{a}
Symmetry	4.15±0.50^{a}	4.35±0.21^{a}	4.35±0.21^{a}	3.65±0.21^{a}	3.64±0.34^{a}
Crumb color	4.45±0.07^{a}	4.10±0.57^{a}	4.10±0.57^{a}	3.98±0.39^{a}	4.30±0.28^{a}
Grain	3.82±0.26^{ab}	4.48±0.39^{a}	4.48±0.39^{a}	3.74±0.20^{ab}	3.56±0.35^{b}
Aroma	4.00±0.01^{a}	3.98±0.32^{a}	3.98±0.32^{a}	3.63±0.53^{a}	3.78±0.03^{a}
Taste	3.77±0.19^{a}	3.73±0.04^{a}	3.73±0.04^{a}	3.98±0.32^{a}	4.00±0.02^{a}
Texture	3.87±0.10^{a}	3.75±0.35^{a}	3.75±0.35^{a}	4.14±0.37^{a}	4.03±0.04^{a}
Overall eating quality	3.85±0.22^{a}	3.80±0.28^{a}	3.80±0.28^{a}	3.75±0.35^{a}	3.83±0.11^{a}

[1] CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100%.
[2] Values are mean±standard deviation. ^{a,b,c}: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

Table 8. Sensory evaluation of breads made from frozen dough with milk proteins and gums subjected to two freeze-thaw cycles

Characteristic	Samples[1,2]				
	Control	CK	CA	WK	WA
Crust color	3.44±0.08^{a}	3.84±0.06^{a}	3.57±0.81^{a}	3.75±0.16^{a}	3.51±0.18^{a}
Break-shred	3.60±0.31^{a}	3.25±0.35^{a}	3.57±0.45^{a}	3.65±0.56^{a}	3.89±0.29^{a}
Symmetry	3.54±0.40^{a}	4.05±0.12^{a}	3.85±0.31^{a}	3.87±0.01^{a}	3.68±0.10^{a}
Crumb color	4.01±0.18^{a}	4.00±0.16^{a}	3.81±0.08^{a}	3.75±0.19^{a}	3.98±0.13^{a}
Grain	3.46±0.11^{a}	3.64±0.36^{a}	3.77±0.33^{a}	3.42±0.41^{a}	3.85±0.40^{a}
Aroma	3.67±0.04^{ab}	3.64±0.19^{ab}	3.40±0.15^{b}	3.84±0.06^{a}	3.70±0.07^{ab}
Taste	3.84±0.06^{a}	3.60±0.41^{a}	3.61±0.15^{a}	3.87±0.01^{a}	3.88±0.02^{a}
Texture	4.00±0.15^{a}	3.84±0.48^{a}	3.75±0.16^{a}	3.77±0.03^{a}	3.81±0.43^{a}
Overall eating quality	3.62±0.34^{a}	3.69±0.18^{a}	3.68±0.25^{a}	3.91±0.04^{a}	3.66±0.13^{a}

[1] CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100%.
[2] Values are mean±standard deviation. ^{a,b,c}: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

Table 9. Sensory evaluation of breads made from frozen dough with milk proteins and gums subjected to three freeze-thaw cycles

Characteristic	Samples[1,2]				
	Control	CK	CA	WK	WA
Crust color	3.88±0.69^{a}	3.75±0.05^{a}	3.82±0.06^{a}	3.94±0.33^{a}	3.71±0.21^{a}
Break-shred	3.86±0.45^{a}	3.93±0.30^{a}	3.05±0.22^{a}	3.88±0.02^{a}	3.71±0.50^{a}
Symmetry	3.85±0.41^{a}	3.54±0.57^{a}	3.44±0.37^{a}	3.86±0.50^{a}	4.06±0.12^{a}
Crumb color	4.15±0.21^{a}	3.89±0.16^{a}	3.95±0.23^{a}	3.93±0.10^{a}	3.97±0.04^{a}
Grain	3.78±0.31^{a}	3.84±0.07^{a}	3.63±0.02^{ab}	3.13±0.28^{b}	3.91±0.33^{a}
Aroma	3.79±0.50^{a}	3.85±0.02^{a}	3.57±0.41^{a}	3.27±0.23^{a}	3.29±0.40^{a}
Taste	3.99±0.22^{a}	3.86±0.20^{a}	3.54±0.05^{a}	3.71±0.21^{a}	4.12±0.46^{a}
Texture	4.36±0.20^{a}	3.98±0.21^{ab}	3.64±0.20^{b}	3.97±0.05^{ab}	3.99±0.22^{ab}
Overall eating quality	4.32±0.45^{a}	3.75±0.05^{abc}	3.41±0.03^{c}	3.89±0.12^{bc}	4.16±0.38^{ab}

[1] CK, casein-κ-carrageenan; CA, casein-sodium alginate; WK, whey-κ-carrageenan; WA, whey-sodium alginate; Control, wheat flour 100%.
[2] Values are mean±standard deviation, ^{a,b,c}: values with different superscripts within rows are significantly different by Duncan's multiple range test at $p<0.05$.

similar to the color values of breads as measured by the Hunter colorimeter.

With regard to bread aged at 5°C for 4 days, breads with milk proteins and gums were generally perceived to have smaller increases in hardness compared to the control. However, the breads received similar scores for texture in the sensory evaluation, probably because the hardness measured by a texturometer was only slightly different, and perhaps to a degree that might not be distinguishable by human senses. In other categories, including crust color, break-shred, symmetry, aroma, grain, and taste, panelists also did not note differences between breads with and without milk proteins and gums. While the addition of milk proteins and gums to dough did not seem to improve sensory properties, their addition caused some negative effects in overall sensory preference.

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