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Heat stability of skim milk containing various levels of micellar calcium phosphate

Elaheh Ahmadi [a], Thom Huppertz [a, b, c], Todor Vasiljevic [a, *]

[a] Advanced Food Systems Research Unit, Institute for Sustainable Industries and Liveable Cities, College of Sport, Health and Engineering, Victoria University, Melbourne, Australia
[b] FrieslandCampina, Amersfoort, the Netherlands
[c] Wageningen University and Research, Wageningen, the Netherlands

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ABSTRACT

This study assessed thermal stability in micellar calcium phosphate (MCP)-adjusted skim milk samples containing 67 (MCP_{67}) or 113 (MCP_{113}) % of the MCP content of control milk (MCP_{100}) at 120 °C for 5 s or 140 °C for 1 s at pH 6.3, 6.6, 6.9 or 7.2. Sample MCP_{67} exhibited the smallest heat-induced reduction in non-sedimentable individual caseins and whey proteins, and only limited heat-induced increases in particle size and turbidity. MCP_{67} samples exhibited the highest levels of non-sedimentable k-casein post-heating, a key factor in heat coagulation. Sample MCP_{113} displayed the strongest heat-induced decrease in non-sedimentable casein, coupled with the highest heat-induced increases in particle size and turbidity, suggesting comparatively lower thermal stability. Moreover, elevated MCP levels in MCP_{113} samples might contribute to micelle instability. Milk pH at heating exhibited a linear correlation with heat stability. Overall, the findings emphasize the substantial influence of MCP and pH on heat-induced alterations in sterilized milk.

1. Introduction

The heat stability of milk relates to its ability to retain its physical and chemical characteristics when it undergoes heat treatment. This characteristic is crucial in various dairy applications, particularly in processes like ultra-high temperature (UHT) treatment and other sterilization treatments (Dumpler, Huppertz, & Kulozik, 2020). Sterilization of milk is used for preservation through either in-container sterilization (115–120 °C for 5–15 min) or by continuous UHT treatment (135–150 °C for 1–10 s) (Dumpler et al., 2020; Lewis, Grandison, Lin, & Tsioulpas, 2011). While sterilization of milk offers benefits in terms of food safety and shelf-life, there are also some challenges and issues that can arise in the industry, which is including protein denaturation, Maillard reaction, vitamin degradation, and sediment formation on the surfaces (Dumpler et al., 2020).

During sterilization of milk, several structural changes occur due to the high temperature and short duration of heating (Dumpler et al., 2020). These changes collectively contribute to the stability of sterilized milk by changing in protein structure. Sterilization causes denaturation of the whey proteins present in milk. The denatured proteins can form aggregates, resulting in changes in the viscosity, texture, and stability of the milk (Qian et al., 2017). The Maillard reaction, which is a non-enzymatic browning reaction, can also occur during UHT treatment. It involves the reaction between lactose and amino acids, resulting in the formation of brown pigments and flavour compounds. It is important to note that these changes are influenced by various factors, including minerals especially calcium, temperature, pH, lactose and amino acid concentrations, reaction time, and the presence of catalysts or inhibitors (Lewis et al., 2011; Qian et al., 2017).

In addition, thermal treatment can also cause instability of milk as a result of the heat-induced aggregation of casein micelles, which can lead to either flocculation or complete gelation of products during or shortly after heat treatment. Over the years, many investigations have focused on the pivotal elements influencing the heat stability of skim milk, such as pH, minerals, κ-casein, and whey proteins (Tessier & Rose, 1964; Fox & Hoynes, 1975; Singh & Fox, 1987; Corredig & Dalgleish, 1996; Anema & Li, 2000, 2003; Wang & Ma, 2020; Ahmadi, Vasiljevic, & Huppertz, 2023). Several studies highlighted the importance of micellar calcium phosphate (MCP, also referred to as colloidal calcium phosphate, CCP) in maintaining micellar structure and its influence on micelle stability during heating (Anema & Li, 2000; Fox & Hoynes,

* Corresponding author.
E-mail address: todor.vasiljevic@vu.edu.au (T. Vasiljevic).

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1975; Singh & Fox, 1987). Regarding the heat stability of skim milk, Fox and Hoynes (1975) demonstrated an inverse relationship between MCP content and heat coagulation time (HCT) in skim milk at 140 °C. Furthermore, the heat coagulation time (HCT) notably exhibited a dependency on pH, indicating optimal stability just above the natural pH of milk (Fox & Hoynes, 1975).

Our previous investigation (Ahmadi et al., 2023) explored into the impact of heat treatment on MCP-adjusted skim milk at temperatures <100 °C across different pH levels. Notably, we observed a strong effect of MCP content on heat-induced changes at temperatures below 100 °C. Such findings could be applicable for some thermal treatments in the dairy sector, e.g., pasteurization of milk or heating of milk for yoghurt manufacture. However, for relevance for e.g., in-container sterilization and UHT treatment, temperatures >100 °C are required. Hence, expanding on this previous study, our objective was to investigate the influence of MCP content on thermal stability and heat-induced changes in skim milk at temperatures >100 °C. Hence, the present study examined the thermal stability of the MCP-adjusted skim milk samples by varying MCP content via a 33% reduction and a 13% increase compared to the initial MCP level in control milk, to observe how it affects the thermal stability of milk. As heat stability of milk is also pH dependent, MCP-adjusted skim milk samples were also heated at different pH levels, 6.3, 6.6, 6.9, and 7.2.

2. Materials and methods

2.1. Sample preparation and fractionation

The experimental design for sample preparation and analysis is illustrated schematically in Fig. 1. Freshly pasteurized skim milk was sourced from Warrnambool Cheese and Butter – Saputo (Warrnambool, Australia) and 0.02% sodium azide was added to prevent bacterial growth. The MCP adjustment in skim milk involved adjusting the pH of the skim milk samples to 6.1 and 7.5 using predetermined quantities of GDL and 1.0 M NaOH, respectively, as described previously (Ahmadi et al., 2023). Subsequently, dialysis procedures were carried out as described previously (Huppertz & Lambers, 2020; Pyne & McGann, 1960). After dialysis, milk pH was adjusted to 6.3, 6.6, 6.9, or 7.2, then, samples were heated at 120 °C for 5 s or 140 °C for 1 s, followed by swift cooling to 20 °C. pH 6.6 was considered as the control pH.

To separate the sedimentable and non-sedimentable phases before and after heat treatment, ultracentrifugation at 100,000 × *g* for 60 min was performed as described previously (Ahmadi et al., 2023).

2.2. Sample analysis

Calcium content of whole samples and ultracentrifugal supernatants was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) with a Shimadzu ICPE-9000 system (Shimadzu Corporation, Kyoto, Japan), based on the methodology described by Bijl, van Valenberg, Huppertz, and van Hooijdonk (2013).

Particle size analysis was analysed using a Zetasizer-Nano series instrument (Malvern Instruments Ltd., Malvern, UK) as previously described (Ahmadi et al., 2023).

The turbidity was determined at 860 nm with a UV–Visible spectrophotometer (Biochrom Ltd, Cambridge, UK), using a 1 mm pathlength quartz cuvette (Ahmadi et al., 2023).

The protein distribution in the serum was assessed using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) analysis of the whole samples and ultracentrifugal supernatants was performed at room temperature using a Shimadzu HPLC system (Model Prominence-i, LC-2030 C, Shimadzu Corporation, Kyoto, Japan), controlled by a Varian 9012 system controller (Agilent Technologies Inc., Santa Clara, CA). This system integrated an RI detector (Varian, 9050) and a C4 column (Aeris WIDEPORE, 150 mm × 4.6 mm, 3.6 μm particle size, 300 Å porosity, Phenomenex, Torrance, USA). Sample preparation followed the method outlined by Aprianita, Vasiljevic, Bannikova, and Kasapis (2014).

2.3. Statistical analysis

Statistical analysis was conducted using a split plot blocked design analysed as a General Linear Model with the MCP level as the main plot and pH adjustment and temperature/time as a

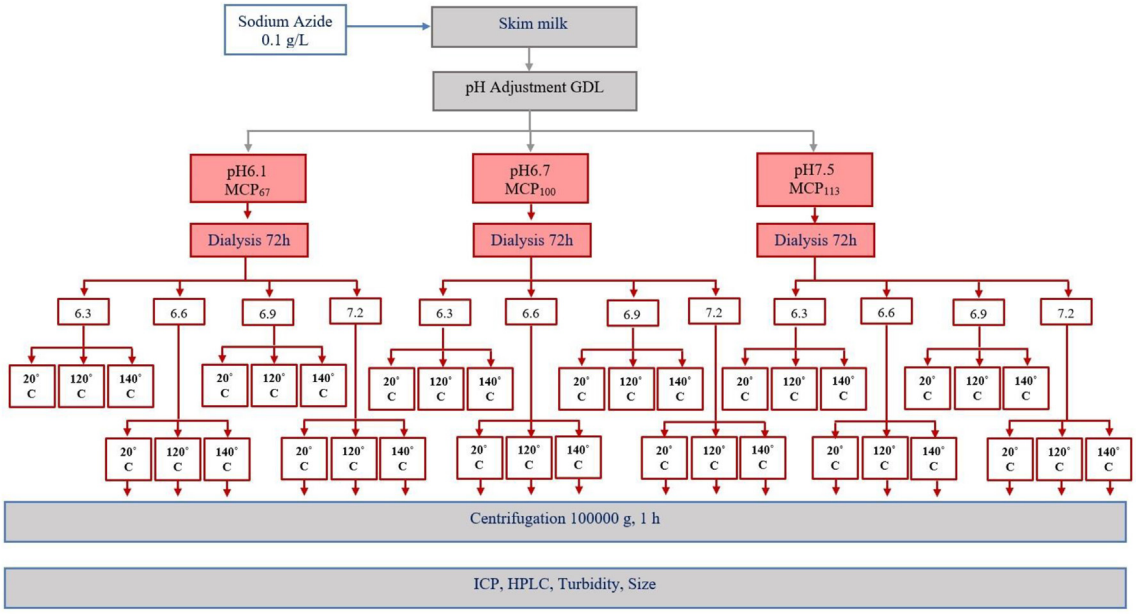

Fig. 1. Experimental design of the study.

subplot. The replications served as a block. The data was analysed using a SAS statistical software (v. 9.1 SAS Institute, Cary, NC, USA). The level of significance was established at $P < 0.05$. The experimental setup was replicated three times.

3. Results

3.1. Heat-induced changes in physicochemical properties of MCP-adjusted milk

The initial average particle size of the unheated control milk (MCP_{100}, pH 6.6) was 161 nm (Table 1) and the particle size distribution of all samples exhibited a primary peak between 50 and 400 nm (Fig. 2). MCP reduction led to a small decrease in particle size and turbidity, whereas the adjustment of milk pH after dialysis did not affect particle size and turbidity in unheated samples (Table 1).

After heat treatment at 120 °C for 5 s and 140 °C for 1 s, differences in particle size and turbidity were observed, with notable effects of pH and MCP content (Table 1). As expected, thermal stability was lowest at pH 6.3, with visible coagulation observed in samples MCP_{100} and MPC_{113} heated at this pH and significant ($P < 0.05$) increases in particle size and turbidity in sample MPC_{67} heated at pH 6.3 (Table 1). Significant increases in particle size and

Table 1
Influence of heat treatment at 120 °C for 5 s or 140 °C for 1 s at pH 6.3, 6.6, 6.9 or 7.2 on the particle size and turbidity of pasteurized skim milk samples with their micellar calcium phosphate (MCP) content adjusted to 67% (MCP_{67}) to 113% (MCP_{113}) of control milk (MCP_{100}). For sample details, see Fig. 1.

MCP content	Adjusted pH	Size (nm)			Turbidity (cm^{-1})		
		unheated	120 °C	140 °C	unheated	120 °C	140 °C
MCP_{67}	6.3	161.7^{Cab}	616.1^{Ba}	862.7^{Aa}	0.28^{Ccd}	1.71^{Ba}	1.89^{Aa}
	6.6	161.9^{Cab}	200.3^{Bbc}	241.3^{Ae}	0.30^{Cc}	0.48^{Bd}	0.70^{Ae}
	6.9	158.9^{Bab}	158.4^{Bf}	305.5^{Ac}	0.33^{Cbc}	0.38^{Be}	1.44^{Ab}
	7.2	156.8^{Bb}	151.1^{Bf}	219.1^{Af}	0.31^{Bc}	0.30^{Bf}	0.57^{Af}
MCP_{100} (control)	6.3	160.9^{Aab}	Coagulated	Coagulated	0.32^{Abc}	Coagulated	Coagulated
	6.6	161.3^{Cab}	210.4^{Bb}	244.4^{Ae}	0.34^{Cbc}	0.54^{Bc}	0.85^{Ad}
	6.9	166.8^{Ca}	179.5^{Bd}	265.5^{Ad}	0.35^{Cb}	0.40^{Be}	1.17^{Ac}
	7.2	168.6^{Ba}	168.3^{Bde}	199.6^{Af}	0.36^{Bab}	0.36^{Be}	0.48^{Ag}
MCP_{113}	6.3	164.2^{Aa}	Coagulated	Coagulated	0.37^{Aab}	Coagulated	Coagulated
	6.6	162.5^{Cab}	193.6^{Bcd}	310.9^{Ac}	0.39^{Ca}	0.70^{Bb}	1.39^{Ab}
	6.9	166.6^{Cab}	180.9^{Bd}	557.9^{Ab}	0.35^{Cb}	0.54^{Bc}	1.32^{Ab}
	7.2	162.2^{Bab}	171.1^{Bde}	209.9^{Af}	0.41^{Ca}	0.48^{Bd}	0.54^{Af}

Lower- and upper-case superscript letters indicate significant difference ($P < 0.05$) within a row and a column, respectively.

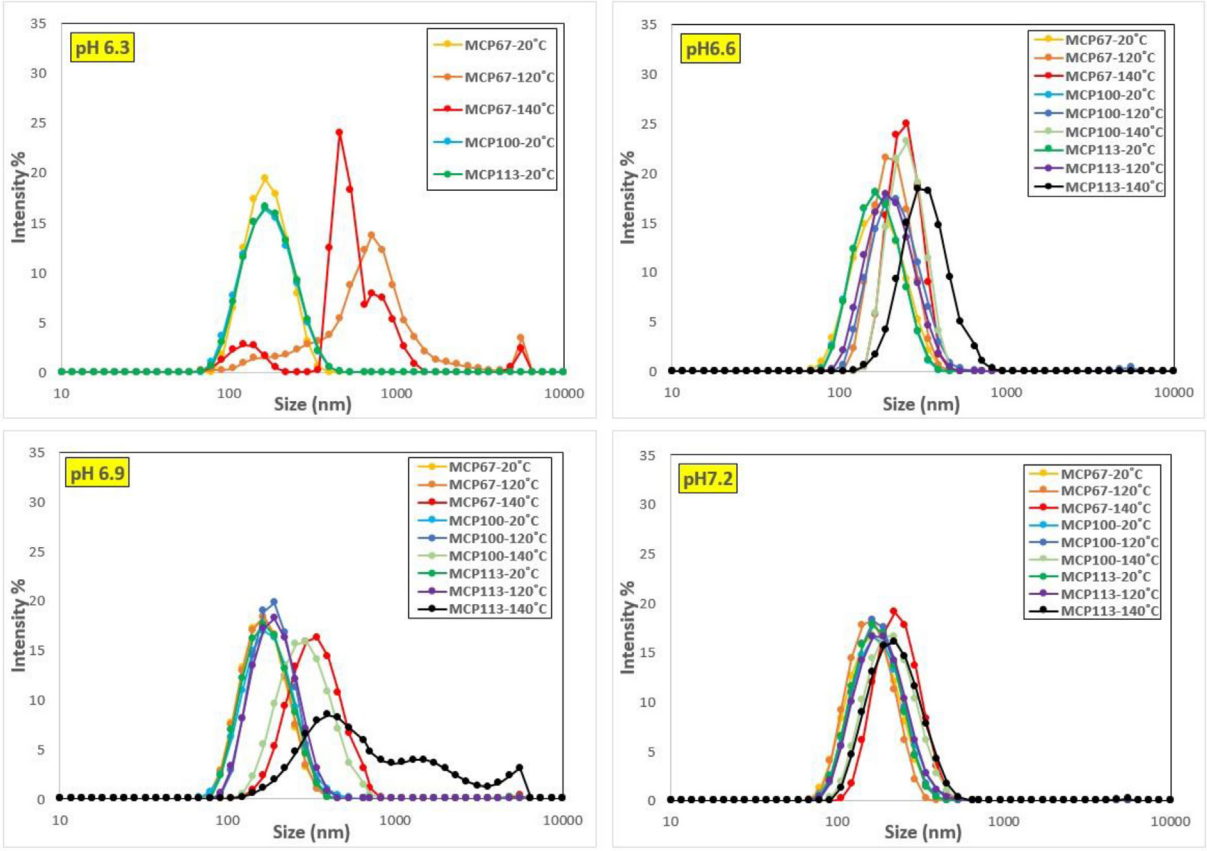

Fig. 2. Influence of heat treatment at 120 °C for 5 s or 140 °C for 1 s at pH 6.3, 6.6, 6.9 or 7.2 on the particle size distribution of pasteurized skim milk samples with their micellar calcium phosphate (MCP) content adjusted to 67% (MCP_{67}) to 113% (MCP_{113}) of control milk (MCP_{100}).

Table 2
Influence of heat treatment at 120 °C for 5 s or 140 °C for 1 s at pH 6.3, 6.6, 6.9 or 7.2 on the total calcium and non-sedimentable (100,000 × *g* for 60 min) calcium content of pasteurized skim milk samples with their micellar calcium phosphate (MCP) content adjusted to 67% (MCP_{67}) to 113% (MCP_{113}) of control milk (MCP_{100}). For sample details, see Fig. 1.

Samples	Adjustment pH	Calcium concentration (mmol L^{-1})					
		Total			Non-sedimentable		
		Unheated	120 °C	140 °C	Unheated	120 °C	140 °C
MCP_{67}	6.3	24.2^{Ac}	24.3^{Ac}	24.6^{Ac}	9.5^{Ab}	5.6^{Cc}	5.9^{Bb}
	6.6	25.4^{Ac}	24.2^{Ac}	25.0^{Ac}	7.5^{Ae}	5.2^{Bd}	5.0^{Ce}
	6.9	24.4^{Ac}	24.2^{Ac}	25.4^{Ac}	5.6^{Ai}	4.5^{Bf}	4.5^{Bf}
	7.2	24.1^{Ac}	23.6^{Ac}	23.9^{Ac}	5.5^{Aij}	4.8^{Ce}	5.0^{Be}
MCP_{100} (Control)	6.3	30.5^{Ab}	Coagulated	Coagulated	9.8^{Aa}	Coagulated	Coagulated
	6.6	30.7^{Ab}	30.2^{Ab}	29.4^{Ab}	7.9^{Ad}	5.5^{Bc}	5.7^{Cc}
	6.9	30.4^{Ab}	29.8^{Ab}	30.3^{Ab}	6.2^{Ag}	4.3^{Bf}	3.8^{Cg}
	7.2	29.8^{Ab}	31.2^{Ab}	30.3^{Ab}	5.4^{Aj}	4.4^{Cf}	5.1^{Bd}
MCP113	6.3	35.4^{Aa}	Coagulated	Coagulated	9.0^{Ac}	Coagulated	Coagulated
	6.6	35.3^{Aa}	34.9^{Aa}	34.5^{Aa}	7.3^{Af}	5.5^{Bc}	5.1^{Cd}
	6.9	35.0^{Aa}	34.7^{Aa}	35.2^{Aa}	5.9^{Ah}	4.4^{Bf}	3.5^{Ch}
	7.2	34.8^{Aa}	34.6^{Aa}	35.2^{Aa}	5.0^{Ak}	4.0^{Bg}	4.9^{Ae}

Lower- and upper-case superscript letters indicate significant difference ($P < 0.05$) within a row and a column, respectively.

turbidity were also observed for all other samples heated at 140 °C. However, for samples heated at 120 °C, no significant heat-induced increases in particle size were observed for all samples at pH 7.2 and for sample MCP_{67} at pH 6.9 (Table 1). Similar trends were observed in particle size distributions (Fig. 2). From the heat-induced changes in particle size and turbidity, it may thus be concluded that the stability of the casein micelles to heat-induced coagulation decreased with increasing MCP content and decreasing pH.

3.2. Heat-induced changes in the calcium distribution in MCP-adjusted skim milk

The effect of MCP-adjustment, pH and heat treatment on the levels of total and non-sedimentable Ca in skim milk is shown in Table 2. In the control sample (MCP_{100} at pH 6.6), total and non-sedimentable Ca content were 30.7 mmol L^{-1} and 7.9 mmol L^{-1}, respectively. Total Ca was ~5 mmol L^{-1} lower after MCP-depletion and ~5 mmol L^{-1} after MCP-enrichment (Table 2). As expected, pH adjustment after MCP adjustment did not affect total Ca content and neither did heat treatment at 120 or 140 °C (Table 2). Non-sedimentable Ca, in contrast, was comparable for all samples at pH 6.6 and decreased with increasing milk pH (Table 2). Heat treatment at 120 and 140 °C caused a significant ($P < 0.05$) reduction in the concentration of non-sedimentable Ca in all samples. In general, heat-induced reductions in non-sedimentable Ca were largest for samples that were heated at pH 6.3 and 6.6. Interestingly, in several instances, non-sedimentable calcium at pH 6.9 was lower than at pH 7.2 (Table 2). This observation may be linked to higher levels of non-sedimentable casein in samples heated at pH 7.2, as well be discussed later.

3.3. Heat-induced changes in the protein distribution of MCP-adjusted skim milk

The proportion of non-sedimentable caseins and whey proteins in the serum relative to total levels in the milk is shown in Table 3 and Table 4, respectively. In the unheated samples, reducing the MCP content significantly ($P < 0.05$) increased the concentrations of non-sedimentable αs_1-, αs_2-, β-, and κ-caseins, whereas MCP-enrichment decreased non-sedimentable levels of these caseins (Table 3). Adjusting the pH (6.3, 6.6, 6.9, or 7.2) had limited impact on the levels of non-sedimentable caseins in unheated samples (Table 3). After heating sample MCP_{100}, the concentration of most non-sedimentable caseins was not strongly ($P > 0.05$) affected, with a notable exception for κ-casein, which showed heat-induced dissociation at pH 6.9 and 7.2 (Table 3). For sample MCP_{67}, heat treatment caused reductions in levels of non-sedimentable α_{s1}-,

Table 3
Influence of heat treatment at 120 °C for 5 s or 140 °C for 1 s at pH 6.3, 6.6, 6.9 or 7.2 on the levels of non-sedimentable caseins (100,000 × *g* for 60 min) pasteurized skim milk samples with their micellar calcium phosphate (MCP) content adjusted to 67% (MCP_{67}) to 113% (MCP_{113}) of control milk (MCP_{100}). For sample details, see Fig. 1.

MCP Adjusted sample	pH	Milk proteins concentration (%)[a]											
		α_{s1}-Casein			α_{s2}-Casein			β-Casein			κ-Casein		
		Unheated	120 °C	140 °C	Unheated	120 °C	140 °C	Unheated	120 °C	140 °C	Unheated	120 °C	140 °C
MCP_{67}	6.3	17.1^{Aab}	9.1^{Bbc}	5.4^{Cde}	11.2^{Ac}	3.6^{Bbc}	2.4^{Bbc}	31.6^{Ab}	16.6^{Bcd}	12.8^{Bf}	29.1^{Abc}	23.6^{Be}	23.5^{Bde}
	6.6	16.7^{Aab}	7.6^{Bcde}	7.4^{Bd}	11.2^{Ac}	3.4^{Bbc}	1.4^{Ccd}	32.8^{Ab}	18.8^{Ccd}	23.0^{Bd}	29.7^{abc}	23.6^{Be}	21.9^{Be}
	6.9	15.5^{Ab}	8.7^{Bbc}	21.8^{Cb}	13.4^{Ab}	3.6^{Bbc}	3.4^{Bb}	39.0^{Aa}	27.6^{Bab}	30.0^{Bbc}	31.6^{Bab}	65.6^{Aa}	69.4^{Aa}
	7.2	18.5^{Ba}	19.8^{Ba}	40.9^{Aa}	18.6^{Aa}	4.0^{Cb}	9.8^{Ba}	42.8^{Aa}	31.3^{Ba}	44.6^{Aa}	34.1^{Ca}	68.3^{Aa}	50.9^{Bb}
MCP_{100} (control)	6.3	10.6^{Ac}	Coagulated	Coagulated	6.4^{Ade}	Coagulated	Coagulated	26.0^{Acd}	Coagulated	Coagulated	27.6^{Abc}	Coagulated	Coagulated
	6.6	9.4^{Ac}	4.3^{Bfg}	2.8^{Bef}	6.3^{Ade}	4.6^{ABb}	3.5^{Bb}	24.1^{ABd}	27.1^{Ab}	22.4^{Bd}	30.0^{Aabc}	15.5^{Bf}	13.8^{Bf}
	6.9	10.3^{Ac}	5.2^{Bef}	3.4^{Bef}	7.1^{Ad}	1.2^{Bd}	1.6^{Bbc}	31.2^{Ab}	20.5^{Bc}	16.6^{Ce}	26.2^{Bbc}	53.9^{Ac}	49.8^{Ab}
	7.2	3.9^{Cd}	10.3^{Bb}	18.0^{Ac}	8.3^{Ad}	1.9^{Bcd}	2.3^{Bbc}	30.5^{Ab}	29.9^{Aab}	33.5^{Ab}	27.4^{Cbc}	61.0^{Ab}	32.0^{Bc}
MCP_{113}	6.3	4.9^{Ad}	Coagulated	Coagulated	5.3^{Ade}	Coagulated	Coagulated	17.2^{Af}	Coagulated	Coagulated	18.7^{Aef}	Coagulated	Coagulated
	6.6	5.8^{Ad}	4.0^{ABfg}	2.0^{Bfg}	5.0^{Be}	8.2^{Aa}	2.5^{Cbc}	20.0^{Bef}	19.6^{Bc}	28.0^{Ac}	20.3^{Aef}	15.3^{Bf}	13.2^{Bf}
	6.9	8.5^{Ac}	2.9^{Bg}	4.9^{Bde}	4.4^{Aef}	0.9^{Bd}	2.0^{Bbc}	23.3^{Bde}	15.4^{Cd}	31.3^{Abc}	22.6^{Bde}	53.5^{Ac}	49.4^{Ab}
	7.2	4.6^{Bd}	6.0^{Bdef}	15.5^{Ac}	5.3^{Ae}	0.6^{Bd}	2.6^{Bbc}	22.2^{Be}	27.7^{Aab}	27.0^{Ac}	17.3^{Cf}	51.9^{Acd}	27.9^{Bcd}

Lower- and upper-case superscript letters indicate significant difference ($P < 0.05$) within a row and a column, respectively.
[a] The percentage of caseins in the serum relative to the original milk.

Table 4
Influence of heat treatment at 120 °C for 5 s or 140 °C for 1 s at pH 6.3, 6.6, 6.9 or 7.2 on non-sedimentable whey proteins (100,000 × *g* for 60 min) in pasteurized skim milk samples with their micellar calcium phosphate (MCP) content adjusted to 67% (MCP_{67}) to 113% (MCP_{113}) of control milk (MCP_{100}).

MCP Adjusted sample	pH	Relative protein concentration (%)[a]					
		α-Lactalbumin			β-Lactoglobulin		
		Unheated	120 °C	140 °C	Unheated	120 °C	140 °C
MCP_{67}	6.3	97.9^{Aa}	34.5^{Bc}	12.7^{Cg}	91.2^{Aa}	34.2^{Bbc}	32.5^{Bd}
	6.6	91.6^{Aa}	52.5^{Ba}	29.3^{Ce}	90.4^{Aa}	35.8^{Bbc}	33.8^{Bd}
	6.9	95.4^{Aa}	39.9^{Cb}	53.1^{Bab}	93.9^{Aa}	59.2^{Ba}	51.8^{Bb}
	7.2	97.7^{Aa}	39.4^{Cb}	56.3^{Ba}	91.9^{Aa}	39.6^{Cb}	70.1^{Ba}
MCP_{100} (Control)	6.3	94.9^{Aa}	Coagulated	Coagulated	90.0^{Aa}	Coagulated	Coagulated
	6.6	96.7^{Aa}	27.0^{Bd}	19.6^{Cf}	98.1^{Aa}	29.3^{Bc}	18.2^{Ce}
	6.9	97.4^{Aa}	38.2^{Cb}	46.1^{Bc}	94.4^{Aa}	30.8^{Cbc}	49.6^{Bb}
	7.2	95.7^{Aa}	34.7^{Bc}	36.2^{Bd}	93.1^{Aa}	30.9^{Cbc}	51.6^{Bb}
MCP_{113}	6.3	94.9^{Aa}	Coagulated	Coagulated	93.1^{Aa}	Coagulated	Coagulated
	6.6	94.2^{Aa}	11.4^{Bf}	11.7^{Bg}	89.5^{Aa}	10.7^{Be}	15.7^{Be}
	6.9	95.6^{Aa}	18.9^{Ce}	42.8^{Bc}	89.7^{Aa}	36.9^{Cbc}	47.9^{Bbc}
	7.2	94.5^{Aa}	20.8^{Ce}	51.4^{Bb}	90.9^{Aa}	23.4^{Ccd}	43.7^{Bbc}

Lower- and upper-case superscript letters indicate significant difference ($P < 0.05$) within a row and a column, respectively.
[a] The proportion of whey proteins in the serum relative to the original milk.

α_{s2}-and β-caseins at pH 6.3–6.9, whereas for non-sedimentable κ-casein, notable heat-induced increases were observed at pH 6.9 and 7.2. For non-sedimentable α_{s1}-casein, a notable heat-induced increase was also observed at pH 7.2 after heat treatment at 140 °C (Table 3). For sample MCP_{113}, the main heat-induced increases in non-sedimentable casein were also observed for κ-casein at pH 6.9 and 7.2 and for α_{s1}-casein at pH 7.2 (Table 3). In the unheated samples, virtually all whey proteins was non-sedimentable (Table 4). In the heated samples, a parallel trend emerges between the non-sedimentable fractions of whey proteins and the pH level, mirroring observations made for κ-casein. As the pH increased, the proportion of non-sedimentable whey proteins also rose (Table 4).

4. Discussion

Heat treatment induces significant structural changes in milk, closely linked to influential factors such as calcium levels and pH (Dumpler et al., 2020; Lewis et al., 2011; Nieuwenhuijse & Huppertz, 2022). Continuing on our previous investigation (Ahmadi et al., 2023), this study expands understanding of the heat stability of three distinct MCP-adjusted skim milk samples: MCP_{67} (33% MCP-depleted), MCP_{113} (13% MCP-enriched), and the control, MCP_{100} to temperatures >100 °C. It is well known that pH plays an important role in the heat-induced changes in milk (Anema, 2021; Nieuwenhuijse & Huppertz, 2022). In the present study, heating at a reduced pH (6.3) resulted in reduced thermal stability of milk, as seen by visible coagulation as well as increase in turbidity and particle size (Table 1). These findings were in a good agreement with those reported previously (Fox & Hoynes, 1975; Singh & Fox, 1987). The reduced micellar charge below the natural milk pH (<6.7) appears to fail in counteracting the heat-induced alterations, leading to thermal instability (Singh & Fox, 1985).

Adjustment of MCP content within the casein micelle has been reported to affect the heat coagulation time (HCT) of skim milk at a set temperature (140 °C) (Fox & Hoynes, 1975; Singh & Fox, 1985). In present study, UHT treatment was applied for a set time (120 °C for 5 s and 140 °C for 1 s; Fig. 1). In line with our previous findings (Ahmadi et al., 2023), MCP reduction resulted in enhanced thermal stability, albeit accompanied by an increase in particle size at high high temperatures (Table 1), which aligns with previous findings (Fox & Hoynes, 1975; Singh & Fox, 1985). For instance, MCP-enhanced skim milk samples displayed a strong increase in particle size and turbidity (Table 1, Fig. 2), and a decrease in non-sedimentable individual caseins at high pH and coagulated at low pH (Table 3). On the other hand, in the MCP-depleted skim milk samples, the impact of UHT treatment was less pronounced, and visible coagulation did not occur, even at the lowest pH. The observed increases in particle size after heat treatment (Table 1, Fig. 2) went beyond the anticipated interactions between whey proteins and casein micelles, pointing to substantial micellar aggregation. In our previous study, conducted at a lower temperature, no significant aggregation or coagulation occurred (Ahmadi et al., 2023). In contrast, coagulation was observed at low pH in the current study; however, the reduction of MCP appeared to prevent heat induced coagulation. This highlights how minor fluctuations in MCP content significantly impact thermal stability of milk. Although variations in MCP content were process-induced in this study, it is important to note that notable variation also occurs naturally between milk from individual cows (Huppertz, Heck, Bijl, Poulsen, & Larsen, 2021). Although heat stability is known to vary widely between milk from individual cows (Davies & White, 1966), the influence of variation in MCP content has not been investigated as a contributing factor to date.

Heat-induced dissociation of κ-casein has often been linked to thermal instability of milk (Anema, 2021; Dumpler et al., 2020; Huppertz, 2016). The heat-induced dissociation of k-casein occurs primarily at elevated pH levels following UHT-treatment, in line with previous findings (Anema & Li, 2000; Singh & Fox, 1985). Interestingly, MCP content had only a minor impact on the heat-induced dissociation of κ-casein (Table 3). Although heat-induced κ-casein dissociation is typically deemed a primary trigger for heat coagulation, our observations with MCP-depleted skim milk samples heated at high pH revealed substantial κ-casein dissociation following heating (Table 3). In addition, the distribution of whey proteins between the sedimentable and non-sedimentable phases closely followed that of κ-casein (Table 4). However, the MCP-depleted skim milk exhibited increased thermal stability, evident by the absence of visible coagulation and minimal impact on particle size and turbidity (Table 1), implying the involvement of other factors in the coagulation process. Considering other factors involved in heat-induced coagulation, MCP-enhanced skim milk, which displayed largest particle size and highest turbidity after heating, contains highest micellar calcium levels. Micellar calcium can be defined as the amount of Ca that sediments on ultracentrifugation (Huppertz & Lambers, 2020) and it can be estimated from the difference between total and non-sedimentable calcium (Table 2). During the heating, Ca and PO_4 tend to precipitate in the

casein micelle (Nieuwenhuijse & Huppertz, 2022). This can lead to the exceeding critical levels of MCP, thereby causing instability in the micelles. Consequently, the heat-induced changes observed in skim milk are influenced by fluctuations in MCP content.

5. Conclusion

The study highlighted the substantial impact of MCP adjustment on alterations induced by ultra-high-temperature treatment, significantly influencing the overall heat stability of milk. 33% MCP-depletion appeared to have the greatest thermal stability among all samples. MCP adjustment led to varying behaviour among individual caseins, influencing their interactions in the soluble and colloidal phases. The pH level of the milk was also observed to have a linear correlation with heat stability during UHT-treatment. Notably, this work provided for the first time an insight into the effect of MCP-adjustment on the impact of ultra-high temperature treatment on skim milk heated at different pH.

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CRediT authorship contribution statement

Elaheh Ahmadi: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Thom Huppertz:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Todor Vasiljevic:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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