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The Influence of High Heat Treatment at Alkaline pH on the Physicochemical Characteristics and Isomerization of Lactose to Lactulose in Skim Milk

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ABSTRACT

The pH of the skim milk was modified, first increased from 6.5 to 7.5, then subjected to high heat treatment at 95°C for 2 min or left unheated for 1 hour. Subsequently, the pH was adjusted back to 6.5, and the samples were analyzed. The concentration of lactulose in skim milk showed a significant increase due to High heat treatment (HHT) ($p < 0.05$), and this effect was more pronounced when the pH was raised from 6.5 to 7.5 before HHT. Raising the pH of both high heat-treated (HHT) and unheated (UH) skim milk from 6.5 to 7.5 before heating or holding led to an extended heat coagulation time (HCT) at pH 7.2 and resulted a 58.7% increase in the dissociation of κ-casein in HHT milk compared to the total κ-casein content. The adjustment of pH had no significant ($p < 0.05$) influence on the chemical composition of the high heat-treated skim milk and ethanol stability in the pH range of 6.2-7.2). Besides heating the sample at 95°C for 2 min resulted in a significant decrease in casein micelle size (CMS). These findings offer a foundation for focusing on the negative impact of alkalinization before high heat treatment on casein dissociation, CMS, thermal and alcohol stabilities. Additionally, it is regarded as a promising approach with the potential to enhance the lactulose content in milk.

Keywords: Defatted milk, lactulose, heat stability, pH at heating, High heat treatment

INTRODUCTION

Milk or recombined milk undergoes a process of intense heat treatment (e.g., temperatures equal to or greater than 85°C for two and ten minutes) when making high and medium-temperature dried milk, various dairy based beverages (such as, UHT milk, flavored milk infant milk formula and recombined evaporated milk), as well as fermented milk products like yogurt (Sanderson, 1999). The application of high-heat treatment (HHT) promotes the structure and viscosity of fermented dairy products such as fermented cheese products and yoghurt (Hinrichs, 2001; Guinee, 2016 and Farkye, 2017). It also increases the reconstituted dried skim milk's thermal stability and increases the length of dairy products' shelf life (Sharma *et al*., 2012). At pH 6.5-6.7, HHT of milk causes a significant amount of the whey protein to become denaturated (Anema and Li, 2003b and Vasbinder and de Kruif, 2003) and interact with κ-casein by exchanging thiol disulfides and forming hydrophobic bonds with it on the outer layer of the casein micelle (Guyomarc'h *et al*., 2003 and Donato and Guyomarc'h, 2009;). Lactulose, which is 4-O-d-galactopyranosyl-d-fructose, is a disaccharide that is closely related to lactose. When milk is thermally preserved, a small amount of it is created by the lactose isomerization process (Marconi *et al*., 2004). A bifido-factor in nutrition, Lactulose is a type of sugar that functions as a prebiotic that is sweeter and more soluble than lactose (Mayer *et al*., 2010). The field of food science has recently introduced the term "prebiotic" which describes a non-digestible component of food that provides specific benefits to the host by promoting the growth and/or activity of a selected group of bacteria in the colon (Darwish *et al*., 2022a,b; Darwish *et al*., 2023; Elbermawi *et al*., 2022a,b and Khojah *et al*., 2022). Lactulose also has a variety of uses in the food and drug industries. Due to its noteworthy medical benefits in treating chronic constipation and portal systemic encephalopathy, lactulose is incorporated into commercial infant formulas and other dairy products. (Zokaee *et al*., 2002 and Mayer *et al*., 2010). Moreover, Lactulose has diverse applications such as serving as a sugar alternative in confectionery items, acting as a sweetener for individuals with diabetes, functioning as a yogurt additive in dairy-based products, and being utilized in various liquid or dried food formulations designed for the elderly population (Mayer *et al*., 2010). Lactulose is currently only made through the alkaline isomerization of lactose for commercial use (Aider and Halleux, 2007). Using a substantial amount of inorganic catalysts is beneficial for achieving high yields of lactulose (Zokaee *et al*., 2002). The management of waste and purification of products in chemical processes can be costly (Mayer *et al*., 2010).

The aim of this study was to assess how altering the pH of skim milk from 6.5 to 7.5 before subjecting it to high heat treatment at 95°C influences its physicochemical and processing properties, including ethanol stability, lactulose content and heat stability.

MATERIALS AND METHODS

Heat treatment and pH adjustment of skim milk

At a temperature of 21°C, defatted milk was prepared to the desired pH level of 6.5 or 7.5 using either 3 N sodium hydroxide or hydrochloric acid. A 5 L batch of pH-altered skim milk was split into two parts. One portion remained unheated (UH) at a temperature of 21°C for one hour, while the other portion was heated for two minutes at 95°C, then decreased to 15°C, and finally allowed to stabilize for 50 minutes at 19°C. After reaching equilibrium, both the heated (HHT) and unheated (UH) samples' pH values were modified

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to 6.5, like the initial pH level of the milk before heat treatment. Subsequently, both samples were examined on the same day to determine the heat coagulation time and the whey production (Lin *et al*., 2016). The remaining portions of each sample were kept at 4°C and subjected to analyses for gross composition, lactulose concentration, ethanol stability and casein micelle properties within 48 hours of heating (Dalgleish and Law, 1988). The skim milk was subjected to a heating process at 40°C for 30 minutes to counteract the effects of cold aging. Subsequently, the temperature of the samples was reduced to 25°C for analysis. Before heating or holding, the HHT and UH skim milk samples having pH levels of either 7.5 or 6.5 were designated as HHT 6.5 and HHT 7.5 and UH 6.5 and UH 7.5, respectively.

Preparation of whey

To eliminate any remaining fat, the serum from the ultracentrifuge of defatted milk at 95,000g for one hour at 25°C was filtered through glass wool (Lin *et al*., 2016).

Analyzing the whey and skim milk's chemical composition

Total solids, casein hydration, fat, lactose, zeta potential and casein micelle size (CMS) were measured in skim milk samples, according to Lin *et al*. (2016), Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to analyze the protein profile in samples of skim milk and whey (Lin *et al*., 2016). To determine the N distribution (total protein, non-protein nitrogen (NPN) and non-casein nitrogen (at pH 4.6) in milk samples, the procedures outlined by AOCS methods (AOCS, 2007) were used. According to Gaines *et al*. (1990), the determination of calcium and phosphorus in milk samples was carried out using atomic absorption spectrophotometry. The formulas used to determine the various N fractions in the samples are below:

(1) True protein in serum (%, w/w) = total protein (%, w/w) − (NPN × 6.38) (%, w/w)

(2) Serum casein (%, w/w) = true protein (%, w/w)] × casein as % of true protein

(3) Denatured whey protein complexed with κ-casein (%,w/w)
= Total protein (%, w/w) − serum casein (%, w/w) − pH 4.6 soluble protein (%, w/w)

RP-HPLC was used to analyze the individual proteins present in milk and whey (Lin et al., 2016). For RP-HPLC analysis, whey protein isolate (WPI), α-La, ß-Lg, k-casein, ß-casein and αS-casein were utilized as calibration standards to determine the protein content. A mixture of HPLC grade water, acetonitrile and trifluoroacetic acid (TFA) was prepared to create the 900:100:1 volume ratio of the hydrophilic mobile phase (A). Similarly, a mixture of HPLC grade water, acetonitrile and TFA was prepared to form the 100:900:1 volume ratio of the hydrophobic mobile phase (B). The milk and whey samples were both diluted before injection using a buffer solution made up of 0.02 M bis-Tris propane, 0.5% (v/v) 2-mercaptoethanol, and 7 M urea.

Thermal stability of milk samples

Using either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide, with pH increments of 0.1, different pH levels of defatted milk samples were achieved at room temperature, ranging from 6.2 to 7.2. The thermal coagulation time at 140°C was measured using the technique outlined by O'Connell and Fox (2000).

Ethyl alcohol stability

At room temperature (21°C), pH adjustments were made to milk samples at intervals of 0.2 pH units, ranging from 6.2 to 7.0, by utilizing 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. To measure ethanol stability, milk samples were mixed with ethyl alcohol solutions with concentrations ranging from 98 to 30% (v/v) at an ethanol-to-milk volume ratio of 4.8:1. After adding the ethanol, the mixture was vibrated for 30 seconds (to agitate it and check for visible flocculation. The smallest amount of ethanol needed to cause flocculation was used to define ethanol stability (Lin *et al*., 2016).

Determination of lactulose

According to Amine *et al*. (2000), lactulose concentrations were measured using a spectrophotometric enzymatic test kit (Sigma Aldrich Co. LLC, US Catalogue Number MAK182).

Statistical analysis

Using SAS 2000's one-way analysis of variance, the average percentages of the chemical composition of the milk samples, CMS, lactulose concentration, alcohol stability, and heat stability were examined. Three independent replicates were used in the analysis. When the main effects were significant, Duncan's Multiple Range Test was used to estimate pairwise comparisons of the means. Principal Component Analysis (PCA) was used for unsupervised clustering to find patterns and outliers in the datasets.

RESULTS AND DISCUSSIONS

Chemical composition of skim milk samples

The HHT skim milk had comparable levels of calcium, phosphorus, protein, and total solids compared with the control (Table 1), indicating that pH adjustment had no impact on the HHT skim milk's gross composition. The levels of lactose in the various milk samples significantly decreased because of the rise in pH before the heat treatment, with the treatment HHT 7.5 showing the greatest reduction in lactose compared to the control (Table 1).

Table 1. The impact of pH on skim milk's chemical composition of skim milk before high heating

Parameters	Treatments			
	UH		HHT skim milk	
	pH 6.5	pH 7.5	pH 6.5	pH 7.5
Skim milk				
Total solids (%, w/w)	8.41 [a]	8.39 [a]	8.40 [a]	8.46 [a]
Total protein (%, w/w)	3.20 [a]	3.24 [a]	3.19 [a]	3.28 [a]
Whey protein (%, w/w)	0.51 [a]	0.50 [a]	0.53 [a]	0.49 [a]
Casein (%, w/w)	2.64 [a]	2.67 [a]	2.57 [a]	2.71 [a]
Denatured WP (% total whey protein)	0 [c]	0 [c]	65.8 [b]	70.2 [a]
Lactose (%, w/w)	4.30 [a]	4.33 [a]	3.2 [b]	1.94 [c]
NPN (% TN)	6.0 [a]	5.98 [a]	5.92 [a]	6.03 [a]
Total P (mg/100g)	90 [a]	91 [a]	88 [a]	90 [a]
Total Ca (mg/100g)	106 [a]	107 [a]	108 [a]	110 [a]
Casein hydration (g water/ g casein)	2.78 [b]	3.18 [a]	2.83 [b]	3.1 [a]
Zeta potential (mV)	-22.4 [a]	-22.8 [a]	-22.9 [a]	- 23.1 [a]

Regarding the statistical effects of pH on samples, there is a significant variation ($p < 0.05$) between values for HHT 7.5 or HHT 6.5 that do not share the same superscript letter within a row.

Raising the pH before applying heat treatment resulted in a notable increase in whey protein denaturation ($p > 0.05$). These findings align with the research conducted by Vasbinder and de Kruif (2003), who observed that elevating milk's pH from 6.7 to 6.9 before heating it for 10 minutes at 80°C resulted in a slight increase in the heat-induced denaturation of ß-lactoglobulin and α-lactalbumin. However, when heated at 80-100°C, the degree of denaturation of major whey protein in the pH between 6.48 to 6.83 remained unaffected (Oldfield *et al*., 2000 and Anema and Li, 2003b). The data presented in Table 1 indicates that there was an

increase in casein hydration as the pH of the HHT milk increased ($p > 0.05$). This phenomenon is likely attributed to the elevated negative charge of casein and the formation of hydrogen bonds between casein and water (Kneifel *et al*., 1991 and Kruif *et al*., 2015). At pH 6.5 and 7.5, HHT significantly decreased casein hydration ($p < 0.05$) compared with unheated skim milk. There are several causes for the decrease in casein hydration, including the dissociation of κ-casein at higher pH levels, hydrophobic casein micelles and whey proteins, the interaction between denatured and the precipitation of calcium phosphate (Rüegg *et al*., 1979).

Casein micelle size (CMS) and zeta potential

In contrast to pH 7.5, which had the contrary impact, CMS increased ($p < 0.05$) after two minutes of heating at 95°C and pH 6.5 (Fig. 1). The fact that HHT 6.5 milk has more sedimentable whey protein than UH 6.5 milk ($p < 0.05$) supports the theory that CMS is increased by interactions between denatured whey proteins and κ-casein on the surface of the micelles when heated at pH 6.6 (Anema and Li, 2003a, 2003b). The concentrations of κ-casein and whey protein in the whey of HHT 7.5 milk samples are greater than those of HHT 6.5 milk samples ($p < 0.05$; Table 2). This corresponds with the observed decrease in casein micelle size (CMS) during high-temperature treatment at pH 7.5 ($p < 0.05$). Contrary to the study's current results, Ménard *et al*. (2005) discovered that raising the pH gradually up to 8.1 before HHT (90°C/ 30 s) raised the CMS. When contrasting the whey of HHT 6.5 or HHT 7.5 skim milk with that of UH 6.5 skim milk, it is evident that the serum of the high heat-treated sample has lower levels of calcium and phosphorus compared with UH 6.5 or UH 7.5. The CMS increased marginally but significantly ($p < 0.05$; Fig. 1) when UH milk's pH was raised before holding. According to Table 1, casein hydration increases with pH, indicating that better water binding is most likely the cause of the increased hydration. These findings align with a study conducted by Sinaga *et al*. (2017), which reported a similar pattern of increased casein micelle size (CMS) in fresh pasteurized defatted milk samples when the pH was raised up to 7.5 and maintained at 4°C for 24 hours.

Zeta potential was unaffected by increasing pH before heating, holding, or heating at 95°C for 2 minutes. This outcome supports the findings of Schmidt and Poll (1986), who discovered that heating casein micelles at 120°C for 10 minutes had little impact on their zeta potential in a milk ultrafiltrate model with a pH range of 6.6 to 7.0.

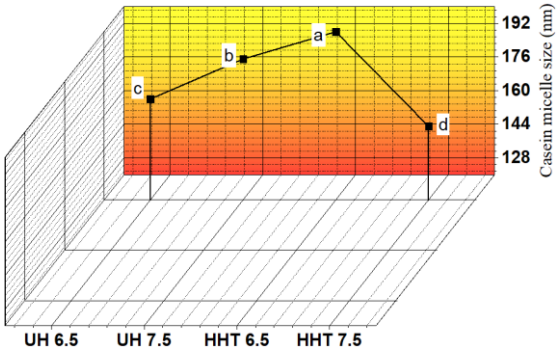

Figure 1. The influence of raising skim milk's pH from 6.5 to 7.5 followed by an hour of ambient temperature incubation (UH) or heating (95°C × 2 min) (HHT) on casein micelle size. Lowercase letters ($p < 0.05$) indicate significant variations in casein micelle size across various pH levels during the same heat treatment or holding at room temperature for 1 hour.

Table 2. The impact of pH on skim milk's chemical composition of whey prior to high heating

Parameters	Treatments			
	UH		HHT skim milk	
	pH 6.5	pH 7.5	pH 6.5	pH 7.5
Whey				
Protein (%, milk protein)	16.91 ^{b}	33.7 ^{a}	17.3 ^{b}	34.2 ^{a}
Protein (%, w/w)	0.65 ^{b}	1.08 ^{a}	0.68 ^{b}	1.12 ^{a}
Casein (% milk protein)	9.3 ^{c}	14.2 ^{b}	10.2 ^{c}	24.2 ^{a}
Casein (% w/w)	0.24 ^{c}	0.34 ^{b}	0.27 ^{c}	0.68 ^{a}
% of total denatured whey protein in serum that is associated with κ-casein	0 ^{c}	0 ^{c}	3.2 ^{b}	52.4 ^{a}
P (% milk P)	41.55 ^{a}	38.1 ^{b}	37.3 ^{c}	35.6 ^{d}
P (mg/ 100 g)	38.6 ^{a}	33.1 ^{b}	33.2 ^{b}	31.7 ^{c}
Ca (% milk Ca)	38.1 ^{a}	30.4 ^{b}	28.3 ^{c}	27.1 ^{d}
Ca (mg / 100 g)	40.8 ^{a}	32.2 ^{b}	31.8 ^{b}	29.7 ^{d}

Regarding the statistical effects of pH on samples, there is a significant variation ($p < 0.05$) between values for HHT 7.5 or HHT 6.5 that do not share the same superscript letter within a row.

Individual casein concentrations in milk serum

The individual caseins concentrations in the whey of both HHT and UH increased as the pH increased before holding or high-heat treatment, expressed as a percentage of the corresponding casein in skim milk (Fig. 2). When the pH was elevated to 7.5 before heating, the percentage of dissociated κ-casein in whey increased by 58.7% of the total amount of κ-casein. The Ca concentration in the whey of both HHT and UH samples reduced on rising pH levels prior to heating or holding ($p < 0.05$), which is the opposite of the latter trend. Our results align with a previous report indicating that when the pH was elevated before heating to levels of pH 7.6 or 8.1, HHT samples contained 60% of the total amount of κ-casein undergoing dissociation (Ménard *et al*., 2005).

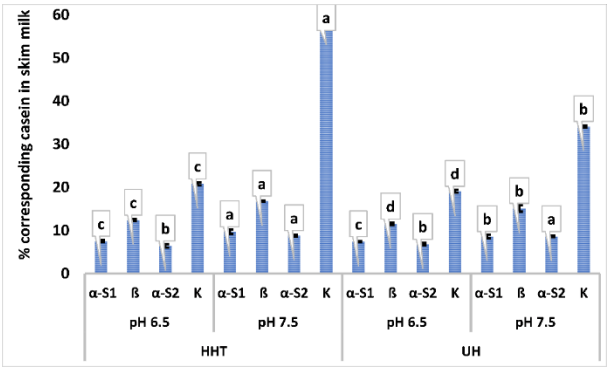

Figure 2. The impact of shifting skim milk's pH from 6.5 to 7.5 on the percentage of different fractions of casein in the whey. Different letters were significant ($p < 0.05$) vs. treatments.

Heat coagulation time of skim milk

The maximum heat coagulation time (HCTmax) during high-temperature treatment was affected by skim milk's pH before heating. HHT caused the HCTmax to shift to smaller values, such as from 6.7 to 6.6 in HHT 6.5 samples and from 6.8 to 6.7 in HHT 7.5 samples (Fig. 3). This resulted in less HCT at pH 6.7 or 6.8 and greater HCT at pH 6.3, 6.4, 6.5, and 7.2. This pattern is in line with earlier studies on how milk with a pH between 6.6 and 7.5 is affected by extreme heat treatment (Lin et al., 2018). The creation of complexes between kappa casein and whey proteins, as well as the drop in calcium concentration in whey, are thought to be caused by the pH of HCTmax's pH level decreasing in HHT milk (Sievanen et al., 2008 and Lin et al., 2018). The heat coagulation time (HCT) at pH 7.2, the pH of the HCTmax, and the HCTmax were all raised when the pH level of high heat-treated or unheated skim milk was raised from 6.5 to 7.5 before heating or holding (Fig. 3). These findings were consistent with the results reported in a previous study conducted by (Lin et al., 2018). However, the results of this study differ slightly from the findings of Singh and Fox (1985). They observed that raising the skim milk's pH from 6.7 to 7.1-7.3 before high-temperature treatment at 140°C for 1 minute caused the pH of HCTmax to drop from 6.5 to 6.4. As shown in Table 1, the rate of casein dissociation increased and the serum calcium concentration decreased in the HHT samples. When the HHT milk's pH was raised before heating, it was also discovered that the rise in the percentage of k-casein and whey protein complexes in the whey was significant ($p < 0.05$) (Table 1). It is unclear how these modifications affect heat stability, so perhaps a more thorough investigation into the physiochemical and compositional modifications that take place during HCT measurement could shed more light on the matter.

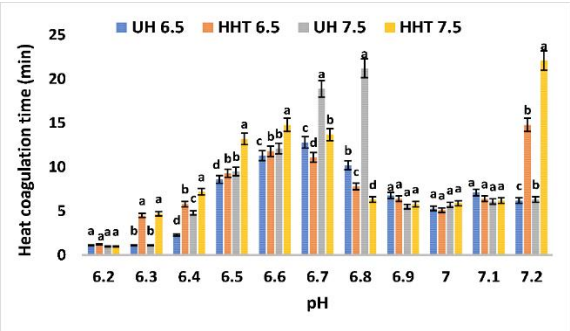

Figure 3. The impact of raising the pH of skim milk from 6.5 to 7.5 followed by an hour at ambient temperature (UH) or heating at 95°C for two minutes (HHT) on the heat coagulation time. Lowercase letters ($p < 0.05$) indicate significant variations in heat coagulation across vs. pH level. Standard deviation of the mean is shown by error bars.

The resistance of skim milk to ethanol

Subjecting skim milk to high heat treatment at pH 6.5 or 7.5 resulted in a significant enhancement of ethanol stability within the pH range of 6.2 to 6.6 ($p < 0.05$), while no noticeable effect was observed at pH 6.8-7.0 (Fig. 4). Previous studies have reported that the beneficial effect of high heat treatment on the ethanol stability of skim milk within the intermediate pH range is attributed to a reduction in the concentration of calcium ions (Horne and Parker, 1981; Mohammed and Fox, 1986 and Lin et al., 2018). Altering the pH before heating or holding the HHT and UH samples had no impact on the ethanol stability within the pH range of 6.2-7.0 ($p < 0.05$). One would anticipate that the milk samples heated at pH 7.5 would exhibit increased dissociation of κ-casein and decreased casein micelle size, rendering the micelle more prone to ethanol-induced flocculation and leading to a denser floc formation and reduced ethanol stability. However, the present results indicate that the lower calcium concentration of whey (as observed in Table 2), and consequently a lower level of free calcium ions, in these samples might have counteracted the impact of CMS and the dissociation of κ-casein.

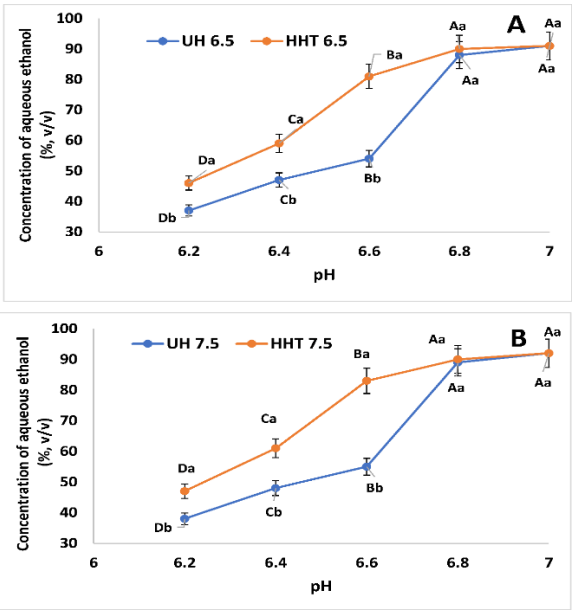

Figure 4. The influence of increasing skim milk's pH from 6.5 to 7.5 on the resistance rate of skim milk samples to ethanol: pH 6.5 (A) and 7.5 (B). Different uppercase letters were significant ($p < 0.05$) *vs.* pH level and different lowercase letter were significant vs. treatments. Standard deviation of the mean is shown by error bars.

Effect of the HHT on lactose isomerization

The influence of shifting the skim milk's pH from 6.5 to 7.5 before heating at 95°C on the lactose conversion into lactulose in defatted milk (Fig. 5). Lactulose concentration in skim milk increased significantly because of HHT ($p < 0.05$), with the impact becoming greater as the pH was raised from 6.5 to 7.5 before HHT. Except for the UH sample at pH 6.5, where lactulose content can be assumed to be absent ($p < 0.05$), raising the pH to 7.5 before holding the UH samples did not lead to a statistically significant ($p < 0.05$) change in the rate of lactulose concentration. The estimation of lactulose content of raw milk has been the subject of numerous reports. According to a report, the isomerization reactions that conversion lactose to lactulose in heated milk. Lactulose can be thought of as being either zero or absent in raw milk, making it a useful indicator for assessing the thermal processing of milk (Morales et al., 2000; Claeys et al., 2001; Pereyra Gonzáles et al., 2003 and Ibrahim, 2016). Lactulose concentrations in commercial milks labelled as pasteurized ranged from 0 mg/l to 5.8 mg/l, according to nd Villamiel et al. (1999), but Feinberg et al. (2006) reported 1.5 1.2 mg/100 ml lactulose for milk heated at 74°C for 30 s. In addition, milk treated at 63°C for 30 minutes contained levels of lactulose of 0.52 mg/100 ml, according to Olano et al. (1989). The observed differences in lactulose content in our study could be

attributed to the interaction between κ-casein and denatured whey protein in the serum, particularly when the pH of the milk is adjusted. To the best of our knowledge, no previous research has investigated the effect of pH adjustment from 6.5 to 7.5 before high heat treatment (95°C) on the lactulose levels in skim milk.

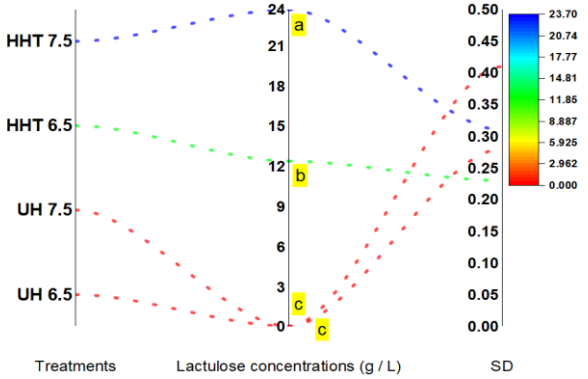

Figure 5. The influences of increasing skim milk's pH from 6.5 to 7.5 followed by an hour of ambient temperature incubation (UH) or heating at 95°C for 2 min (HHT) on the amount of lactulose in skim milk. Different letters were significant ($p < 0.05$) vs. lactulose concentration in skim milk.

Multivariate Analysis of UH and HHT Parameters

Table 3 (varimax rotated PC factor loadings) displays the Varimax rotated factor loadings, which indicate correlations between the original characteristic measurements and the PC. Strong influences are indicated by loadings with an absolute value higher than 0.560 (indicated in bold type). Additionally, PCA generates factor score values (Table 4) that identify each treatment's position along each of the Varimax rotated PC.

Principal component analysis (PCA) of thermal stability, ethanol stability, Individual serum casein (α_{S1}-, α_{S2}-, ß- and κ-casein), CMS and lactulose concentration of UH 6.5, UH 7.5, HHT 6.5 and HHT 7.5 explained 89.0% of the variability on 2PC (Fig. 6A). PC1 (57.92%) included thermal stability at pH 6.3, 6.4, 6.5, 6.5 and 7.2, ethanol stability at pH 6.2, 6.4, 6.6 and 6.8 and Individual serum casein (α_{S1}-, α_{S2}-, ß- and κ-casein). However, the second aspect (31.07%) was mainly linked to the stability of heat at pH levels of 6.2, 6.7, 6.8, 6.9, 7.0, and 7.1, as well as the stability of ethanol at pH 7.0 and the concentration of lactulose (Fig. 6A). The treatments were divided into four categories. On PC1's left side, Groups 1 and 2 were discovered. In contrast, the third and fourth groups were located on the PC1's positive side. The initial group was distinguished by its high heat stability at pH 6.7 and 6.8, along with a high CMS. On the other hand, group 2 displayed the highest heat stability values at pH 6.2, 6.9, and 7.1 (Fig. 6A). The thermal stability at pH 6.3, 6.4, 6.5, 6.6, 7.0 and 7.2, ethanol stability at pH 6.2, 6.4, 6.6 and 7.0, individual serum casein (ß-casein and k-casein) and high concentration of lactulose were detected in group 3 compared with other groups. However, group 4 had serum levels of S1- and S2-casein that were very high (Fig. 6A). Correlations were established between the 22 studied attributes (Table 5). The concentration of lactulose exhibited a strong positive correlation with heat stability at pH 6.4, 6.5, 6.6, 7.0, and 7.2, as well as with ethanol stability at pH 7.0 and the concentration of k and ß-caseins in milk serum (Fig. 6B; Table 5). Additionally, a weak positive correlation was observed between lactulose concentration and ethanol stability at pH 6.2, 6.4, 6.6, and 6.8, as well as the concentration of α-$_{S1}$ in milk serum (Fig. 6B; Table S3). On the other hand, lactulose concentration displayed a negative correlation with heat stability at pH 6.2, 6.9, and 7.1, the concentration of α-$_{S2}$ casein in milk serum, and CMS (Fig. 6B; Table 5).

Table 3. Principal component factor loadings for UH and HHT milk attributes using Varimax rotation.

Attributes	F1	F2	F3
HS 6.2	0.006	0.894	0.099
HS 6.3	0.882	0.108	0.010
HS 6.4	0.877	0.049	0.074
HS 6.5	0.739	0.159	0.102
HS 6.6	0.715	0.190	0.095
HS 6.7	0.104	0.838	0.058
HS 6.8	0.373	0.429	0.198
HS 6.9	0.111	0.750	0.138
HS 7.0	0.142	0.774	0.084
HS 7.1	0.316	0.352	0.332
HS 7.2	0.974	0.005	0.021
ES 6.2	0.921	0.062	0.017
ES 6.4	0.933	0.058	0.008
ES 6.6	0.895	0.092	0.013
ES 6.8	0.820	0.001	0.179
ES 7.0	0.126	0.874	0.001
α-$_{S1}$	0.741	0.185	0.074
α-$_{S2}$	0.522	0.427	0.051
ß	0.996	0.003	0.001
κ	0.935	0.007	0.058
CMS	0.219	0.024	0.757
Lactulose	0.393	0.556	0.051

The bolded values represent the factor for each variable for which the squared cosine is the largest.

Table 4. Principal component factor scores for UH and HHT milk samples using Varimax rotation.

Treatments	F1	F2	F3
UH 6.5	-4.111	-1.575	-1.782
UH 7.5	1.579	-3.313	1.704
HHT 6.5	-2.501	3.430	1.381
HHT 7.5	5.032	1.458	-1.303

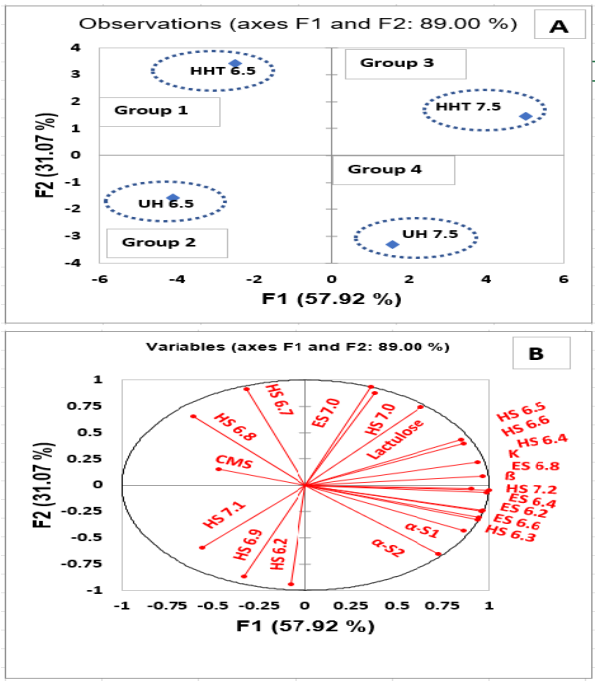

Figure 6. (A) Principal component analysis (PCA) biplot representing the impact of adjusting skim milk's pH between 6.5 and 7.5 before heating or holding on the characteristics of milk samples. (B) The analysis of correlation variables.

Table 5. The parameter of the studied milk sample response to treatments are correlated using the Pearson's correlation matrix.

Variables	HS 6.2	HS 6.3	HS 6.4	HS 6.5	HS 6.6	HS 6.7	HS 6.8	HS 6.9	HS 7.0	HS 7.1	HS 7.2	ES 6.2	ES 6.4	ES 6.6	ES 6.8	ES 7.0	α_{S1}	α_{S2}	ß	κ	CMS	Lac
HS 6.2	1																					
HS 6.3	0.267	1																				
HS 6.4	-0.198	0.835	1																			
HS 6.5	-0.547	0.644	0.806	1																		
HS 6.6	-0.577	0.620	0.804	0.999	1																	
HS 6.7	-0.764	-0.579	-0.035	0.011	0.052	1																
HS 6.8	-0.430	-0.744	-0.307	-0.406	-0.368	0.904	1															
HS 6.9	0.729	-0.066	-0.604	-0.513	-0.545	-0.775	-0.529	1														
HS 7.0	-0.953	0.036	0.468	0.767	0.792	0.614	0.217	-0.780	1													
HS 7.1	0.425	-0.391	-0.814	-0.536	-0.556	-0.500	-0.302	0.916	-0.567	1												
HS 7.2	-0.057	0.936	0.870	0.866	0.848	-0.419	-0.714	-0.213	0.351	-0.429	1											
ES 6.2	0.200	0.996	0.879	0.684	0.663	-0.507	-0.691	-0.153	0.105	-0.467	0.946	1										
ES 6.4	0.179	0.996	0.877	0.705	0.684	-0.511	-0.707	-0.147	0.125	-0.452	0.958	0.999	1									
ES 6.6	0.246	1.000	0.850	0.657	0.633	-0.556	-0.727	-0.094	0.057	-0.416	0.940	0.998	0.998	1								
ES 6.8	0.091	0.904	0.956	0.631	0.622	-0.220	-0.386	-0.431	0.191	-0.733	0.835	0.932	0.921	0.914	1							
ES 7.0	-0.905	0.029	0.545	0.670	0.699	0.747	0.407	-0.937	0.949	-0.768	0.279	0.110	0.119	0.054	0.302	1						
α_{S1}	0.423	0.977	0.786	0.482	0.457	-0.606	-0.686	-0.016	-0.132	-0.386	0.841	0.969	0.960	0.976	0.909	-0.090	1					
α_{S2}	0.631	0.916	0.594	0.289	0.257	-0.777	-0.768	0.241	-0.368	-0.149	0.727	0.886	0.876	0.907	0.771	-0.349	0.964	1				
ß	-0.021	0.957	0.934	0.827	0.811	-0.360	-0.627	-0.302	0.322	-0.552	0.984	0.975	0.980	0.964	0.921	0.308	0.891	0.762	1			
κ	-0.233	0.856	0.859	0.942	0.928	-0.293	-0.643	-0.305	0.508	-0.454	0.983	0.876	0.892	0.863	0.771	0.415	0.731	0.590	0.952	1		
CMS	0.166	-0.403	-0.168	-0.619	-0.597	0.502	0.774	-0.301	-0.293	-0.330	-0.598	-0.374	-0.410	-0.393	-0.061	0.000	-0.232	-0.242	-0.444	-0.649	1	
Lactulose	-0.826	0.322	0.691	0.908	0.925	0.426	0.005	-0.771	0.958	-0.665	0.598	0.387	0.405	0.342	0.449	0.914	0.158	-0.085	0.581	0.723	-0.374	1

CONCLUSION

The alcohol stability and heat coagulation time were influenced by the pH during the high heat treatment of milk samples. Changes in κ-casein dissociation, denaturation of whey proteins, and a reduction in the concentration of soluble Ca in the serum were associated with these outcomes. For those who use skim milk powder in applications like recombined milks and heated milk-based beverages, the ability to control lactulose content, ethanol stability and heat coagulation time in defatted milk is crucial. Additionally, this approach could offer a new avenue for increasing lactulose content in milk through its use in lactose isomerization to lactulose, potentially leading to the development of innovative techniques.

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تأثير المعالجة الحرارية العالية عند درجة pH القلوية على الصفات الفيزيوكيميائية ومعدل تحول اللاكتوز إلى اللاكتولوز في اللبن الفرز

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الملخص

تم تعديل الرقم الهيدروجيني للحليب الخالي من الدسم، حيث تمت زيادته أولًا من 6.5 إلى 7.5، ثم تعرض للمعالجة الحرارية العالية عند 95 درجة مئوية لمدة دقيقتين أو تركه بدون تسخين لمدة ساعة واحدة. وبعد ذلك، تم تعديل الرقم الهيدروجيني مرة أخرى إلى 6.5، وتم تحليل العينات. أظهر تركيز اللاكتولوز في الحليب الخالي من الدسم زيادة كبيرة بسبب المعاملة الحرارية العالية (P <0.05)، وكان هذا التأثير أكثر وضوحًا عندما تم رفع الرقم الهيدروجيني من 6.5 إلى 7.5 قبل المعاملة الحرارية العالية. أدى رفع الرقم الهيدروجيني لكل من الحليب الخالي من الدسم المعالج بالحرارة العالية (HHT) وغير المسخن (UH) من 6.5 إلى 7.5 قبل التسخين أو الاحتفاظ به إلى تمديد وقت التخثر الحراري (HCT) عند الرقم الهيدروجيني 7.2 وأدى إلى زيادة بنسبة 58.7٪ في تفكك الشق كابا كازين κفي حليب HHT مقارنة بإجمالي محتوى الكابا كازين. لم يكن لتعديل الرقم الهيدروجيني أي تأثير كبير (P <0.05) على التركيب الكيميائي للحليب خالي الدسم عالي المعالجة بالحرارة وثبات الإيثانول في نطاق الرقم الهيدروجيني (6.2-7.2). إلى جانب تسخين العينة عند 95 درجة مئوية لمدة دقيقتين أدى إلى انخفاض كبير في حجم ميسيلات الكازين CMS. توفر هذه النتائج أساسًا للتركيز على التأثير السلبي للقلوية قبل المعالجة الحرارية العالية على تفكك الكازين، وCMS، والثبات الحراري والكحولي. بالإضافة إلى ذلك، فهو يعتبر نهجًا واعدًا مع إمكانية تعزيز محتوى اللاكتولوز في الحليب.

الكلمات الدالة: حليب منزوع الدسم، اللاكتولوز، ثبات الحرارة، الرقم الهيدروجيني عند التسخين، المعاملة الحرارية العالية