Effects of Heating Conditions on Physicochemical Properties of Skim Milk Powder during Production Process

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To investigate the effects of production-related heat treatments on physicochemical properties of skim milk powder, four kinds of skim milk powder were prepared under different heating conditions. The degree of heating was expressed as the integrated caloric content added to the powders. The four skim milk powders were dispersed in water, and the physicochemical properties of them were compared. Solution turbidity increased with an increase in integrated caloric content, but the protein content in the supernatant decreased; the latter characteristic indicated that severe heating conditions resulted in aggregate formation of denatured whey protein molecules. The content of sulfhydryl groups and surface hydrophobicity of skim milk solutions were closely related to the heating conditions in the production process. SDS-PAGE analysis revealed that β -lactoglobulin and α -lactalbumin were main proteins involved in aggregate formation. The powder that had been treated with the most severe heating condition was found to be the most suitable for making bread.

Keywords: skim milk powder, heat treatment, protein denaturation, surface hydrophobicity, bread, loaf volume, protein aggregation

Introduction

Skim milk powder is used as an ingredient in many food products, including cheese, yogurt, milk beverages, bread, and cakes (Mulvihill and Ennis, 2003). Skim milk powder imparts on food products not only a milky flavor, but also desirable physical properties, thereby satisfying preference requirements. The nutritional value is also expected to be increased through the addition of skim milk powder, because it is rich in nutrients such as lactose, proteins, and calcium.

Chemical and physical changes in skim milk powder components are necessary during the production process. In particular, heat treatment, which is essential for inactivat-

ing microorganisms, tends to induce protein denaturation. Whey proteins in milk have been found to be more sensitive to heat denaturation than caseins (Kruif, 1999; Erdam and Yuksel, 2005). The main components of whey proteins, β -lactoglobulin and α -lactalbumin are globular proteins with rigid conformations (Erdam and Yuksel, 2005). Heating induces the unfolding of these rigid conformations, thereby facilitating the aggregation of denatured molecules. Furthermore, the presence of a free sulfhydryl group and disulfide bonds in the β -lactoglobulin molecule is preferable for polymer formation via intermolecular disulfide linkage between β -lactoglobulin molecules or between β -lactoglobulin and other proteins (Cho *et al.*, 2003; Dalgleish, 1990; Dannenberg and Kessler, 1988b; Haque and Kinsella, 1988; Haque *et al.*, 1987; Jang and Swaisgood, 1990; Singh and Fox, 1985; Smits and Brouwershaven, 1980). Therefore, β -lactoglobulin and α -lactalbumin denaturation in milk and various model systems has been widely studied (Corredig and Dalgleish,

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1999; Dannenberg and Kessler, 1988a; Parris *et al.*, 1991). Numerous reports have found that heating milk to temperatures $> 60^{\circ}\text{C}$ leads to whey protein denaturation, thereby facilitating their interaction with each other or with κ -casein to form aggregates (Anema and Li, 2000, 2003a, 2003b; Corredig and Dalgleish, 1999; Dannenberg and Kessler, 1988a; Guyomarc'h *et al.*, 2003; Pearse *et al.*, 1985; Smits and Brouwershaven, 1980; Vasbinder *et al.*, 2003; Vasbinder and Kruif, 2003).

Some reports have also found that protein denaturation and protein aggregate formation in skim milk powder can be achieved by heating during the production process. Anema and Li (2003a) observed that casein micelle size changed when skim milk was subjected to heat treatment; they speculated that this change was due to the association between whey proteins and casein micelles. Erdem and Yuksel (2005) confirmed the formation of a complex between heat-denatured whey proteins and casein micelles in skim milk. However, no systematic study has investigated the relationship between the degree of heat treatment and the physicochemical changes of proteins in skim milk powder.

In this study, we compared the physicochemical properties of four skim milk powders that were subjected to heat treatment under different conditions. Both heating temperature and time were strictly controlled, and the extent of heating was expressed as the amount of integrated calories added to the skim milk powders. Turbidity and solubility were measured for comparison of the colloidal properties of the skim milk powders. The denaturation degree was assessed by determining the surface hydrophobicity and content of the sulfhydryl groups. The suitability of the skim milk powders as an ingredient in bread was also tested.

Materials and Methods

Materials and chemicals The four skim milk powders

were supplied by Snow Brand Milk Products Co., Ltd. (Tokyo, Japan), and categorized into four types super-high heat (SH), high heat (H), normal heat (N), and low heat (L), according to the heat treatment conditions applied. The degree of heating was expressed as the amount of integrated calories added to skim milk during the production process. The heating condition for type N skim milk is the factory standard condition and its integrated caloric content was assumed to be 1 (Table 1). The amounts of integrated calories added to the other types were expressed as the relative content to that of the N type. Whey protein nitrogen index values were also shown in Table 1. Flour, dry yeast, sugar, and salt were purchased from a local market. Reagent-grade chemicals were purchased from Nakarai Tesque Inc. (Kyoto, Japan).

ζ -potential measurements Skim milk solutions were diluted with water to obtain a final solid concentration of 0.2%. Diluted solutions were then injected into the measurement chamber of an ELS-Z1 particle electrophoresis instrument (Otsuka Co., Tokyo, Japan), and ζ -potential was determined.

Determination of turbidity Skim milk solutions (0.1% powder in distilled water) were shaken overnight at room temperature; absorbance was then measured at OD 550 nm, using a UV spectrometer (Shimadzu Corp., Kyoto, Japan).

Measurement of protein content in supernatant of skim milk solutions Skim milk solutions (0.1% powder in distilled water) were shaken overnight at 20°C . Each solution (1 mL in a 1.5-mL Eppendorf tube) was centrifuged at $103,000 \times g$ for 20 min at 20°C , and the obtained supernatant was diluted twice with distilled water. The protein content in the supernatant was determined using the method of Lowry *et al.* (1951).

Measurement of surface hydrophobicity The surface hydrophobicity of the skim milk solutions (0.2% powder in distilled water) was estimated using a 1-anilino-8-naphthalenesulfonic acid (ANS)-binding fluorometric assay, accord-

Table 1. Relative content of integrated calories and whey protein nitrogen index of skim milk powder.

Type of skim milk powder	Relative content of integrated calories	Whey protein nitrogen index (%)
Super high heat (SH)	4.4	100.0 ± 5.8
High heat (H)	3.2	39.0 ± 5.6
Normal heat (N)	1.0	13.6 ± 1.2
Low heat (L)	0.4	11.9 ± 1.0

Type N skim milk powder was prepared under the standard factory condition. The amount of integrated calories added to type N was assumed to be 1.0. The amount of integrated calories added to the other skim milk powders were expressed as the relative content to that of type N.

* Whey protein nitrogen index was determined according to the method of Kuramoto, *et al.* (1959). Whey protein nitrogen index in SH was regarded as 100%.

Each value represent the mean $\pm$ SD (n=3, minimum).

ing to Kato and Nakai (1980). Each skim milk solution was diluted 50, 83.5, 125, and 250 times with distilled water, and then incubated at 30°C for 10 min; ANS was then added to the solution to obtain a final concentration of 0.13 μ M. The mixture was incubated at 30°C for another 70 s, and the fluorescence of the mixture was immediately measured with a fluorescence spectrophotometer (F-3000; Hitachi High-Tech-nologies Corp., Tokyo, Japan). The excitation and emission wavelengths were 390 and 470 nm, respectively. The initial slope of the fluorescence intensity versus protein concentration plot was used as an index of surface hydrophobicity.

Measurement of SH content The amount of SH content in each skim milk solution (10% powder in distilled water) was determined using the method of Ellman (1959).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis A 1.0-mL aliquot of 0.125 M Tris-HCl buffer (pH 6.8) containing 4% SDS and 20% glycerol was added to an equivalent amount of each skim milk solution (1% powder in distilled water). After agitation, 2-mercaptoethanol (2-ME) was added to the mixture to obtain a final concentration of 10% (v/v); the mixture was then heated to 100°C for 3 min. SDS-PAGE was carried out on a slab gel (Laemmli, 1971), and protein bands were stained with Coomassie Brilliant Blue R-250.

Baking procedure Water absorption of each blended flour was determined with a farinograph, using 50.0 g flour (AACC Method 54-21, 1995). Flour (263.2 g), skim milk powder (16.8 g), dry yeast (9.0 g), sugar (15.0 g), salt (3.0 g), and water (estimated with a farinograph at 500 BU) were mixed in a National Automatic Bread Maker (SD-BT3; Matsushita Electric Ind. Co., Ltd., Japan). For the control, skim milk powder was displaced by the same weight of flour. For the first proof, the dough was maintained at 30°C with the following time profile: 15 min for the first mixing, 50 min as rest, 5 min for the second mixing, and 70 min for fermentation. Mixing (time and temperature) was carefully controlled by the bread maker. The dough was removed from the bread

maker and divided into 120-g portions, rounded, molded, and placed in baking pans (AACC Method 10-10A, 1995). The dough was further proofed for 22 min at 38°C and baked at 210°C for 30 min in a drying oven (2-2050; Isuzu Seisakusho, Co., Ltd., Japan). After baking, the bread was removed from the pan and cooled for 1 h at room temperature.

Measurement of bread loaf volume After cooling for 1 h at room temperature, the volume of each loaf of bread was measured by the rapeseed displacement method (Goesaert *et al.*, 2008).

Statistical analysis Unless otherwise stated, each result is presented as the mean $\pm$ standard deviation (SD) of at least triplicate samples. Significant difference was evaluated using a Student's *t*-test.

Results and Discussion

Determination of turbidity The turbidity of 0.1% skim milk solutions containing types SH, H, N, and L were 0.123 ± 0.007 ($n = 6$), 0.108 ± 0.010 ($n = 6$), 0.077 ± 0.003 ($n = 6$), and 0.066 ± 0.005 ($n = 6$), respectively (Table 2). There were significant differences among all samples ($p < 0.05$). A close relationship between turbidity (Table 2) and integrated caloric content or whey protein nitrogen indexes (Table 1) was found, i.e., the greater the amount of integrated calories, the higher the solution turbidity. Proteins — particularly whey proteins in milk — are very sensitive to heat, and readily form aggregates of denatured molecules. Such aggregates are not completely solubilized in aqueous media without some reagent such as urea, SDS, or 2-ME. Thus increased turbidity of the type SH, H, and N solutions should reflect the presence of larger aggregates that were induced by heating during the production process.

Measurement of protein content in supernatant of skim milk solution As protein aggregates are likely to be formed by heating during the production process of skim milk powder, the amount of native protein molecules should decrease via this thermal stress. To assess the content of native pro-

Table 2. Physicochemical properties of the solutions containing four types of skim milk powder.

	SH	H	N	L
Turbidity (OD550nm)	0.123 ± 0.007^a	0.108 ± 0.010^b	0.077 ± 0.003^c	0.066 ± 0.005^d
Amount of soluble protein (mg/mL)	0.220 ± 0.006^a	$0.237 \pm 0.011^{a,b}$	$0.251 \pm 0.012^{b,c}$	0.283 ± 0.022^c
Content of sulfhydryl group (μ moles/mL)	0.053 ± 0.002^a	0.073 ± 0.006^b	0.085 ± 0.011^b	0.133 ± 0.012^c
Surface hydrophobicity	775.13 ± 6.77^a	764.23 ± 7.07^a	787.51 ± 12.9^a	692.22 ± 28.99^b

Each value represent the mean $\pm$ SD. They were measured 3 times, except for turbidity ($n=6$). Different letters (a-d) in the same column indicate a significant difference of $p < 0.05$.

tein molecules in the skim milk powders, the solutions were centrifuged and the resulting supernatants were analyzed. The protein content in the supernatants of 0.1% skim milk solutions containing types SH, H, N, and L were 0.220 ± 0.006 ($n = 3$), 0.237 ± 0.011 ($n = 3$), 0.251 ± 0.012 ($n = 3$), and 0.283 ± 0.022 mg/mL ($n = 3$), respectively (Table 2). A relationship between protein content (Table 2) and integrated caloric content or whey protein nitrogen indexes (Table 1) was found, i.e., the lower the amount of protein found in the solution supernatant, the more the integrated caloric content of the skim milk powder suffered. This indicates that protein denaturation progressed largely with increasing thermal stress.

Measurement of sulfhydryl group content Major proteins in skim milk powder, such as κ -casein and β -lactoglobulin, comprise sulfhydryl groups. Sulfhydryl groups are very susceptible to oxidation, giving rise to products like cystenic acid. Heating may accelerate the oxidation rate of these groups, especially following protein denaturation by heating. Globular β -lactoglobulin contains one embedded sulfhydryl group heating induces the unfolding of the globular conformation, thereby exposing this group. This exposed sulfhydryl group is potentially more chemically reactive — i.e., more liable to undergo various reactions such as oxidation and sulfhydryl-disulfide interchange reactions. Therefore, the content of the sulfhydryl groups may serve as an index of protein denaturation in skim milk powder.

Therefore, the free sulfhydryl group is greatly correlated with the functional properties of skim milk powder, including those that facilitate curd formation, emulsification, and bread-making. The content of the sulfhydryl groups in 10% skim milk solutions containing types SH, H, N, and L were 0.053 ± 0.002 ($n = 3$), 0.073 ± 0.006 ($n = 3$), 0.085 ± 0.011 ($n = 3$), and 0.133 ± 0.012 ($n = 3$) μ moles/mL, respectively (Table 2), there was a significant difference between the values of types SH and L ($p < 0.01$).

There was a relationship between the sulfhydryl group content (Table 2) and integrated caloric content or whey protein nitrogen indexes (Table 1), i.e., the fewer the sulfhydryl groups, the higher the integrated caloric content of the skim milk powder. As revealed by the results of protein solubility (Table 2) with respect to denaturation state (in decreasing order: types SH, H, N, and L), a higher integrated caloric content should result in more exposed sulfhydryl groups on the molecular surface, concomitant with protein denaturation. In turn, the probability of oxidation damage to the sulfhydryl groups is increased.

As the resulting aggregates as determined via turbidity (Table 2) were most likely formed via disulfide and non-covalent bonding, which can be confirmed by SDS-PAGE

analysis, the sulfhydryl groups exposed by thermal treatment might be partially involved in an inter-molecular sulfhydryl-disulfide reaction. The total content of the sulfhydryl groups does not change before and after the sulfhydryl-disulfide reaction. Therefore, the oxidation process that directly results in a disulfide bond from two cysteine groups may also contribute to the protein aggregate formation in skim milk powder.

Measurement of surface hydrophobicity Surface hydrophobicity is a good index of protein denaturation, especially for globular proteins. Embedded hydrophobic areas are exposed to the protein molecular surface by various treatments including heating results in an increase in surface hydrophobicity. Since ANS is a hydrophobic probe that preferentially binds to the hydrophobic area at the protein molecular surface to produce fluorescence, it is often used to measure the increase in protein surface hydrophobicity following heating. Therefore, the hydrophobicity of the four skim milk solutions was determined by ANS binding methods and compared with one another.

The surface hydrophobicity of skim milk solutions of types SH, H, N, and L were 775.13 ± 6.77 ($n = 3$), 764.23 ± 7.07 ($n = 3$), 787.51 ± 12.95 ($n = 3$), and 692.22 ± 28.99 ($n = 3$), respectively (Table 2). Although there were no significant differences among types SH, H, and N, the hydrophobicity for type L was significantly lower than that of the other types ($p < 0.01$). This result suggests that surface hydrophobicity was decreased by lowering the integrated caloric content from normal levels during the production process (see Table 1), but was unaffected by increasing it.

Solubility differed among the four skim milk powders, and is known to have a close relationship with surface hydrophobicity of proteins (Hayakawa and Nakai, 1985). Contrary to this ANS hydrophobicity showed a good relationship with the insolubility of proteins ($r = 0.592$, $p < 0.001$). According to our finding, solubility should decrease with an increase in the amount of integrated calories added to the skim milk powders; although, the surface hydrophobicity was similar for skim milk solutions of types SH, H, and N.

To investigate another important factor affecting solubility, the charge frequency of protein molecules, the ζ -potential of 0.2% skim milk solutions was measured. There were no significant differences between types SH, H, N, and L skim milk solutions (-18.2 ± 1.2 , -17.6 ± 1.1 , -17.7 ± 1.1 , and -18.0 ± 0.7 , respectively), indicating that charge frequency does not contribute to differences in solubility.

Although heating normally induces an increase in the surface hydrophobicity of proteins, further heating sometimes induces a decrease. This phenomenon is thought to be due to the interaction between the hydrophobic area exposed by

heating and the resultant shielding of the exposed area from the hydrophobic probe (Nakai *et al.*, 1995). The heating condition for type N skim milk solution appears to be sufficient to expose the hydrophobic area to the protein molecular surface and further heating only induces aggregation of denatured protein molecules. Although not significantly different, the slight decrease in the hydrophobicity of types SH and H solutions compared to type N (Table 2) supports this speculation.

Surface hydrophobicity appears to be specific to globular proteins. In this study, increases in surface hydrophobicity were due to conformational changes in globular proteins from whey fractions, such as β -lactoglobulin, α -lactalbumin and bovine serum albumin. However, it is also likely that denatured globular proteins could bind to casein micelles, thereby facilitating aggregate formation of casein micelles and whey proteins. In this case, the exposed surface area resulting from further heating may interact with casein micelles, and thus not contribute to an increase in surface hydrophobicity.

SDS-PAGE analysis To determine which protein is involved in aggregate formation, the four skim milk powders were subjected to SDS-PAGE. To identify each band, major proteins such as α -casein, β -casein, κ -casein, β -lactoglobulin and α -lactalbumin obtained from Sigma Chemical Co. (St Louis, MO, USA) were applied for comparison. Electrophoresis was performed in the absence (Fig. 1, lanes A-I) and presence (lanes J-R) of 2-ME. The purchased κ -casein was shown to be polymerized via a disulfide bond during the manufacturing process, as a trace band was present in the absence of 2-ME (lane E) and a major band in the presence of

2-ME (lane N).

In the absence of 2-ME, huge substances unable to enter the stacking gel were observed for all four skim milk powders (lanes A-D). The intensity of these aggregates was determined densitometrically to be 113.8 ± 8.9 ($n = 4$), 116.4 ± 11.4 ($n = 4$), 109.1 ± 12.4 ($n = 4$) and 70.2 ± 20.9 ($n = 4$), for types SH, H, N, and L, respectively. These aggregates almost completely disappeared in the presence of 2-ME (lanes J-M), suggesting that they form via disulfide linkage.

In the absence of 2-ME, the intensity of the α -, β -, and κ -casein bands remained relatively unchanged for all samples (lanes A-D). The intensity of the α / β -casein band (these bands could not be determined separately by the densitometer) and the κ -casein band was approximately 200 and 50, respectively. In contrast a significant difference in the amount of β -lactoglobulin and α -lactalbumin was seen between the four skim milk powders (lanes A-D). These results

Table 3. Amounts of α -lactalbumin and β -lactoglobulin in the supernatant of skim milk solutions.

	α -Lactalbumin	β -Lactoglobulin
SH	$32.6 \pm 12.8^{a,b}$	$37.4 \pm 7.9^{a,b}$
H	19.4 ± 6.1^a	31.6 ± 2.7^a
N	33.6 ± 6.0^b	48.5 ± 4.0^b
L	90.0 ± 2.5^c	66.1 ± 6.4^c

Amounts of α -lactoalbumin and β -lactoglobulin were densitometrically determined from the results of SDS-PAGE (Fig. 1 lane A~D). Each value is represented as the mean $\pm$ SD ($n=3$). Different letters in the same column indicate a significant difference of $p < 0.05$.

Fig. 1. SDS-PAGE of proteins in skim milk powder.

Proteins in all four skim milk powders were analyzed by SDS-PAGE. SH, H, N and L gave lanes A, B, C and D. To identify each band, major milk proteins, including κ -casein (lane E), α -casein (lane F), β -casein (lane G), β -lactoglobulin (lane H), and α -lactalbumin (lane I), were analyzed. Lanes J-R, 2-ME was added to A-I to final concentration of 10%, respectively.

are shown in Table 3. For both proteins, the amount was the largest for type L skim milk powder, followed by type N. Although the amounts of these proteins were higher for type SH than for type H, comparison of the results (Tables 1 and 3) suggests that the decreasing the integrated caloric content retains α -lactalbumin and β -lactoglobulin. Thus decreases in α -lactalbumin and β -lactoglobulin may be involved in aggregates via disulfide linkage, because these proteins were restored by the addition of 2-ME (lanes J-M).

The results of SDS-PAGE showed the close relationship between aggregate formation via disulfide linkage and heat denaturation of skim milk powders. Even for type L skim milk powder, with the mildest heat treatment aggregates were present (lane D), only dissociating in the presence of 2-ME (lane M), indicating that the heat process induced protein denaturation and subsequent protein aggregation via disulfide bonding (as well as noncovalent bonding). However, the degree of aggregate formation changed according to the conditions of the production process (Table 1). Large aggregates formed in the case of type N, H, and SH skim milk powders, and the intensity of α -lactalbumin and β -lactoglobulin was decreased by an increase in integrated caloric content. The increase in integrated caloric content during the production process therefore resulted in aggregate formation mainly comprising α -lactalbumin and β -lactoglobulin, concomitant with decreases in these proteins as their monomeric form in skim milk powders.

β -Lactoglobulin molecules are readily polymerize via intermolecular disulfide linkages with each other or other protein molecules (Cho *et al.*, 2003; Dalgleish, 1990; Dannenberg and Kessler, 1988b; Haque and Kinsella, 1988; Haque *et al.*, 1987; Jang and Swaisgood, 1990; Singh and Fox, 1985; Smits and Brouwershaven, 1980). Embedded sulfhydryl groups are exposed to the molecular surface at heat treatments $> 60^{\circ}\text{C}$; it also becomes more susceptible to

a sulfhydryl-disulfide interchange and/or oxidation reaction (Haque and Kinsella, 1998; Smits and van Brouwershaven, 1980; Singh and Fox, 1985; Dannenberg and Kessler, 1988a; Dalgleish, 1990; Jang and Swaisgood 1990; Haque *et al.*, 1987; Cho *et al.*, 2003). Much evidence suggests that β -lactoglobulin after heat denaturation can interact with κ -casein via disulfide and noncovalent bonding, thereby also affecting the casein micelle size in milk (Anema and Li, 2003a). In this study, β -lactoglobulin molecules denatured by heat treatment likely play a major role in aggregate formation in skim milk powders during the production process. That is an exposed sulfhydryl group in denatured β -lactoglobulin may trigger polymerization via intermolecular disulfide linkage with another β -lactoglobulin or κ -casein, causing aggregate formation.

The amount of both β -lactoglobulin and α -lactalbumin decreased in the SDS-PAGE patterns of skim milk powders, especially in types SH, H, and N (Fig. 1, lanes A-C). Most α -lactalbumin molecules recover their initial native structure after thermal treatment (Bernal and Jelen, 1985; Barel *et al.*, 1972). Because of such thermal reversibility, α -lactalbumin is thought to be more heat resistant than β -lactoglobulin (Ruegg *et al.*, 1977). α -Lactalbumin has four intramolecular disulfide bonds, but no sulfhydryl groups. Therefore, a sulfhydryl-disulfide interchange reaction does not occur when the α -lactalbumin molecule alone is heated; this characteristic contributes to the thermal reversibility of this protein. The reason for the decrease in α -lactalbumin in types SH, H, and N on SDS-PAGE patterns (lanes A-C) may be due to the intermolecular interaction of α -lactalbumin and β -lactoglobulin. The exposed and more reactive sulfhydryl group in denatured β -lactoglobulin molecules could attack disulfide bonds in α -lactalbumin, thereby triggering intermolecular polymerization between the two proteins. Such polymerization has been reported in a mixed system of

Fig. 2. Appearance of cross sections of bread loaves containing 6% skim milk powder.

Loaf volumes of bread made from dough containing types SH (B), H (C), N (D), and L (E) skim milk powder compared to that of bread made from dough containing no skim milk powder (A).

Table 4. Loaf volume of breads made without or with one of four types of skim milk powder.

Type	No skim milk powder	SH	H	N	L
Loaf volume (cm ³)	318.6 ± 4.7	299.4 ± 12.6 ^a	284.4 ± 11.2 ^a	270.1 ± 14.3 ^{a,b}	257.6 ± 4.0 ^b

Each value is represented as the mean ± SD (n=3). Different letters indicate a significant difference of $p < 0.05$.

α -lactalbumin and β -lactoglobulin adsorbed at the oil-water interface (Dickinson and Matsumura, 1991); following interfacial denaturation, β -lactoglobulin could be polymerized with α -lactalbumin at the oil-water interface of emulsions.

Measurement of bread loaf volume In bakeries, use of whey protein products generally reduces loaf volume (Mulvihill and Ennis 2003). Milk proteins do not have properties similar to those of wheat gluten and therefore cannot replace the wheat protein in large part in bakery products; however, they could be used as nutritional supplements and for functional effects in cereal-based products. The effects of adding skim milk powder to dough for bread-making was investigated. Examination of the cross sections of breads (Fig. 2) demonstrated that bread without added skim milk powder rose the most, and such rise decreased in bread with added skim milk powder relative to the extent of heating (decreasing in order of SH, H, N, and L).

The loaf volumes of breads made with skim milk powder of types SH, H, N, and L were 299.4 ± 12.6 (n = 3), 284.4 ± 11.2 (n = 3), 270.1 ± 14.3 (n = 3), and 257.6 ± 4.0 (n = 3) cm³, respectively (Table 4). There was a significant difference between volumes for types SH and L ($p < 0.01$), and the loaf volume was inversely proportional to the integrated caloric content added to the skim milk powder (Table 1). This difference in loaf volume may be attributed to differences in the content of disulfide groups and surface hydrophobicity of the skim milk powders. In the dough-making process, hydrophobic interactions and sulfhydryl-disulfide interchange reactions play important roles in the formation of thread-like polymers of gluten (Shewry and Tatham, 1997). These polymers are thought to interact with each other through hydrogen bonding, additional hydrophobic interactions, and sulfhydryl-disulfide interchanges, resulting in physical entanglements that are created during the mixing process to form a continuous sheet-like film of protein that has the unique ability to retain fermentation gases (Mecham *et al.*, 1963; Tsen, 1969; Bloksma, 1975).

When cysteine or cystine (comprising the disulfide bridge) glutathione is added to gluten, the result is the same: softer gluten is formed (Kieffer *et al.*, 1983). The softening effect is thought to be due to a reduction in disulfide bonds. The changes in stress-relaxation behavior and stickiness of gluten treated with cysteine could be the result of a decreased

number of disulfide linkages in the gluten network (Mita and Bohlin, 1983).

Taken together the decrease in loaf volume due to the addition of type L skim milk powder to dough (Table 4) may be due to the larger amount of sulfhydryl groups in this type. The sulfhydryl groups in skim milk powder act as reduction reagents and promote the cleavage of cross linkages in dough. In contrast, the reduction in loaf volume was larger for dough containing type SH skim milk powder (Fig. 2B and Table 4), partially because of the low content of sulfhydryl groups in this type. Increasing the sulfhydryl content leads to the larger surface hydrophobicity that contributes to the loaf volume. It is also likely that proteases in L type skim milk were not inactivated by mild heating conditions during production process thereby attacking gluten and decreasing the loaf volume of the breads.

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