Heat stability of reconstituted, protein-standardized skim milk powders

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ABSTRACT

We determined the effects of standardization material, protein content, and pH on the heat stability of reconstituted milk made from low-heat (LH) and medium-heat (MH) nonfat dry milk (NDM). Low-heat and MH NDM were standardized downward from 35.5% to 34, 32, and 30% protein by adding either edible lactose powder (ELP) or permeate powder (PP) from skim milk ultrafiltration. These powders were called standardized skim milk powders (SSMP). The LH and MH NDM and SSMP were reconstituted to 9% total solids. Furthermore, subsamples of reconstituted NDM and SSMP samples were set aside to measure heat stability at native (unadjusted) pH, and the rest were adjusted to pH 6.3 to 7.0. Heat stability is defined as heat coagulation time at 140°C of the reconstituted LH or MH NDM and SSMP samples. The entire experiment was replicated 3 times at unadjusted pH values and 2 times at adjusted pH values. At an unadjusted pH, powder type, standardization material, and protein content influenced the heat stability of the samples. Heat stability for reconstituted LH NDM and SSMP was higher than reconstituted MH NDM and SSMP. Generally, decreased heat stability was observed in reconstituted LH or MH SSMP as protein content was decreased by standardization. However, adding ELP to MH SSMP did not significantly change its heat stability. When pH was adjusted to values between 6.3 and 7.0, powder type, standardization material, and pH had a significant effect on heat stability, whereas protein content did not. Maximum heat stability was noted at pH 6.7 for both reconstituted LH NDM and SSMP samples, and at pH 6.6 for both reconstituted MH NDM and SSMP samples. Furthermore, for samples with adjusted pH, higher heat stability was observed for reconstituted LH SSMP containing PP compared with reconstituted milk from LH SSMP containing ELP. However, no statistical difference was observed in the heat stability of reconstituted milk from MH NDM and MH SSMP

samples. We conclude that powder type (LH or MH) and effect of standardization material (ELP or PP) can help explain differences in heat stability. The difference in the heat stability of powder type may be associated with the difference in the pH of maximum heat stability and compositional differences in the standardization material (ELP or PP).

Key words: heat stability, nonfat dry milk, protein standardization

INTRODUCTION

Milk and milk products are heat-treated to increase shelf life and ensure their safety for human consumption. Therefore, it is important that milk and milk powders used in various food applications be heat stable. Heat stability is defined as the ability of milk to withstand high temperatures without flocculation, gelation, or protein separation (Fox, 1982).

Heat stability of milk is mainly a function of its milk protein stability (Fox and Morrissey, 1977; O'Connell and Fox, 2003; Singh, 2004) and may be affected by protein content. Natural fluctuations in milk protein content (Harland et al., 1955; Ng-Kwai-Hang et al., 1987; Heck et al., 2009) have been reported throughout the seasons. Thus, protein standardization can be used to achieve more consistent protein content in dairy products (Jensen, 1988; Rattray and Jelen, 1996; Hardham, 1998; Sikand et al., 2008) and perhaps improve heat stability.

Milk protein standardization refers to the adjustment of protein content by the addition of small quantities of either retentate or permeate obtained from milk ultrafiltration (UF; Kiesecker and Healey, 1996). Per Codex Alimentarius 207 (Codex Alimentarius, 1999), milk-derived permeate or lactose can be used to standardize protein content in the manufacturing of skim milk powder (SMP) for various food applications. Milk-derived permeate contains minerals, nonprotein nitrogen, and lactose, whereas lactose contains 99% lactose and trace minerals. Green et al. (1984) found that low-molecular-weight components comprising nonprotein nitrogen appeared to be accounted for primarily by urea, AA, and ammonia. A positive cor-

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relation between nonprotein nitrogen and heat stability was observed in bovine milk (Robertson and Dixon, 1969) and goat milk (Mukherjee, 1993). Higher urea levels have been shown to increase the heat stability of milk (Robertson and Dixon, 1969; Muir and Sweetsur, 1976), which suggests that nonprotein nitrogen fraction plays a role in milk heat stability. Previous studies (Ratnay and Jelen, 1996) using UF milk permeate to standardize milk protein content demonstrated high heat stability. Permeate is a coproduct of milk or whey protein concentrate manufacture, and it is possible that nonprotein nitrogen fraction in permeate used for milk protein standardization may improve heat stability.

Throughout the years, few studies have been conducted regarding milk protein standardization on heat stability. Newstead (1977) reported no influence of protein concentration on the heat stability of evaporated milk; however, during the majority of a season, enhanced evaporated milk heat stability was observed when permeate with low salt content was used for standardization. Therefore, it has been suggested that salt concentration appeared to play an important role in evaporated milk heat stability. Ratnay and Jelen (1996) observed higher heat stability for skim milk protein content standardized with permeate from UF milk or permeate from UF sweet whey generated from skim milk treated with chymosin compared with unstandardized milk in the pH range of 6.4 to 7.1. The increased heat stability of protein-standardized milk was attributed to low calcium content. Protein-standardized skim milk with UF permeate from acid whey was observed to have decreased heat stability. This decreased heat stability was attributed to an increased ratio of soluble calcium to protein when compared with the UF permeate generated from the skim milk or sweet whey.

We hypothesized that protein standardization of NDM with edible lactose powder (**ELP**) may increase the heat stability of reconstituted standardized skim milk powder (**SSMP**) samples because ELP contains mainly lactose and very small amounts of minerals. However, protein standardization of NDM with permeate powder (**PP**) may increase the heat stability of reconstituted SSMP because PP contains nonprotein nitrogen.

Although some previous studies have been done on the heat stability of protein standardization of milk with UF permeate with respect to salt and protein concentration (Newstead, 1977; Anema et al., 1993; Ratnay and Jelen, 1996), at this time, limited information is available on the effects of protein standardization of low-heat (**LH**) and medium-heat (**MH**) NDM with either PP or ELP on heat stability. No single comprehensive study that compares the heat stability of different grades of milk powders in which protein contents

are standardized with either ELP or PP is currently available.

The objective of this research was to determine the heat stability of reconstituted milk made from LH and MH NDM and SSMP at unadjusted and adjusted pH values. We provide 2 models describing how heat stability responds to differences in powder type, standardization material, and pH: the first is reconstituted milk from LH or MH NDM and SSMP at its unadjusted pH, and the second is at pH adjusted to values from 6.3 to 7.0.

MATERIALS AND METHODS

Materials, Sample Preparation, and Experimental Design

Four 55-pound commercial bags of LH and MH NDM powder, ELP, and PP were obtained. The composition of these commercial ingredients is shown in Table 1. The samples were named LH-NDM when LH NDM powder was used to reconstitute milk and MH-NDM when MH NDM powder was used to reconstitute milk. Protein content ($\% \text{total N} \times 6.38$) in LH or MH NDM powder was standardized downward from 35.5% to 30, 32, and 34%, either by adding a predetermined quantity of ELP or by adding PP (Sikand et al., 2008). These powders were called SSMP. Under Codex Alimentarius 207 (Codex Alimentarius, 1999), the standard limit for milk powders and standardization with milk permeate is up to 34% protein on an SNF basis. However, we studied reconstituted milk from LH SSMP and reconstituted milk from MH SSMP at or below 34% protein content to observe the trends of protein standardization of NDM powder on heat stability. A randomization procedure for standardizing the protein content of LH and MH NDM powder with ELP or PP was followed by blending LH or MH NDM with ELP or PP, respectively. The blended SSMP samples were named according to the level of protein (30, 32, and 34%) and material of standardization (ELP, PP); for example, when LH NDM powder was standardized with ELP to 30, 32, or 34% protein content, standardized samples were reconstituted to contain 9% TS and were referred to as LH30-ELP, LH32-ELP, and LH34-ELP, respectively. Collectively, these reconstituted standardized samples containing ELP (independent of protein content) were referred to as LH-ELP. Similarly, when LH NDM was standardized with PP to 30%, 32, or 34% protein content, standardized samples were reconstituted to contain 9% TS and were referred to as LH30-PP, LH32-PP, and LH34-PP, respectively. Collectively, these reconstituted standardized samples containing PP (independent of protein content), were referred to

Figure 1. Flow diagram for low-heat NDM (LH-NDM) powder standardization with edible lactose powder (ELP) or permeate power (PP) to different protein contents.

as LH-PP. Furthermore, LH reconstituted standardized skim milk powder samples containing either ELP or PP, collectively, were referred to as LH-SSMP. Figure 1 is a flow diagram for LH NDM powder standardization used in this study. Table 2 contains the abbreviation or identification names used for reconstituted LH or MH NDM and SSMP samples.

Analytical Methods

Samples of LH NDM powder and MH NDM powder were analyzed for total nitrogen, moisture, ash, and fat content. Composition analyses were performed using the following AOAC (1995) methods: total nitrogen (991.20; 33.2.11), moisture (990.20; 33.2.44), ash (945.46; 33.2.10), and fat content (989.05; 33.2.26). Total calcium was determined by atomic absorption

(model 3030B, Perkin-Elmer, Norwalk, CT) spectroscopy (Brooks et al., 1970).

Heat Stability

The reconstitution of LH and MH NDM and SSMP powder samples was performed in random order. The reconstitution was completed by stirring the samples with a magnetic stir bar in a beaker for 4 h at room temperature. After 4 h, 50 mL of each sample was set aside to measure heat stability at the unadjusted pH. The remaining samples were adjusted to pH 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 by dropwise addition of either 1 M HCl or 1 M NaOH. Samples were stored overnight in a refrigerator. These samples were brought to room temperature the next day and readjusted for pH as necessary.

Table 1. Composition of commercial low-heat and medium-heat NDM powder, edible lactose powder (ELP), and permeate powder (PP; n = 3)¹

Sample type	CP (wet basis)	Moisture	Ash	Fat	Lactose ²
LH-NDM	35.4 ± 0.28	4.5 ± 0.07	7.68 ± 0.02	0.95 ± 0.07	51.47
MH-NDM	35.5 ± 0.60	3.35 ± 0.28	7.75 ± 0.01	0.88 ± 0.13	52.52
ELP	ND	1.75 ± 0.21	0.15 ± 0.10	0.00 ± 0.0	98.14
PP	2.40 ± 0.28	3.73 ± 0.28	7.69 ± 0.02	0.06 ± 0.03	86.12

¹Values given as percentage mean ± SD. CP = total nitrogen × 6.38; ND = not detectable; LH-NDM = low-heat NDM; MH-NDM = medium-heat NDM.

²Lactose calculated by difference.

Table 2. Identification terms used for reconstituted NDM powders and standardized milk powders

Low heat powders	Medium heat powders	Description
LH-NDM	MH-NDM	Low heat (LH) or medium heat (MH) NDM with approximately 35% protein content
LH-SSMP	MH-SSMP	LH or MH standardized skim milk powder (SSMP) independent of standardization material
LH-PP	MH-PP	LH or MH SSMP with permeate powder (PP)
LH34-PP	MH34-PP	LH or MH SSMP with PP to contain 34% protein content
LH32-PP	MH32-PP	LH or MH SSMP with PP to contain 32% protein content
LH30-PP	MH30-PP	LH or MH SSMP with PP to contain 30% protein content
LH-ELP	MH-ELP	LH or MH SSMP with edible lactose powder (ELP)
LH34-ELP	MH34-ELP	LH or MH SSMP with ELP to contain 34% protein content
LH32-ELP	MH32-ELP	LH or MH SSMP with ELP to contain 32% protein content
LH30-ELP	MH30-ELP	LH or MH SSMP with ELP to contain 30% protein content

Several methods have been reported to measure the heat stability of milk, such as the ethanol stability test (Sommer and Binney, 1923), oil bath method (Davies and White, 1966), and the viscosity measurement (Foissy and Kneifel, 1984; de Wit et al., 1986; Kieseker and Aitken, 1988; Nieuwenhuijse et al., 1988). Of these methods, the oil bath method, although somewhat subjective, remains a widely used tool for heat stability in academic research. As has been shown by previous researchers (Fox, 1982; Rattray and Jelen, 1996; Singh, 2004; Sievanen et al., 2008) for unconcentrated milk, a temperature of 140°C is a relevant temperature for testing the heat stability of milk. At this temperature, milk coagulates in a reasonable timeframe for doing an assay and is also below the flash temperature of the commonly used oils.

The heat coagulation time (**HCT**) was determined following the method described by Davies and White (1966) with some modifications: 2.5 mL of milk was heated in tightly capped, 100-mm-long glass tubes, each with a 13-mm i.d. The tubes were fitted in a rocker and immersed in an oil bath containing heated mineral oil controlled at 140°C. The HCT was recorded as the time between immersing the samples in the hot oil bath and the onset of visible clots.

Statistical Analyses

Results were evaluated using ANOVA and multiple linear regression in both Minitab (Minitab Inc., State College, PA) and Data Desk (Data Description Inc., Ithaca, NY) statistical software. Possible predictors of heat stability included in the model were powder type (LH, MH), protein level (30, 32, 34, and 35.5%), standardization material (ELP and PP), and pH. All experiments for LH-NDM, MH-NDM, LH-SSMP, and MH-SSMP at unadjusted pH were performed in triplicate and repeated twice at adjusted pH values.

The heat stability of the aforementioned samples at unadjusted pH and adjusted pH values was analyzed

separately using ANOVA with HCT as the response, and powder type, standardization material, protein content, pH, and replicates as factors. In some analyses, data for LH-NDM and MH-NDM was excluded to create a factorial treatment structure that would allow for the study of possible interaction effects between protein levels (30, 32, and 34%) and standardization material (ELP and PP).

RESULTS AND DISCUSSION

The pH of LH-NDM, MH-NDM, LH-SSMP, and MH-SSMP samples was measured. It was observed that the pH of LH-PP or MH-PP was lower than the pH of the LH-NDM or MH-NDM, whereas the pH of LH-ELP or MH-ELP was higher than the pH of LH-NDM or MH-NDM. Therefore, the pH of PP and ELP powders was determined by reconstituting PP and ELP in water to contain 6% TS; UF permeate usually contains TS in the range of 5.5 to 6.0. After reconstitution, the pH of the permeate sample was observed to be 5.26, whereas the pH of the ELP was determined to be 5.53. This led to further investigation with the manufacturer of PP. It was determined that the manufacturer adjusted the pH of the milk before UF, which resulted in acidic permeate. Furthermore, we discovered that it is not uncommon in the industry to make this adjustment.

During the course of this project, we analyzed PP obtained from the UF of skim milk with no pH adjustment of milk. In this analysis, it was observed that the pH of this reconstituted PP containing 6% TS was 6.7 and LH30-PP had enhanced heat stability when compared with the reconstituted LH-NDM (unstandardized milk; Table 3). However, for the current project, we used commercial permeate.

Heat Stability at Unadjusted pH

Reconstituted LH Milk. Mean HCT value for LH-NDM was higher than the HCT values obtained for LH-

Table 3. Heat stability of reconstituted milk made from low-heat NDM (LH-NDM) and reconstituted milk made from standardized low-heat skim milk powder containing permeate (LH30-PP) adjusted to different pH values (n = 2)

pH	Heat coagulation time (min; mean $\pm$ SD)	
	LH-NDM	LH30-PP
unadjusted pH (6.7)	24.0 $\pm$ 0.40	28.0 $\pm$ 0.42
6.4	9.41 $\pm$ 0.14	12.0 $\pm$ 0.0
6.5	11.8 $\pm$ 0.91	14.2 $\pm$ 0.40
6.6	16.0 $\pm$ 2.12	22.6 $\pm$ 0.70
6.7	22.6 $\pm$ 1.35	27.5 $\pm$ 0.35
6.8	8.0 $\pm$ 0.40	9.2 $\pm$ 0.14
6.9	8.6 $\pm$ 0.78	8.4 $\pm$ 0.10
7.0	12.0 $\pm$ 2.72	14.8 $\pm$ 1.59

PP or LH-ELP. It was noted that a decrease in protein content resulted in a decrease in HCT values. Mean ($\pm$ SD) HCT for LH-NDM and LH30-PP was observed to be 24.5 ($\pm$ 0.61) and 16.3 ($\pm$ 0.16) min, respectively. Mean ($\pm$ SD) HCT for LH-NDM and LH30-ELP was observed to be 24.5 ($\pm$ 0.61) and 20.60 ($\pm$ 1.38) min, respectively. The higher heat stability of LH-NDM may be attributed to the maximum heat stability at native pH (6.7), whereas the low heat stability of LH30-PP may be attributed to its low pH (6.52) or the high pH (6.75) of LH30-ELP. Similar observations for maximum heat stability at a native pH have been reported (Holt et al., 1978). These authors reported in an extensive survey that the heat stability of bulk Scottish milk and the pH of maximum stability coincided with the native pH. For LH-NDM or LH-SSMP reconstituted samples, maximum heat stability was observed to be at native pH and less heat stability was observed when the pH of the samples was changed by standardization to either lower or higher pH.

Reconstituted MH Milk. Mean HCT value for MH-NDM was slightly higher than the HCT values obtained for reconstituted MH-ELP. The mean ($\pm$ SD) HCT value for MH-NDM was 9.3 ($\pm$ 0.13) min, whereas HCT values for MH34-ELP, MH32-ELP, and MH30-ELP were 9.01 ($\pm$ 0.43), 8.43 ($\pm$ 0.12), and 7.9 ($\pm$ 0.4) min, respectively. Higher mean $\pm$ SD HCT values were observed for MH-PP compared with MH-ELP. The effect of protein content was dependent upon standardization material. Mean ($\pm$ SD) HCT values for MH34-PP, MH32-PP, and MH30-PP were 19.2 ($\pm$ 0.20), 18.5 ($\pm$ 0.96), and 14.3 ($\pm$ 0.15) min, respectively. At unadjusted pH, HCT values for MH-NDM and MH-SSMP were lower than for LH-NDM and LH-SSMP respectively. The difference in heat stability at unadjusted pH of the LH-NDM, LH-SSMP, MH-NDM, and MH-SSMP samples may be attributed to the difference in the preheating of milk before powder manufacturing. It has been reported that preheating conditions shift maximum heat stability to

Table 4. The ANOVA of heat coagulation time at unadjusted pH (controls excluded)

Source	df	F-ratio	P-value
Treatment ¹	3	453.06	<0.0001
Protein level	1	51.31	<0.0001
pH	1	0.28	0.6002
Treatment $\times$ protein level	3	3.42	0.0327
Replicate	2	2.62	0.0926
Error	25		
Total	35		

¹Treatment = powder type and standardization material.

an acidic value (Rose, 1961; Newstead et al., 1975). A higher heat treatment is given to MH NDM powders to denature whey protein compared with the heat treatment given to LH NDM powders.

Statistical Analysis. For analysis at unadjusted pH, protein and pH were treated as continuous factors because they had linear relationships with HCT over the range of unadjusted pH and protein values in the experiment. For simplicity in some analyses, the powder type and standardization material factors were combined into 1 treatment factor with 4 levels: LH-PP, LH-ELP, MH-PP, and MH-ELP.

The ANOVA of HCT at unadjusted pH for LH-SSMP and MH-SSMP is shown in Table 4. At unadjusted pH, HCT was significantly related to protein content ($P < 0.0001$) and treatment ($P < 0.0001$), which is a combination of powder type and standardization material. After accounting for differences in protein among the samples, unadjusted pH did not significantly affect HCT ($P = 0.60$). Because LH or MH NDM powder standardization with PP or ELP changed the protein content and (upon reconstitution) pH values of both

Figure 2. Effect of protein content on the heat coagulation time (HCT, adjusted for replicate differences) of reconstituted milk made from low-heat standardized skim milk powder and medium-heat standardized skim milk powders with permeate powder (LH-PP and MH-PP, respectively) or edible lactose powder (LH-ELP, MH-ELP).

Table 5. Effect of each 1% decrease in protein on mean heat coagulation time (min)

Treatment ¹	Estimate ²	Lower limit ²	Upper limit ²	SD ³
LH-PP	-1.63	-0.59	-2.67	a
MH-PP	-1.37	-0.54	-2.20	a
LH-ELP	-0.69	-0.22	-1.15	ab
MH-ELP	-0.25	0.23	-0.73	b

¹Treatments: LH-PP = reconstituted milk made from low-heat standardized skim milk powder with permeate powder; MH-PP = reconstituted milk made from medium-heat standardized skim milk powder with permeate powder; LH-ELP = reconstituted milk made from low-heat milk standardized skim milk powder with edible lactose powder; MH-ELP = reconstituted milk made from medium-heat standardized skim milk powder with edible lactose powder.

²Estimate is the change in mean heat coagulation time for each 1% decrease in protein; limits are the 99% confidence bounds associated with the estimate.

³Samples sharing the same letter do not have a statistically significant difference in the effect of protein on heat coagulation time.

powders, it is assumed that standardization material, protein level, and pH level are all related. This makes it more difficult to determine which of these factors affects HCT; however, based on this analysis, protein has a much more significant effect on HCT than unadjusted pH. This could be due to the small range of pH values present in the samples (e.g., with increasing level of PP standardization in LH NDM, pH ranged from 6.7 to 6.52 in LH-NDM and LH30-PP, respectively).

A linear regression analysis based on the model from Table 4 was used to estimate the effect of these factors on HCT. Because the effect of protein content on HCT depends on powder type and standardization material, a separate estimate of the protein content effect is given for each combination of powder type and standardization material. Estimates of the effect of a 1% decrease in protein content caused by protein standardization in LH-PP, MH-PP, LH-ELP, and MH-ELP are summarized in Table 5. For example, a 1% decrease in protein (LH-PP) caused a decrease between 0.59 to 2.67 min in mean HCT. All estimates given in Table 5 are with 99% confidence. The letters on the right indicate samples that had a significantly different effect of protein on HCT. Samples that share the same letter did not have significantly different protein effects. Using estimates shown in Table 5, we see that whereas a decrease in protein content reduced HCT in 3 of the treatments, protein content had no significant effect on HCT in MH-ELP.

Figure 2 is a partial regression plot of the protein effect on HCT for each of the 4 treatments. The variables in Figure 2 have been adjusted for the presence of different replicates and pH values in our model. The variable on the vertical axis is the deviation of the model adjusted HCT from its mean. The horizontal axis is

the deviation of the model adjusted protein from its mean. (The small variation in protein level within each treatment group is a result of the statistical adjustment for having natural pH in the model.)

Because HCT changes with protein standardization, different protein levels will have a different mean HCT. As an example, results in Table 6 show the mean predicted HCT for samples with 32% protein and 6.7 pH, values near the center of the protein and pH ranges. The letters on the right indicate which samples had a significantly different mean HCT for 32% protein and 6.7 pH. Samples that share the same letter were not significantly different based on Tukey intervals with 99% simultaneous confidence. At pH 6.7, LH32-ELP had significantly greater heat stability than MH32-ELP or MH32-PP; MH32-ELP has significantly lower heat stability than the 3 other treatments.

Heat Stability at Adjusted pH

We selected a pH range of 6.3 to 7.0 because this pH range accounts for a typical bovine pH (6.6–6.7). It also accounts for the variability that can be associated with quality and heating effect (Walstra and Jenness, 1984).

Reconstituted LH Milk. The LH-NDM and LH-SSMP exhibited an increase in heat stability as pH increased from 6.3 to 6.7. The minimum heat stability for both LH-NDM and LH-SSMP was found to be at pH 6.9. Additionally, a heat stability increase was observed as pH increased to 7.0. Maximum heat stability for both LH-NDM and LH-SSMP was observed to be at pH 6.7, as shown in Figure 3. Similar observations for maximum heat stability at native pH during most of the year have been reported (Holt et al., 1978) over the pH range of 6.5 – 7.4 and over the pH range of 6.3–7.1 (Sindhu and Singh, 1987). Both LH-NDM and LH-

Table 6. Model estimated mean heat coagulation time (HCT; min) at 32% protein and 6.7 pH for each treatment

Treatment ¹	Mean HCT	SD ²
LH-ELP	22.10	a
LH-PP	18.06	ab
MH-PP	17.79	b
MH-ELP	8.60	c

¹LH-PP = reconstituted milk made from low-heat standardized skim milk powder with permeate powder; MH-PP = reconstituted milk made from medium-heat standardized skim milk powder with permeate powder; LH-ELP = reconstituted milk made from low-heat milk standardized skim milk powder with edible lactose powder; MH-ELP = reconstituted milk made from medium-heat standardized skim milk powder with edible lactose powder.

²Samples sharing the same letter do not have a statistically significant difference in HCT at 32% protein and 6.7 pH.

Figure 3. Effect of pH on the heat coagulation time (HCT) of reconstituted milk made from low-heat (LH) and medium-heat (MH) powder averaged across standardization material and powder type.

SSMP showed a typical type A milk HCT pH profile. It is well established that 2 types of milk exist: type A and type B (Tessier and Rose, 1964). In a typical type A milk, heat stability first increases with an increase in pH from 6.3 to 6.7 and then decreases at or up to 6.9, followed by an increase as the milk reaches pH 7.0; in type B milk, HCT increases with respect to pH (Rose, 1961; Fox, 1982).

Although statistically not significant, a trend was observed toward higher heat stability for SSMP with an increasing amount of PP in maximum heat stability region. For example, a higher HCT value was observed for LH30-PP followed by HCT values of LH32-PP and LH34-PP compared with LH-NDM. Similar observations were made by Rattray and Jelen (1996) in skim milk protein standardized with skim milk UF permeate or sweet whey UF permeate over a pH range of 6.4 to 7.1; however, our results differed at pH minima. Kelly (2006) reported similar results. In addition, Rattray and Jelen (1996) reported that the higher heat stability of milk protein standardized with either skim milk UF permeate or sweet whey UF permeate may be attributed to its low total calcium. From Table 7, it is clear that LH-SSMP has reduced total calcium compared with the total calcium in LH-NDM. However, the ratio of total calcium to protein was slightly higher in LH-PP compared with LH-ELP. Permeate contains nonprotein nitrogen and minerals when compared with lactose; therefore, we attributed the enhanced heat stability of LH-PP over adjusted pH to the buffering capacity of the nonprotein nitrogen fraction of permeate powder compared with ELP. A positive correlation between nonprotein nitrogen and heat stability was reported (Robertson and Dixon, 1969). The addition of urea in normal milk comprises more than 50% of the nonprotein nitrogen fraction and leads to an increase in the

Table 7. Total calcium content of low-heat NDM (LH-NDM) and low-heat standardized skim milk powder with edible lactose powder (LH-ELP) or low-heat standardized skim milk powder with permeate powder (LH-PP)¹

Sample ²	Total calcium (mg/L; mean $\pm$ SD)
LH-NDM	1,100 $\pm$ 7.1
LH34-ELP	1,060 $\pm$ 10.34
LH32-ELP	994 $\pm$ 4.24
LH30-ELP	942 $\pm$ 5.66
LH34-PP	1,070 $\pm$ 12.0
LH32-PP	1,014 $\pm$ 5.66
LH30-PP	964 $\pm$ 11.3

¹n = 3.

²LH-NDM = reconstituted low-heat NDM powder; LH34-ELP, LH32-ELP, LH30-ELP = reconstituted milk made from low-heat standardized skim milk powder with edible lactose powder to contain 34, 32, and 30% protein content, respectively; LH34-PP, LH32-PP, LH30-PP = reconstituted milk made from low-heat standardized skim milk powder with permeate powder to contain 34, 32, and 30% protein content, respectively.

heat stability (de Koning et al., 1974). Enhanced heat stability of LH-PP was observed around maximum heat stability and no effect was observed at pH 6.8 or 6.9. Similar to our results, Muir and Sweetsur (1977) reported that urea increased the HCT at maximum heat stability and no effect was seen at minimum heat stability until a high level of urea (>40 mg/100 mL of milk) was used. Fox et al. (1980) suggested that the higher heat stability of milk containing urea may be due to the buffering capacity of urea following thermal decomposition. A higher heat stability in protein-standardized LH-PP compared with LH-ELP was observed. Rajput et al. (1984) reported that heat-induced acidity (HIA) of milk decreased with added levels of urea (0, 25, 50, 200 mg/100 mL) and was attributed to low degradation of urea with increased heating time (2, 5, and 10 min) at 140°C. The decreased HIA by urea contributed to the increased heat stability of milk. Furthermore, in a milk salt solution, the HIA was decreased by the addition of urea (25 mg/100 mL of milk) followed by a combination of urea and lactose (25 mg/100 mL of milk and 4.6% lactose) and increased by lactose (4.6% lactose).

Similarly, we observed less reduction in pH (original pH – pH after coagulation of cold samples) for reconstituted PP (pH = 6.7) standardized samples compared with the ELP standardized samples. Fox (1981) reported a decrease in pH as the primary cause of milk coagulation during heating. Some factors responsible for a decrease in pH upon heating include formation of organic acids from decomposition of lactose during heating, release of CO₂, precipitation of calcium phosphate, and release of H⁺ (Walstra and Jenness, 1984).

A slightly lower heat stability for SSMP with an increasing amount of ELP was observed in maximum heat

stability region compared with LH-NDM. Although statistically not significant, a trend toward higher heat stability was observed for LH-NDM, followed by LH34-ELP, LH32-ELP, and LH30-ELP. This trend was applicable for all pH values except in the region of minimum (pH = 6.8 or 6.9). Similar observations of enhanced heat stability at pH minima were made by Fox and Hearn (1978) in milk diluted with water (40 mL of water/100 mL of milk).

Reconstituted MH Milk. Both MH-NDM and MH-SSMP showed a typical type A milk HCT–pH profile similar to LH-NDM and LH-SSMP. However, maximum heat stability for MH-NDM and MH-SSMP was found to be at pH 6.6. We observed pH values 6.7, 6.65, 6.59, and 6.52 for MH-NDM, MH34-PP, MH32-PP and MH30-PP respectively. Thus, the lowering of the pH value from 6.7 to 6.59 actually increased the heat stability as it brought the pH value down to an optimal pH value for maximum heat stability. However, pH values were found to increase slightly with an increase in the degree of NDM powder protein standardization with ELP (e.g., pH values of 6.72, 6.73, and 6.75 were observed for MH34-ELP, MH32-ELP, and MH30-ELP, respectively). Therefore, enhanced heat stability was observed for MH-PP samples around maximum heat stability (pH = 6.6) and the reverse trend was observed at pH minima. However, for MH-ELP samples, a slight increase in heat stability at pH minima was observed, similar to LH-ELP.

Statistical Analysis. Results in Table 8 show the ANOVA for HCT in LH-NDM, LH-SSMP, MH-NDM, and MH-SSMP at adjusted pH. The combinations of powder type and standardization material ($P = 0.0003$), powder type and pH ($P < 0.0001$), and standardization material and pH ($P < 0.0001$) were found to affect heat stability significantly. Because the protein level for the LH-NDM or MH-NDM differed from LH-SSMP or MH-SSMP, the effect of protein content on HCT had to be compared across the 7 combinations of protein and standardization (unstandardized/35.5%, ELP/34%, ELP/32%, ELP/30%, PP/34%, PP/32%, and PP/30%). To accomplish this in the statistical model, the protein factor was nested within the standardization factor. Mean heat stability did not differ significantly across these 7 protein groups ($P = 0.3466$). Newstead (1977) reported that alternating the protein concentration had no effect on heat stability during any season. Anema et al. (1993) reported that increased protein concentration at a constant mineral concentration had negligible effect on HCT. Similarly, no correlation between the protein content and HCT was observed in goat milk (Mukherjee, 1993). Based on our results, when pH is adjusted to values between 6.3 and 7.0, we conclude that decreasing protein content with an increasing level

Figure 4. Effect of powder type and standardization material on the heat coagulation time (HCT) of reconstituted milk made from low-heat (LH) standardized skim milk powder and medium-heat (MH) standardized skim milk powder samples averaged across adjusted pH. Standardization: C = control/unstandardized; ELP = edible lactose powder; PP = permeate powder.

of standardization does not significantly influence heat stability.

Figure 4 shows the effect of powder type and standardization material on HCT from the ANOVA in Table 8. Tukey intervals with 99% simultaneous confidence were used for LH-NDM, LH-PP, LH-ELP, MH-NDM, MH-PP, and MH-ELP in Table 9. The LH-SSMP samples had a higher mean HCT than the MH-SSMP samples for all standardization material. Among the LH NDM powder samples, LH-PP had a significantly higher mean HCT than LH-ELP. No significant difference in heat stability was observed between the MH-NDM and MH-PP or MH-ELP.

In Table 10, Tukey intervals with 99% simultaneous confidence were used to separate the powder type by pH treatment combinations. Independent of protein standardization, the LH-SSMP had a higher mean HCT than MH-SSMP at pH 6.4, 6.7, 6.8, and 6.9. Although the MH-SSMP had a slightly higher mean HCT

Table 8. The ANOVA of heat coagulation time in pH-adjusted samples (controls included)

Source	df	P-value
Replicate	1	<0.0001
Powder type	1	<0.0001
Standardization material	2	<0.0001
Protein level (standardization material)	5	0.3466
pH	7	<0.0001
Powder type × standardization material	2	0.0003
Powder type × protein level	4	0.3925
Powder type × pH	7	<0.0001
Standardization material × pH	14	<0.0001
Protein × pH	35	0.2346
Error	143	
Total	221	

Table 9. Model estimated mean heat coagulation time (HCT; min) for each pairing of powder and standardization for pH-adjusted samples

Treatment ¹	Mean HCT	SD ²
LH-PP	14.15	a
LH-NDM	12.95	ab
LH-ELP	11.92	b
MH-PP	8.707	c
MH-NDM	8.649	c
MH-ELP	8.328	c

¹LH-PP = reconstituted milk made from low-heat standardized skim milk powder with permeate powder; LH-NDM = reconstituted milk made from low-heat NDM; LH-ELP = reconstituted milk made from low-heat milk standardized skim milk powder with edible lactose powder; MH-PP = reconstituted milk made from medium-heat standardized skim milk powder with permeate powder; MH-NDM = reconstituted milk made from medium-heat NDM; MH-ELP = reconstituted milk made from medium-heat standardized skim milk powder with edible lactose powder.

²SD: Samples sharing the same letter do not have statistically significant differences.

than LH-SSMP at pH 6.6, this was not statistically significant. At all other pH levels tested, there was no significant difference between the HS of the reconstituted LH-SSMP and MH-SSMP.

For LH-NDM and LH-SSMP samples, mean HCT increased significantly at each pH interval until HCT peaked at pH 6.7. Mean HCT significantly decreased in each pH interval from 6.7 to 6.9 before the mean HCT increased again from pH 6.9 to 7.0. For MH-NDM and MH-SSMP samples, mean HCT did not increase significantly between pH 6.3 and 6.4. The mean HCT increased rapidly from pH 6.4 to a peak of pH 6.6. The mean HCT decreased significantly from pH 6.6 to 6.8, did not change significantly between pH 6.8 and 6.9, and increased from pH 6.9 to 7.0. Independent of standardization material, maximum heat stability for MH-SSMP lies at a lower pH when compared with LH-SSMP. This may be attributed to a higher preheating condition given to MH NDM compared with LH NDM during its manufacture. Similarly, preheating unconcentrated milk at 90°C for 10 min shifted the maximum pH-HCT profile to a more acidic pH, as was observed by Tan-Kintia and Fox (1999).

Figure 5. Effect of standardization material and pH on the heat coagulation time (HCT) of reconstituted milk made from low-heat (LH) or medium-heat (MH) nonfat dry milk powder (LH-NDM and MH-NDM, respectively), LH or MH standardized skim milk powder with edible lactose powder (LH-ELP and MH-ELP, respectively), or standardized skim milk powder with permeate powder (LH-PP, MH-PP) samples. Results averaged across LH and MH samples. Standardization: C = control (unstandardized); ELP = edible lactose powder; PP = permeate powder.

Figure 5 shows the effect of standardization material and pH on HCT. In Table 11, Tukey intervals with 99% simultaneous confidence were used to separate the HCT levels based on standardization material within each pH value. Because of the large number of combinations, differences in HCT across pH levels were not tested. The Tukey intervals show that samples standardized with PP had higher mean HCT than those standardized with ELP only at pH 6.5 and 6.6. At all other pH levels, no significant difference was observed between mean HCT among the standardization material.

Although these differences are not noted on Table 11, the Tukey intervals also revealed that mean HCT for samples standardized with PP, independent of type of powder, peaked at pH 6.6. For unstandardized and ELP standardized samples, mean HCT increased between pH 6.5 and 6.6, but was not significantly different between pH 6.6 and 6.7. It is difficult to differentiate between HCT at pH 6.6 and 6.7 for the LH-NDM or MH-NDM and LH-ELP or MH-ELP; the true peak HCT could be at either pH 6.6 or 6.7 or somewhere in between.

Table 10. Model estimated mean heat coagulation time (min) for each pairing of powder and adjusted pH

Reconstituted sample ¹	pH							
	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7
LH-NDM	2.1440 ^{ae}	9.889 ^d	12.82 ^b	17.53 ^{ch}	23.81	12.960 ^b	9.119 ^d	15.80 ^{hi}
MH-NDM	0.7926 ^a	2.009 ^a	12.86 ^b	18.91 ^c	8.28 ^{df}	4.847 ^{eg}	6.412 ^{fg}	14.38 ^{bi}

^{a-i}Samples sharing the same letter do not have statistically significant differences.

¹LH-NDM = reconstituted milk made from low-heat NDM; MH-NDM = reconstituted milk made from medium-heat NDM.

Table 11. Model estimated mean heat coagulation time (min) for each pairing of standardization and adjusted pH (differences evaluated for each pH triplet only)

Type of standardization ¹	pH							
	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7
SSMP-PP	2.6560 ^a	6.119 ^b	14.46 ^c	20.19 ^e	17.05 ^g	8.959 ^h	7.102 ⁱ	14.90 ^j
C	1.1170 ^a	6.154 ^b	12.74 ^{cd}	18.53 ^{ef}	16.19 ^g	7.861 ^h	7.777 ⁱ	16.03 ^j
SSMP-ELP	0.6317 ^a	5.573 ^b	11.32 ^d	15.93 ^f	14.90 ^g	9.896 ^h	8.418 ⁱ	14.34 ^j

^{a-j}Samples sharing the same letter do not have statistically significant differences within the same pH level.

¹SSMP-PP = standardized skim milk powder containing permeate powder; C = control (not standardized);

SSMP-ELP = standardized skim milk powder containing edible lactose powder.

CONCLUSIONS

Based on our observations, we conclude that heat stability is influenced by the type of NDM powder (LH or MH) and standardization material (PP or ELP). In some situations, PP, besides containing minerals that cause destabilization of the milk system during heating, also contains a nonprotein nitrogen fraction that may impart heat stability to the milk system in the maximum heat stability region. Of the options studied, our results indicate that LH-PP provides the best heat stability for reconstituted milk.

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