Centrifuged Liquid and Breadmaking Properties of Frozen-and-Thawed Bread Dough

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ABSTRACT

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Breadmaking properties (bread height, mm, and specific volume, cm^3/g) showed marked deterioration when bread dough was frozen and stored at –20°C for one day. However, these properties of bread dough baked after storage for three to six days were not further deteriorated as compared with that baked after one day of storage. A large amount of liquid was oozed from the frozen-and-thawed bread dough. The liquid was separated from the bread dough by centrifugation (38,900 × *g* for 120 min at 4°C), and collected by tilting the centrifuge tube at an angle of 45° for 30 min. There was a strong correlation between the amount of centrifuged liquid and breadmaking properties (bread height and specific volume). The mechanism responsible for the oozing of liquid in frozen-and-thawed bread dough was studied. The presence of yeast and salt in bread dough was suggested to be closely related to the amount of centrifuged liquid, and fermented products particularly had a large effect on the amount of centrifuged liquid.

Frozen dough is widely used in the breadmaking industry, and it is generally believed that the use of frozen dough will increase in the future. The use of frozen dough has the advantage of allowing breadmaking to be discontinuous, obviating the need for the process to continue uninterrupted from start to finish. However, there are two problems with the use of frozen dough that hinder its use in the breadmaking industry. One is related to the effects of freezing on baker's yeast and has been studied by many researchers (Kulp 1995), resulting in the development of freeze-resistant yeast strains. Kline and Sugihara (1968) proposed that release of disulfide-reducing substances into the dough from dead yeast cells caused a decrease in gassing power and gradual loss of dough strength. Progress being made in yeast biochemistry and the underlying genetic elements responsible for freeze-tolerance traits may soon offer opportunities to exploit the tools of biotechnology to create improved strains of yeast for frozen dough (Casey and Foy 1995). Another problem is related to deterioration in the bread dough itself caused by the freezing, and this also has been studied by many researchers. According to Variano-Marston et al (1980), the structure of gluten in frozen dough was damaged by formation of ice crystals. And Wolt and D'Appolonia (1984) suggested that protein quality (strength) was important for bread production from frozen dough. Inoue and Bushuk (1991, 1992) and Inoue et al (1994) reexamined the extensigraphic patterns of frozen doughs and confirmed the weakening of doughs containing yeast observed by Variano-Marston et al (1980) during frozen storage and successive freeze-thaw cycles. However, the mechanism of this deterioration has not been determined. We studied the relationship between free water oozed from frozen-and-thawed dough and the dough's breadmaking properties. Furthermore, we investigated the relationship between amount of liquid centrifuged from dough and the presence of salt and fermented products.

MATERIALS AND METHODS

Wheat Flour

Seven different wheat flours were used in this experiment: Kameria (Nisshin Flour Milling Co. Ltd., Japan), Red Knight and Alps (Nitto Flour Milling Co. Ltd., Japan), Midoriuzumaki (Masuda Flour Milling Co. Ltd., Japan), Horoshiri (Ebetsu Flour Milling Co. Ltd., Japan), and Kitakami and Nanbujigona (Fukin Flour Milling Co. Ltd., Japan). Protein conversion was N × 5.7. Ash was determined by Approved Method 08-01 (AACC 2000) at 14.0% mb

Preparation of bread dough and freezing-and-thawing, and baking were as reported in Seguchi et al (1997). Baking absorption of each flour was determined in a farinograph with 300 g of flour (Approved Method 54-21, AACC 2000). Freeze-resistant yeast (Kaneka Co. Ltd., Japan) was used in this experiment. Wheat flour (290 g), compressed yeast (8.7 g), sugar (14.5 g), salt (2.9 g), and water (estimated from farinograph at 500 BU) were mixed in a computer-controlled automatic bread maker (National SD-BT6, Matsushita Electric Ind. Co. Ltd., Japan) with the first proof for 2 hr 20 min at 30°C. The time consisted of 15 min for the first mixing, 50 min of rest, 5 min for the second mixing, and 70 min of fermentation. The bread dough was divided into 120-g pieces, rounded, molded, and placed in baking pans (Approved Method 10-10A, AACC 2000). The bread dough was further proofed for 22 min at 38°C. This was the complete bread dough. The baking pan in which the bread dough (120 g) was placed was put into a plastic film bag, frozen, and stored at –20°C for one, three, and six days. For the thawing, the baking pan was placed in a refrigerator at 5°C for 16 hr, which was adequate for bread dough to thaw completely. After thawing, one part of the bread dough was used for breadmaking and the other was subjected to determination of free water oozed from bread dough. The thawed dough was baked at 210°C for 30 min in an oven (model DN-63, Yamato Scientific Co. Ltd., Japan). After baking, the bread was removed from the pan and cooled for 1 hr at a room temperature of 26°C and relative humidity of 43%. Bread height (mm), weight (g), and volume (cm^3) were measured, and the crumb grains were evaluated visually. As a control, nonfrozen and unstored bread dough of each flour was subjected to same breadmaking.

The amount of liquid oozed from bread doughwas determined. The thawed bread dough was removed from its pan. No water drops were observed in the pan or plastic film bag. The dough was centrifuged at 38,900 × *g* for 120 min at 4°C (Hitachi himac CR 21G, Hitachi Koki Co., Ltd. Japan), after which the centrifuge tube was titled at an angle of 45° for 60 min in a cold room (4°C), and the supernatant (centrifuged liquid) was collected in a dropwise manner and recorded as mL/100 g of dough. The centrifuged liquid after dialysis against water was subjected to protein and carbohydrate determinations by the method of Lowry et al (1951) and the phenol sulfate method, respectively.

Various combined bread doughs were prepared. Salt (2.9 g) and Kameria wheat flour (290 g), sugar (14.5 g) and flour, yeast (8.7 g) and flour, yeast, sugar and flour, yeast, salt and flour, sugar, salt and flour, yeast, sugar, salt and flour (complete) were blended, respectively, and were mixed with 210 mL of water in a bread maker.

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They were then treated in the same manner as above. All dough samples were frozen-and-thawed after one day of storage, and the amount of liquid centrifuged from each was determined. Protein and carbohydrate in the liquid were also determined as above.

Cultured fluid/flour dough were prepared, and centrifuged liquid was determined. Compressed yeast (4.35 g), sugar (7.25 g), and salt (1.45 g) were mixed with 105 mL of water in 300-mL conical flasks and incubated at 35°C for 140 min with slight shaking. This was the complete cultured fluid. Then, the mixtures were boiled for 5 min to kill all the yeast and cooled with tap water. Kameria wheat flour (145 g) was mixed with above whole cultured fluid in a farinograph bowl at 30°C for 10 min and referred to as cultured fluid/flour dough. This cultured fluid/flour dough represented dough with presence of yeast and fermentation products. Cultured fluid/flour dough (120 g) was centrifuged at 38,900 × *g* at 4°C for 120 min. The centrifuge tubes were tilted at an angle of 45°for 30 min in a cold room (4°C), and centrifuged liquid was collected. Protein and carbohydrate in the liquid were also determined as above. Various combined cultured fluid/flour doughs were prepared with salt (1.45 g) and Kameria wheat flour (145 g), sugar (7.25 g), and flour, yeast (4.35 g) and flour, yeast, sugar and flour, yeast, salt and flour, and yeast, sugar, salt and flour (complete) were mixed with 105 mL of water, respectively, and treated in the same manner as above. All cultured fluid/flour doughs were frozen-and-thawed after one day of storage, and the liquid centrifuged from them was determined. Protein and carbohydrate in the liquid were also determined as above.

Time course of liquid centrifuged from cultured fluid/flour dough was determined by incubating cultured fluids at 35°C for 0, 70, 140, 210, and 280 min, and were treated as above. The relationship between incubation time of cultured fluid and the amount of the centrifuged liquid was investigated.

Statistical Analysis

A statistical software package (SPSS Inc., Chicago, IL.) was used for statistical analyses. Experiments were repeated four times and the results were averaged. Analysis produced significant *F* values by analysis of variance, followed by Duncan's multiple range test for comparison of means.

RESULTS AND DISCUSSION

Breadmaking Properties from Frozen-and-Thawed Bread Dough

Kameria, Red Knight, Alps, Midoriuzumaki, Horoshiri, Kitakami, and Nanbujigona wheat flours were used in this experiment. The protein and ash contents of these wheat flours are listed in Table I. Table II lists the changes in breadmaking properties (bread height, mm, and specific volume, cm^3/g) with increasing storage time of the bread dough at –20°C. These breadmaking properties showed marked deterioration after one day of frozen storage of the bread

TABLE I
Protein and Ash Contents[a] of Wheat Flour

Wheat Flour	Protein[b] (%, db)	Ash (%, db)
Kameria	13.6 ± 0.3	0.40 ± 0.02
Red Knight	14.8 ± 0.3	0.37 ± 0.00
K-Alps	10.1 ± 0.2	0.34 ± 0.05
Midoriuzumaki	14.5 ± 0.1	0.47 ± 0.01
Horoshiri	10.4 ± 0.1	0.36 ± 0.00
Kitakami	10.7 ± 1.1	0.53 ± 0.00
Nanbujigona	11.2 ± 0.0	0.49 ± 0.00

[a] Values represent means and standard deviation of duplicates.
[b] N × 5.7.

TABLE III
Effect of Centrifugation on the Centrifuged Liquid (mL), Protein, and Carbohydrate (mg)

Minutes at 38,9000 × *g*	Centrifuged Liquid (mL/100 g of dough)	Protein Conc. (mg/mL)	Carbohydrate Conc. (mg/mL)
60	0.71 ± 0.31	14.3 ± 1.0	55.3 ± 2.4
90	3.24 ± 0.80	13.4 ± 0.7	50.6 ± 2.9
120	7.39 ± 0.91	12.2 ± 0.2	48.6 ± 2.8

TABLE II
Results of Breadmaking and Centrifuged Liquid

	Days of Freezing			
	0	1	3	6
Red Knight				
Bread height (mm)	83.83 (1.76)a	66.37 (2.71)b	65.73 (1.53)b	59.57 (1.77)c
Bread SV (cm^3/g)	4.18 (0.09)a	3.15 (0.03)b	3.51 (0.21)c	2.70 (0.15)d
Liquid (mL/100 g of dough)	1.05	3.91	3.75	4.39
Kameria				
Bread height (mm)	86.50 (2.35)a	67.13 (5.78)bf	64.23 (4.91)cef	57.67 (2.89)de
Bread SV (cm^3/g)	4.99 (0.17)a	2.89 (0.40)be	3.40 (0.22)cf	2.92 (0.16)def
Liquid (mL/100 g of dough)	2.20	4.30	4.45	4.70
Alps				
Bread height (mm)	83.4 (0.1)a	64.2 (2.7)b	70.8 (1.8)c	59.4 (1.0)d
Bread SV (cm^3/g)	3.50 (0.01)a	2.38 (0.02)b	2.22 (0.04)c	2.65 (0.13)d
Liquid (mL/100 g of dough)	7.46 (0.46)a	9.67 (0.09)b	8.63 (0.38)c	10.2 (0.20)b
Midoriuzumaki				
Bread height (mm)	88.0 (1.5)a	64.9 (1.3)befg	74.0 (6.4)cf	59.3 (2.1)deg
Bread SV (cm^3/g)	3.74 (0.02)a	2.13 (0.7)b	3.35 (0.08)c	2.44 (0.05)d
Liquid (mL/100 g of dough)	6.58 (0.47)a	8.42 (0.12)bde	8.15 (0.35)adf	8.95 (1.06)cef
Horoshiri				
Bread height (mm)	80.3 (4.2)a	66.5 (2.1)bf	57.2 (6.2)cef	51.3 (0.2)de
Bread SV (cm^3/g)	3.26 (0.17)a	2.46 (0.16)b	2.12 (0.06)c	1.87 (0.01)c
Liquid (mL/100 g of dough)	0.00 (0.00)a	0.28 (0.18)b	0.38 (0.04)b	0.40 (0.0)b
Kitakami				
Bread height (mm)	79.5 (1.9)a	59.8 (0.8)b	57.7 (3.8)b	60.3 (0.9)b
Bread SV (cm^3/g)	3.38 (0.01)a	2.52 (0.09)b	2.17 (0.13)c	2.48 (0.02)b
Liquid (mL/100 g of dough)	0.90 (0.10)a	9.67 (0.09)b	9.19 (0.94)c	8.12 (1.30)c
Nanbujigona				
Bread height (mm)	88.00 (0.28)a	66.05 (1.48)b	62.40 (0.42)c	58.10 (0.85)d
Bread SV (cm^3/g)	4.43 (0.54)a	3.37 (0.00)b	2.13 (0.01)c	2.19 (0.08)c
Liquid (mL/100 g of dough)	0.39 (0.97)a	12.58 (1.66)b	13.15 (1.06)b	13.70 (1.69)b

dough compared with nonfrozen and unstored bread dough. However, no further deterioration was observed after three to six days of frozen storage. Figure 1 shows the appearance of sectioned breads baked from bread doughs stored at 0 (nonfrozen), one, three, and six days made from Kameria and Red Knight wheat flours.

Centrifuged Liquid in Frozen-and-Thawed Bread Dough

It was qualitatively observed that liquid oozed from dough was increased by freezing-and-thawing, and the increased amount of liquid may be related to the deterioration of breadmaking properties baked with frozen-and-thawed bread dough. Then, we thought that it was necessary to measure the amount of the oozed liquid from bread dough by centrifugation. Relevant studies are presented in the literature (Mauritzen and Stewart 1965, 1966; MacRitchie 1976; Larsson and Eliasson 1996; Rasanen et al 1997). However, to measure a similar amount of the liquid that naturally oozed from dough, we excluded the high gravity of ultracentrifugation conditions. We designed a centrifugation condition of 38,900 × *g* for 120 min at 4°C to obtain the amount of the centrifuged liquid. Resulting centrifugation was performed at steady state. The centrifuged liquid from nonfrozen and unstored bread dough was rather viscous and contained high protein (12.2–14.3 mg/mL) and carbohydrate (48.6–55.3 mg/mL) concentrations (Table III). The protein and carbohydrate composition of the centrifuged liquid was not so affected by the centrifugation parameters. After one day of frozen storage, the amount of free water obtained from thawed dough samples was markedly increased, and the amounts from doughs stored for three to six days were slightly increased or decreased (Table II). All wheat flours showed the same trends. There was a marked correlation between the increase in the amount of liquid centrifuged from frozen-and-thawed doughs and the deterioration in their breadmaking properties (Table IV). The deterioration of the breadmaking properties of frozen-and-thawed bread doughs compared with nonfrozen and unstored doughs may be due to decreases in the water binding ability of a bread dough caused by freezing-and-thawing.

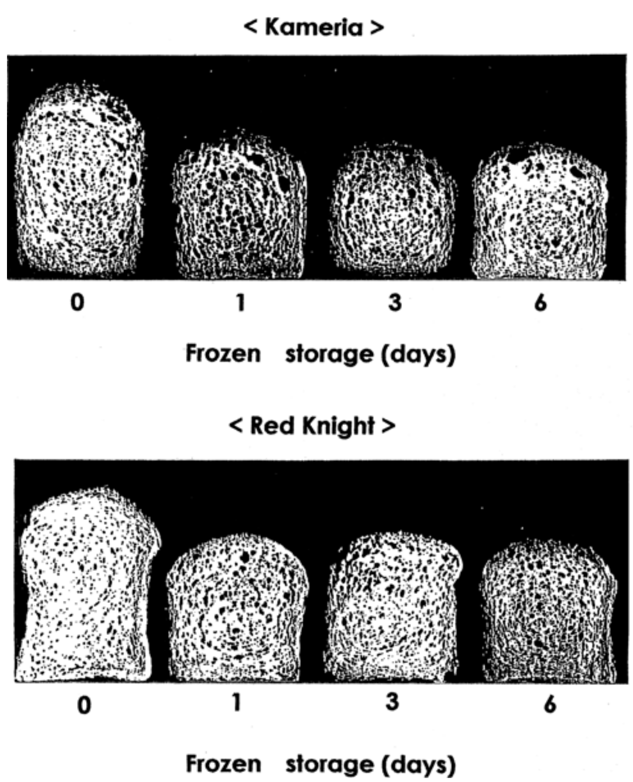

Fig. 1. Appearance of sectioned bread baked from bread doughs of Kameria and Red Knight wheat flours after 0 (nonfrozen), 1, 3 or 6 days in frozen storage.

Effects of Bread Dough Components on the Amount of Centrifuged Liquid

It is necessary to determine which components of bread dough affect the amount of centrifuged liquid. Figure 2 lists the bread dough components (except for wheat flour) and their combinations, and shows the effects of the presence of each component and their combinations on the amount of centrifuged liquid. When the amount of centrifuged liquid from the nonfrozen and unstored complete bread dough was set as 100%, the nearest value, 85%, was obtained in the nonfrozen and unstored doughs with the combination of yeast and salt, followed by 42% with the combination of salt and sugar, and 27% with salt alone. Others were all <5%. From these results, we concluded that salt was an important component in bread dough for the centrifuged liquid. Yeast also contributed to the centrifuged liquid when it was mixed with salt and, in this case, yeast could use wheat carbohydrates as a carbon source even if sugar is absent, and the fermented products could contribute to the amount of centrifuged liquid. These changes in the amount of the centrifuged liquid with bread dough components were also observed in frozen-and-thawed dough samples. When samples were frozen-and-thawed after one day of storage, each bread dough showed almost the same trends. However, in case of yeast and yeast+sugar bread doughs, the amount of liquid centrifuged from the frozen-and-thawed dough was greater than those from nonfrozen and unstored doughs. To make clear the mechanism of centrifuged liquid from bread dough in the presence of salt and yeast, a new experiment with cultured fluid/flour dough, in which wheat flour was excluded in the incubation period of 2 hr 20 min and added afterward, was designed. The amounts of protein and carbohydrate in a centrifuged liquid from each combined bread dough with or without freezing-and-thawing were determined and compared with the amount of centrifuged liquid from each (Table V). Results of these correlation coefficients (*r*) indicates that the amount of the protein and carbohydrate in each bread dough is highly related to the amounts of the centrifuged liquid, and suggests that there are no higher or lower extraction of protein and carbohydrate in each centrifuged liquid. In bread dough, wheat flour was mixed in the bread dough and incubated for 2 hr 20 min at 38°C and wheat flour enzymes such as amylases, proteases could be activated in dough. So, it was possible that those enzymes in wheat flour could affect the amount of centrifuged liquid. However, the amount of the resulting centrifuged liquid from the nonfrozen complete cultured fluid (yeast+ sugar+salt)/flour dough was almost the same as that from nonfrozen complete bread dough (yeast+sugar+salt+flour), which indicated that the enzymes in wheat flour were not related to the amount of liquid released. Then, it was ascertained that the presence of wheat flour in incubation time did not relate to the amount of centrifuged liquid. So, cultured fluid/flour dough was used in the experiments described below.

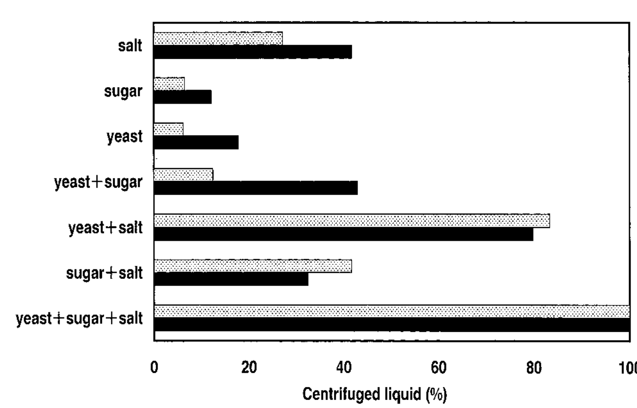

Fig. 2. Effects of bread dough components and their combinations on the amount of centrifuged liquid. Nonfrozen and unstored (□); after one day of frozen storage (■). Kameria wheat flour was used.

Centrifuged Liquid from Cultured Fluid/Flour Dough

As various bread dough samples were listed in Fig. 2, the various cultured fluid/flour dough made with various components (yeast, salt, and sugar) and their combinations are listed, and the amounts of the liquid centrifuged from each respective nonfrozen and unstored cultured fluid/flour dough sample are indicated in Fig. 3. The greatest difference in the amount of centrifuged liquid between nonfrozen and unstored cultured fluid/flour dough and bread dough with the same combinations of components was observed when yeast and salt were combined (yeast+salt) (Figs. 2 and 3). In cultured fluid/flour dough, the amount of centrifuged liquid from nonfrozen and unstored cultured fluid/flour dough was ≈36% compared with nonfrozen and unstored complete cultured fluid/flour dough (100%) (Fig. 3), while in nonfrozen and unstored bread dough made with yeast and salt, it was ≈85% compared with nonfrozen and unstored complete bread dough (100%) (Fig. 2). Sugar was omitted in both cases, so it was suggested that yeast could use wheat carbohydrates in bread dough where the yeast was visible, but could not use yeast in the cultured fluid/flour dough because wheat flour was omitted in incubation time. These observations suggested that the products of fermentation may be key materials in increasing the amount of centrifuged liquid. When they were frozen-and-thawed after one day of storage, each dough sample showed almost the same trends. Yeast+sugar cultured fluid/flour dough increased remarkably by freezing and thawing. Salt alone or in combination with sugar in the cultured fluid/flour doughs showed higher levels of centrifuged liquid than the other combinations examined for both nonfrozen and frozen-and-thawed doughs. The amounts of protein and carbohydrate in the centrifuged liquid from each combined cultured fluid/flour dough, with or without freezing-and-thawing were determined and compared with the amount of centrifuged liquid (Table V). Results of these correlation coefficients (*r*) indicates that the amount of protein and carbohydrate in each cultured fluid/flour dough is highly related to the amount of the centrifuged liquid, and suggests that there are also no higher or lower extraction results of protein and carbohydrate in each centrifuged liquid.

Effects of Fermented Products and Salt in Cultured Fluid/Flour Dough on Centrifuged Liquid

It has not yet been determined whether the liquid centrifuged from cultured fluid/flour dough was increased by the presence of increased yeast metabolic products. So the relationship between incubation time (0, 70, 140, 210, and 280 min) of cultured fluid and the amount of the centrifuged liquid was investigated. In Fig. 4B, the amount of centrifuged liquid increased linearly with incubation time of cultured fluid, which suggested that the increase in free water was due to increases in the amounts of fermented products made by the yeast. Furthermore, the effect of level of salt addition on the centrifuged liquid was also observed (Fig. 4A, C, and D). When salt level was increased twofold in cultured fluid (Fig. 4A), the amount of centrifuged liquid was increased to a greater extent than that from normal level salt cultured fluid/flour dough (Fig. 4B). However, the centrifuged liquid increased even when incubation time was zero, which demonstrated an increasing effect of salt alone. When salt was omitted from the culture fluid (Fig. 4D), no increase in amount of centrifuged liquid was observed for any level of cultured fluid incubation time. However, when salt was added to the cultured fluid at the same time as mixing with wheat flour (Fig. 4C), the amount of centrifuged liquid from the dough was very similar to that from doughs made with normal cultured fluid as indicated in Fig. 4B. These observations indicated that fermented products are present after each cultured fluid incubation time without salt and have the ability to increase the amount of liquid centrifuged from doughs. However, no centrifuged liquid was observed without the presence of salt.

Effect of Salt in Cultured Fluid/Flour Dough on Centrifuged Liquid

The relationship between salt and centrifuged liquid was examined in more detail. As shown in Fig. 5, the addition of salt to cultured fluid at the same time as mixing with wheat flour increased

TABLE IV
Correlation Coefficients Between the Amount of Centrifuged Liquid, and Bread Height, and Specific Volume

	Bread Height	Specific Volume
Kameria	–0.828	–0.855
Red Knight	–0.963	–0.947
Alps	–0.938	–0.662
Midoriuzumaki	–0.912	–0.752
Horoshiri	–0.880	–0.939
Kitakami	–0.926	–0.950
Nanbujigona	–0.885	–0.854

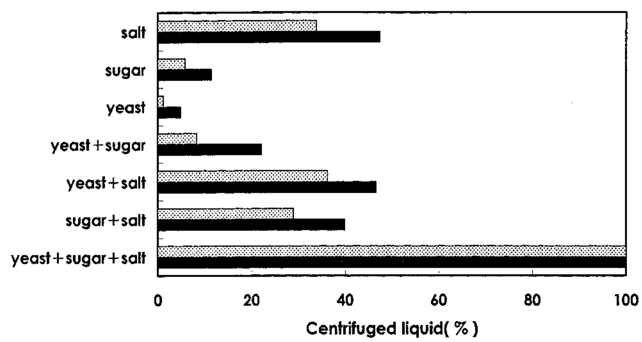

Fig. 3. Effects of cultured fluid/flour dough components and their combinations on the amount of centrifuged liquid. Nonfrozen and unstored (□); after one day of frozen storage (■). Kameria wheat flour was used.

TABLE VI
Correlation Coefficients Between the Amount of Centrifuged Liquid (mL) and Protein (mg) and Carbohydrate (mg) from Salt-Added Cultured Fluid/Flour Dough

	Protein (mg)	Carbohydrate (mg)
Liquid (mL)	0.9720	0.9597

TABLE V
Correlation Coefficients Between the Amount of Centrifuged Liquid (mL) and Protein (Prot, mg) and Carbohydrate (Carb, mg) from Various Combined Bread Doughs and Cultured Fluid/Flour Doughs

	Bread Dough				Cultured Fluid/Four Dough			
	Nonfrozen		Frozen		Nonfrozen		Frozen	
	Prot (mg)	Carb (mg)	Pro (mg)	Carb (mg)	Prot (mg)	Carb (mg)	Pro (mg)	Carb (mg)
Bread dough								
Nonfrozen liquid (mL)	0.9769	0.9977						
Frozen liquid (mL)			0.9407	0.9739				
Cultured fluid/flour dough								
Nonfrozen liquid (mL)					0.9673	0.9103		
Frozen liquid (mL)							0.9869	0.9177

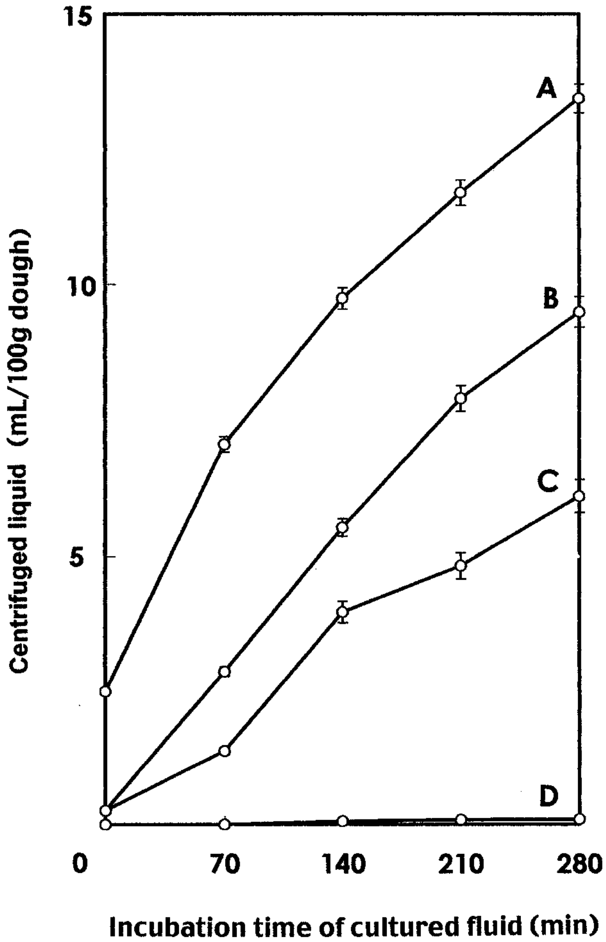

Fig. 4. Effects of fermented products and salt in cultured fluid/flour dough on the amount of centrifuged liquid. **A,** 2.90 g of salt added before incubation; **B,** 1.45 g of salt added before incubation; **C,** 1.45 g of salt added after incubation; **D,** no salt added. Kameria wheat flour was used.

the amount of centrifuged liquid to 3.63 g/145 g of wheat flour, while further addition was associated with a gradual decrease. These observations suggested that when salt was added to the doughs, loosely bound water was moved from flour to salt, while further addition of salt may change the nature of the flour and salt-bound water was reabsorbed into the wheat flour. Addition of 29 g of salt resulted in complete loss of liquid release. The amount of protein and carbohydrate in centrifuged liquid from each salt adding culture fluid/flour dough were determined, and compared with the amount of centrifuged liquid. The correlation coefficients (*r*) (Table VI) indicates high value between them, which indicates that the concentrations of the extracted protein and carbohydrate did not change by salt addition.

CONCLUSIONS

The breadmaking properties (bread height, mm, and specific volume, cm^3/g) of breads baked from frozen-and-thawed doughs were markedly deteriorated. Centrifuged liquid which was observed on the frozen-and-thawed dough samples could be quantitatively analyzed, and high inverse correlations between the breadmaking properties and centrifuged liquid were present. There is evidence that the amount of centrifuged liquid is highly related to both salt and fermented products in the bread dough.

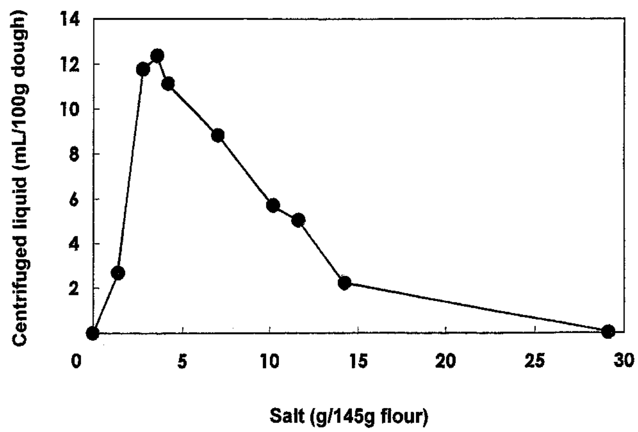

Fig. 5. Effects of salt in cultured fluid/flour dough on the amount of centrifuged liquid. Salt was added with wheat flour after cultured fluid incubation. Kameria wheat flour was used.

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