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Effect of heat treatment during skim milk powder manufacture on the compositional and processing characteristics of reconstituted skim milk and concentrate

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ABSTRACT

The effects of key manufacturing steps (heat treatment, evaporation and spray drying) during the manufacture of low- and high-heat skim milk powders (SMP) on the physico-chemical and processing characteristics of milk, and concentrates of varying total solids (TS) levels prepared by reconstituting the milk powders, were evaluated. Milk heat treatment had the most pronounced effect, with an increase in severity of heat treatment from 72 °C × 15 s to 120 °C × 120 s, prior to evaporation resulting in higher heat coagulation time (HCT) at pH 6.3–6.6 and ethanol stability (ES) at pH 6.2–6.6, and a marked deterioration of rennet-induced coagulability. Increasing TS of the milk on reconstitution from 9.4 to 25% reduced HCT at pH >6.3 and ES at pH 6.6–7.0, increased ES at pH 6.2–6.4, and led to partial recovery of rennet-coagulability. The results highlight how heat treatment may be used to customise the functionality of SMP to different applications.

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1. Introduction

Apart from its use in formulated foods such as sauces, custards, ice-cream and processed cheese products, skim milk powder (SMP) is extensively reconstituted to skim milk with different levels of total solids (e.g., 9–30%), for applications such as milk-based beverages, condensed milks, and recombined milks for cheese or yoghurt manufacture (Gilles & Lawrence, 1982; IDF, 19; Lagrange, Whitsett, & Burris, 201599). SMP is classified as low, medium- or high-heat SMP according to the heat treatment applied to skim milk prior to evaporation and drying (Martin, Williams, & Dunstan, 2007). Typical heat treatments are 70–72 °C for 15 s for low-heat SMP, and 120 °C for 60–120 s, or 90 °C for 300 s (Kelly, O'Connell, & Fox, 2003). High-heat SMP is used as an ingredient in bakery, sweetened condensed milk, and confectionery products such as UHT recombined concentrated milk, toffee, caramel, fudge and milk chocolate (Aitken, Agustin, & Clarke, 1999; Stewart et al., 2017). Low-heat powder is also used extensively in food formulation, including applications such as recombined milk for cheese manufacture, milk solids standardisation in products such as cheese milk, yoghurt and fermented milk products (Patel, Anema, Holroyd, Singh, & Creamer, 2007).

For all types of SMP, the stages of manufacture include heat treatment of the milk, evaporation to ~45–50% total solids (TS) and spray drying to ~97% TS. Heat treatment, depending on the severity (temperature and time) and milk pH, affects the extent of whey protein denaturation, the binding of denatured whey protein to the casein micelle and the partitioning of components (salts, whey protein and caseins) between the serum and colloidal phases of milk (Donato & Guyomarc'h, 2009). These changes affect milk processing characteristics such as rennet gelation (Guinee et al., 1997; Pomprasirt, Singh, & Lucey, 1998), acid-induced gelation (Vasbinder, Alting, & de Kruif, 2003a), heat stability (Sievanen,

Huppertz, Kelly, & Fox, 2008), syneresis of acid-induced and rennet-induced-milk gels (e.g., yoghurt, cheese), and can result in altered cheese texture and functionality (Rynne, Beresford, Kelly, & Guinee, 2004).

Studies on the impact of heat treatment on the ethanol stability (ES) of skim milk concentrates are scarce, though the separate effects of heat treatment (Horne & Parker, 1981; Mohammed & Fox, 1986) and concentration (Horne & Parker, 1983) have been investigated. ES is of relevance in alcoholic milk-based beverages (e.g., cream liquor, eggnog and coquito) as an indicator of the resistance of the milk protein to aggregation and, hence, emulsion stability. Martin et al. (2007) reported that the casein micelle sizes in low-, medium- and high-heat treated skim milk increased during evaporation to 45% TS, and remained high in high-heat SMP on reconstitution. Singh and Creamer (1991) found that the heat coagulation time (HCT) of concentrated milk (prepared by diluting evaporated milk to 20% TS) in the pH region 6.3 to 6.6 increased significantly on increasing severity of heat treatment from 72 °C × 15 s to 120 °C × 180 s. Similarly, an increase in heat treatment from 110 °C × 120 s to 120 °C × 180 s affected the heat stability of reconstituted milk (9.7% TS), as evidenced by a shift in the HCT/pH curve to lower pH and the concomitant increase in HCT at pH 6.5–6.6 and reduction at pH 6.8–7.1 (Singh & Creamer, 1991).

The objective of the current study was to evaluate the impact of heat treatment, evaporation and spray drying on the partitioning of milk proteins and minerals between serum and colloidal phases, rennet gelation, HCT and ES of the resultant milk samples, and concentrates prepared by reconstitution of the SMP.

2. Materials and methods

2.1. Manufacture of low heat and high heat skim milk powder

Skim milk powder was manufactured at Moorepark Technology Limited (Cork, Ireland). Milk was separated at 55 °C (Westfalia Model MM1254 Separator; Westfalia, Germany) and the skim milk (≤0.1% fat) was pasteurised using a plate heat-exchanger (APV Pasilac SSP pilot plant, APV DK 8600, Silkeborg, Denmark) at 72 °C × 15 s (low heat, LH) or using a MicroThermics® pilot-scale tubular heat-exchanger (MicroThermics, Raleigh, NC, USA) at 120 °C for 120 s (high heat, HH). The pasteurised skim milk was cooled directly to 4 °C, held at 4 °C overnight, heated to 50 °C, stirred for 30 min, concentrated to 45% TS (Anhydro Falling Film Evaporator Type F, SPX Flow Technology Danmark A/S, Soeborg, DK-2860, Denmark) and spray-dried (Anhydro Spray Dryer, SPX Flow Technology Danmark A/S) using centrifugal disc atomisation at inlet and outlet air temperatures of 180 and 85 °C, respectively. The resultant LH- and HH-skim milk powders were each produced on two separate occasions (trials), with both powder types being produced from the same milk on each occasion.

2.2. Preparation of skim milk samples

Samples taken during powder manufacture included: skim milk, heat-treated skim milk, evaporated skim milk (45% TS) and powder. Samples of low heat-treated skim milk, evaporated skim milk and powder are denoted as LHSM, LHE and LHP, respectively, and the corresponding high heat-treated samples as HHSM, HHE and HHP, respectively (Table 1).

The LHE and HHE samples were diluted with distilled water at 25 °C and stirred (Model RW16; IKA-Werke GmbH, Staufen im Breisgau, Germany) at 750 rpm for 30 min to give skim milk with 9.4% TS, denoted as LHE-SM and HHE-SM, respectively (Table 1).

Skim milk samples (9.4% TS), denoted LHP-SM and HHP-SM, were also prepared by reconstitution of the LHP and HHP in distilled water. The powder was dispersed in distilled water (50 °C), held in a water bath (50 °C) while stirring at 750 rpm for 30 min and stored at 4 °C for 22 h to allow hydration of the protein; prior to analysis, the reconstituted skim milk samples were warmed to 40 °C and held for 30 min to reverse the cold-aging, and then cooled to 25 °C for analysis (Dalgleish & Law, 1988).

2.3. Compositional analysis of skim milk and serum

Skim milk samples were assayed for TS and fat using the CEM SMART Trac II (CEM, Matthews, NC, USA), lactose using the FOSS MilkoScan™ FT+ (N. Foss Electric A/S, Hillerød, Denmark) and ionic calcium [Ca^{2+}], using a sensION+ 9660C Calcium Combination Ion Selective Electrode (Hach Lange, Barcelona, Spain), as described by Lin, Kelly, O'Mahony, and Guinee (2017).

Serum was prepared by ultracentrifugation of skim milk at 100,000 × *g* at 25 °C for 1 h and filtration of the supernatant, as described by Lin et al. (2017). Skim milk and serum were analysed for total protein, non-casein nitrogen (NCN), non-protein nitrogen (NPN), calcium (Ca), phosphorus (P), and protein profile using reversed-phase high performance liquid chromatography (RP-HPLC) using methods described previously by Lin et al. (2017).

The analysis scheme used to isolate the different nitrogen (N) /protein fractions of the HH samples is shown in Fig. 1A; the measurements performed on the different samples and the parameters derived are shown in Fig. 1B. The true protein content of the serum was calculated as the difference between total (crude) protein of serum and NPN (expressed as protein). Total serum casein was calculated as the product of true protein in the serum and casein as a proportion of true protein in the serum, as measured by RP-HPLC.

On pH adjustment of the serum to pH 4.6, serum-soluble casein (κ-, β-, α_S-caseins) and denatured whey protein, assumed to be complexed with κ-casein in the form of serum-soluble aggregates (Mollé, Jean, & Guyomarc'h, 2006), precipitate. Hence, the total protein concentration of the pH 4.6 soluble filtrate corresponds to native whey protein and NPN. The concentrations of serum-soluble casein and denatured whey protein/κ-casein aggregates were, thus, calculated as the difference between the total protein content of the serum and that of the pH 4.6 soluble filtrate. The difference in concentration between that of the latter (serum-soluble casein and denatured whey protein/κ-casein aggregates) and the serum casein corresponds to denatured whey proteins contributing to serum-soluble aggregates. The equations used in the calculation of the different N fractions in the serum phase are below:

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

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

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

Denatured whey protein complexed with κ-casein on the casein micelle (%, w/w) = Total denatured whey protein (%, w/w) – denatured whey protein complexed with dissociated κ-casein (%, w/w) (4)

2.4. Physico-chemical characteristics of skim milk samples

Casein micelle size, expressed as z-average (nm), and the apparent zeta potential of skim milk samples were determined using a Malvern Zetasizer Nanoseries Nano-ZS

(Malvern Instruments Ltd, Malvern, UK), as described by Lin et al. (2017). Casein hydration was measured by lyophilisation of the pellet obtained on ultracentrifugation, and expressed as g water g^{-1} sedimented casein (Lin et al., 2017).

2.5. Preparation of skim milk concentrates

The LHP and HHP powders were reconstituted in distilled water for the preparation of concentrated milks with 9.4–25% TS, using a similar procedure to that used for the LHP-SM and HHP-SM skim milk samples. The concentrates from the LHP and HHP are denoted LHP-SMC and HHP-SMC, respectively (Table 1).

2.6. Calcium ion concentration of skim milk concentrates

The LHP-SMC and HHP-SMC samples, at 25% TS, were adjusted to pH values in the range 6.2 to 7.0, at 0.2 pH unit intervals. The [Ca^{2+}] of the pH-adjusted concentrates was immediately measured, as described in section 2.3.

2.7. Rennet gelation of skim milk and skim milk concentrates

Samples of skim milk concentrates with 9.4–25% TS were adjusted to pH 6.55 and inoculated with chymosin (single strength Chy-Max® plus, 200 IMCU mL^{-1}; Chr. Hansen, Hørsholm, Denmark), which had been diluted 20-fold with distilled water, at a level of 0.103 mL g^{-1} protein. Milk samples were tested for rennet gelation properties at 31 °C using dynamic low-amplitude strain oscillation rheometry in a controlled-stress rheometer (Carri-Med, type CSL^2_{500}, TA instruments, New Castle, DE, USA) at a strain of 0.025 and a

frequency of 1 Hz, as described by Lin et al. (2017). The storage modulus, G', was measured dynamically as a function of time over 1 h (G'_{60}); the gelation time (GT) was defined as the time for G' to reach a threshold value of ≥0.2 Pa and the maximum curd firming rate as the maximum slope of the G'/time curve.

2.8. HCT of skim milk and skim milk concentrates

Samples of skim milk (SM, LHSM, HHSM, LHE-SM, HHE-SM, LHP-SM and HHP-SM) and skim milk concentrates with 15–25% TS (LHP-SMC, HHP-SMC) were adjusted to pH values in the range from 6.2 to 7.2 (in increment of 0.1 pH unit) at room temperature using 0.1 N HCl or NaOH. The HCT of the skim milk and skim milk concentrate samples was measured at 140 and 120 °C, respectively, as described by Lin et al. (2017). Preliminary trials indicated that skim milk concentrates with 15–25% TS were sometimes prone to instantaneous coagulation at 130 or 140 °C depending on pH, while concentrates with ≥25% TS gelled/solidified instantly at temperatures ≥120 °C.

2.9. ES of skim milk concentrates

Skim milk concentrates with 9.4–25% TS were prepared by reconstitution of SMP and adjusted to pH values in the range 6.2 to 7.0 at 0.2 pH unit intervals. The ES was tested by blending 1 mL of sample with aqueous ethanol solutions of different concentrations (30–98%) while keeping the ethanol-to-protein ratio constant. The mixture of aqueous ethanol and sample was mixed for 30 s before inspection for visible flocculation.

2.10. Statistical analysis

Data were analysed using a randomised complete block design, which incorporated the skim milk samples (SM, LHSM, HHSM, LHE-SM, HHE-SM, LHP-SM, HHP-SM) or skim milk concentrate (LHP-SMC and HHP-SMC) and 2 replicate blocks (samples from the 2 separate bathes of SMP or evaporated milk made on different days). Analysis of variance (ANOVA) was carried out using a general linear model (GLM) procedure of SAS 9.3 (SAS Institute, 2011) and the effects of treatment (stage of manufacture: heat treatment, evaporation and drying) and replicate on each response variable was determined. Tukey's multiple-comparison test was used for paired comparison of treatment means and the level of significance was determined at $P < 0.05$.

Regression analysis was performed to investigate potential correlations between G'_{60} and TS in the skim milk concentrates.

3. Results

3.1. Gross composition of skim milk samples

The composition of the skim milk samples (SM, LHSM, HHSM, LHE-SM, HHE-SM, LHP-SM, HHP-SM; Table 1) is shown in Table 2. As expected, all samples had similar levels of TS, lactose, total protein, casein, NPN (% total N), total Ca and P. Increasing the heat treatment of the skim milk prior to evaporation led to a significant increase in whey protein denaturation from ~5% of total whey protein on heating at 72 °C × 15 s to 80% at 120 °C × 120 s (Table 2).

The concentration of ionic Ca, $[Ca^{2+}]$, in the unheated skim milk from trials 1 (2.1 mM) and 2 (~5.0 mM) differed markedly. The values, though very different, reflect the range reported in the literature for bovine milk (~1–5 mM) (Kelly, Keogh, O'Keeffe, & Phelan,

1982; White & Davies, 1958). Hence, the value of [Ca^{2+}] was normalised to 100 for the skim milk in both trials 1 and 2, to facilitate statistical analysis. HH treatment led to a significant reduction in [Ca^{2+}], but low heat treatment had no effect, as reflected by the similar [Ca^{2+}] in the SM and LHSM samples (Table 2). During the manufacture of both LHP and HHP, evaporation led to a reduction in [Ca^{2+}], while drying resulted in restoration to a level equal to that of the LHSM and HHSM samples, respectively. The mean [Ca^{2+}] value of the HHSM, HHE-SM and HHP-SM were significantly lower than those of the corresponding samples of LHSM, LHE-SM and LHP-SM (Table 2).

3.2. Physico-chemical properties of skim milk samples

All skim milk samples showed a mono-modal particle size/number distribution. Casein micelle size increased significantly during the manufacture of both LHP and HHP, with the increase occurring during evaporation for the former, and increased during milk heat treatment and evaporation for the latter (Table 2). Particle sizes for the LHSM, LHE-SM and LHP-SM were significantly lower than those of the corresponding HHSM, HHE-SM and HHP-SM. The zeta potential and hydration of all skim milk samples ranged from –20.6 to –2.9 mV and from 3.02 to 3.19 g water g^{-1} casein, respectively, and were not significantly affected by heat treatment, evaporation or drying.

3.3. Composition of the sera from skim milk samples

The concentrations of serum β-lactoglobulin A (β-Lg A), β-lactoglobulin B (β-Lg B) and α-lactalbumin (α-La) in the HHSM, HHE-SM and HHP-SM milk from the milk heated at 120 °C were ~19–22, 13–18, and 24–38%, respectively, of the level in the unheated skim

milk, SM; the corresponding levels in the LHSM, LHE-SM and LHP-SM samples were ~96–99, 95–100 and 96–100%, respectively. This result is consistent with the increase in whey protein denaturation on intensifying milk heat treatment (Table 2). Evaporation and drying did not induce denaturation of whey proteins during the preparation of the SMP, as evidenced by the similar levels of whey proteins in the serum (expressed as a % of the unheated SM) in the heated skim milk, evaporate and reconstituted SMP for both the LH and HH treatments.

The concentration of serum caseins, α_S-, β- or κ-casein, expressed as % of the corresponding casein in skim milk, was not affected by heat treatment (72 °C × 15 s), evaporation or drying during the manufacture of LHP, as indicated by the similar values in the SM, LHSM, LHE-SM and LHP-SM. In contrast, heat treatment (120 °C × 120 s) during the manufacture of HHP resulted in significant increases in the levels of serum casein and κ-casein (Table 2, Fig. 2A). For both the LH and HH milk samples, the level of serum κ-casein (%κ-casein in milk) was higher than that of serum β- or α_S-casein (Fig. 2A). Nevertheless, owing to the different concentrations of the individual caseins in milk, the proportions of different serum caseins, expressed as % of total serum casein, were not significantly affected by heat treatment, evaporation or drying during the manufacture of the LHP or HHP (Fig. 2B)

In contrast to serum casein, the concentrations of serum Ca and P decreased significantly during the manufacture of HHP, as seen on comparing the SM and HHP-SM skim milk samples (Table 2); the reduction was observed entirely during the heating step (120 °C × 120 s), with no further reduction during evaporation and drying. In the manufacture of LHP, serum Ca and P were not affected by heat treatment (72 °C × 15 s), decreased during evaporation, and increased during drying. Nevertheless, the levels of serum Ca and P in the LHP-SM and SM were similar, indicating no overall influence during the

manufacture of LHP. Consequently, serum Ca and P levels in the LHP-SM were significantly higher than that of the HHP-SM.

3.4. Calcium ion content of skim milk concentrates

The $[Ca^{2+}]$ of the LH concentrates (LHP-SMC) at pH 6.2–7.0 increased slightly, but significantly, with increasing % TS; an opposite effect was found for the HH concentrates (HHP-SMC) (Fig. 3A). The $[Ca^{2+}]$/casein ratio decreased with TS, with the magnitude of the difference between the low (9.4%) and high (25%) TS concentrate decreasing as pH increased (Fig. 3B). For both concentrates, the $[Ca^{2+}]$ decreased with increasing pH (Fig. 3A).

3.5. Rennet gelation of skim milk and skim milk concentrates

The changes in gel strength, G', of the LH- and HH- skim milk samples with time after rennet addition are shown in Figs. 4A and 4B, respectively. The values of G'_{60} of LH samples from trial 2 were notably higher than those from trial 1, an effect most likely associated with higher concentrations of protein and $[Ca^{2+}]$ of the SM in trial 2.

G' deteriorated during the heat treatment and evaporation stages of LHP manufacture, but recovered during drying, as shown by the similar magnitude of G' with time in the LHSM and LHP-SM milk samples. HH treatment irreversibly impeded rennet coagulability, as indicated by the failure of the HHSM, HHE-SM, HHP-SM to undergo gelation.

Increasing TS was paralleled by a significant reduction in GT and increases in gel-firming rate and G'_{60} of both the LHP-SM and HHP-SM samples (Fig. 5A–D). G' increased with increasing TS in the LHP-SM samples, with regression analysis indicating a power law dependency of G'_{60} on TS (LH: r = 0.98, n = 8), where $G'_{60} = \text{total solids}^n$, and the exponent *n*

was 2.4 (Fig. 5E). The increase in G' of the LHP-SMC samples with TS reflects the increase in the concentration of casein contributing to the structure of the calcium phosphate *para*-casein gel network, and the attendant increase in its stress-bearing capacity. While there was no improvement in the rennet coagulability on increasing TS from 9.4 to 15%, G' increased linearly at a rate of ~8.5 Pa g^{-1} TS with a further increase in TS from 15 to 20–25% (Fig. 5F). Hence, while the rennet gelation characteristics of the reconstituted LH- and HH- powders improved with increasing TS concentration, the rate of increase in G'_{60} was markedly lower in the latter than the former.

3.6. Heat stability of skim milk and skim milk concentrates

The HCT/pH curves for SM and the LH- and HH skim milk samples are shown in Fig. 6A–D. All curves displayed the typical type A profile, with a maximum (HCT_{max}) and a minimum (HCT_{min}). The processing steps during the manufacture of LHP had little, or no, effect, as seen from the similar profiles of the SM, LHSM, LHE-SM and LHP-SM samples. In contrast, HH treatment during the manufacture of HHP reduced the pH of HCT_{max} by 0.1 and broadened the pH region of HCT_{min}, as observed by comparing the SM and HHSM samples. Otherwise, evaporation and drying during the manufacture of HHP had little impact on the heat stability characteristics of skim milk, as seen by the similarity of the HCT/pH profiles for the HHSM, HHE-SM and HHP-SM samples. High-solids recombined milks, which generally have relatively low pH compared with skim milk, are frequently subjected to heating (e.g., pasteurisation and sterilisation); consequently, the HCT/pH profile of reconstituted skim milk with varying TS is of interest.

The HCT/pH profiles of milk samples with TS of 9.4 to 25% at 120 °C are shown in Fig. 7A–D. The HCT of the HHP-SMC from trial 2 was higher than that of trial 1 at

corresponding pH values, probably because of the slightly higher protein content and [Ca^{2+}] of milk from trial 2. Nevertheless, the trend in HCT with TS was similar for both trials. At 9.4% TS, the HCT of HHP-SM showed a typical type A profile, with a distinct HCT_{max} at 6.5 and HCT_{min} at 6.7–6.8, whereas that of the LHP-SM increased continuously on increasing pH to pH 6.9 and then decreased slightly as pH was further increased to 7.0. Compared with the LHP-SM (9.4% TS), the HCT of the HHP-SM skim milk was 35 to 100 min higher than that of the LHP-SM at pH 6.3–6.5 and ~20 to 34 min lower at pH 6.7–6.9.

The HCT of both the LHP-SMC and HHP-SMC samples at pH values ≥6.4 decreased on increasing TS from 9.4 to 25% (Fig. 7A–D). A major difference between the LHP-SMC and HHP-SMC samples was the higher HCT of HHP-SMC concentrates (20 and 25% TS) at pH values 6.3–6.6. Hence, while the HCT of the LHP-SMC with 20–25% TS was very low (<10 min) at all pH values, that of the corresponding HHP-SMC was quite high in the pH region 6.3–6.6, e.g., 90 (trial 1) and 77 min (trial 2) at pH 6.4 and 90 (trial 1) and 55 min (trial 2) at pH 6.5 (Fig. 7B). The results clearly indicate that increasing the severity of the heat treatment of the skim milk prior to powder manufacture enhances the heat stability of high-solids skim milk concentrates, or conversely enables reconstitution of skim milk powder to higher TS while retaining adequate heat stability at pH 6.3–6.6 during thermal processing of recombined milks.

3.7. ES of skim milk concentrates

The ethanol concentration/pH profiles of the skim milk concentrates (LHP-SMC and HHP-SMC) samples with TS ranging from 9.4 to 25% are shown in Fig. 8A–D. The stability of all samples to ethanol increased with increasing pH. The ES of the HHP-SMC samples was numerically higher than that of the corresponding LHP-SMC samples at pH ≤6.6, but

similar at pH 6.8 and 7.0; however, the magnitude of the differences between the corresponding LH and HH samples in the pH region 6.2–6.6 was significant ($P < 0.05$) at some pH values only, as indicated by different lower-case superscripts (a, b) (Fig. 8A–D). The ES of the LHP-SMC and HHP-SMC samples at pH values 6.2 and 6.4 increased with TS, while the ES at pH 6.6–7.0 decreased (Fig. 8E, F).

4. Discussion

The manufacture of SMP involves heat treatment, evaporation and drying. The separate and combined effects of each step on the properties of reconstituted milk prepared from the SMP were evaluated in the current study. The severity of the heat treatment of milk prior to evaporation and drying during the manufacture of skim milk powder had a major influenced on the properties of reconstituted milk prepared from the powder. The level of heat treatment affected the partitioning of caseins, whey protein and minerals between the serum and the sedimented phase, rennet gelation, HCT and ES. By comparison, the evaporation and drying stages of skim milk powder manufacture had little, or no, effect on the characteristics of reconstituted milk. Hence, the properties of reconstituted skim milk are quite similar to those of the unheated skim milk for low heat SMP.

Increasing the severity of heat treatment of the skim milk prior to evaporation led to a significant increase in whey protein denaturation and casein micelle size, and reductions in the concentrations of whey proteins, Ca and P in the serum. The reduction in serum Ca and P suggests that calcium phosphate which precipitates during high heat treatment does not fully re-solubilise on cooling (Singh, Roberts, Munro, & Teo, 1996; van Hooydonk, de Koster, & Boerrigter, 1987).

In contrast to the trend for serum whey protein, the concentration of serum casein increased significantly with HH treatment, mainly as a consequence of an increase in the concentration of serum κ-casein (% total κ-casein). This increase in serum □-casein and denatured whey protein complexed with the κ-casein confirms the results of previous studies showing a significant increase in the extent of dissociation of κ-casein from the micelle into the serum as the temperature during heat treatment was increased, e.g., from 60 to 120 °C (Anema & Li, 2015; Ménard, Camier, & Guyomarc'h, 2005). It has been shown that the dissociated κ-casein interacts with denatured whey protein in the serum to form serum-soluble aggregates or particles (Donato & Guyomarc'h, 2009; Ménard et al., 2005; Mollé et al, 2006). Using a combination of chymosin-induced precipitation and capillary electrophoresis, Vasbinder et al. (2003a) determined the proportions of β-Lg and α-La that complexed with dissociated κ-casein (in the serum) and non-dissociated κ-casein (on the casein micelle) in milk as the pasteurisation temperature was increased from 70 to 90 °C (for 10 min) at native pH; the level of β-Lg denaturation increased from ~2 to 95% of total β-Lg, and the level of serum casein increased from <5 to 10 % of total casein. Simultaneously, the proportions of total β-Lg involved in the formation of serum-soluble aggregates or associated with the casein micelle increased from ~2 to 25% or 1 to 65 % of total, respectively. Hence, the proportions of denatured β-Lg that form serum-soluble aggregates or reacted with the casein micelle were ~28 and 72% of total denatured whey protein. In the current study, the proportion of denatured whey protein interacted with dissociated κ-casein was estimated at ~14% of total denatured whey protein in the HH-SMP; this estimate was based on the difference between the whey protein content of the HH-SMP serum and the filtrate obtained on pH-adjustment of the serum to pH 4.6. The interaction of most of the denatured whey protein (~86%) with casein micelle was supported by the significantly higher casein micelle size in the HH skim milk samples. Likewise, Martin et al (2007) reported progressive

increases in whey protein denaturation and the hydrodynamic diameter of the casein micelle on increasing milk heat treatment from 79 °C for 5 s to 90 °C × 30 s or 120 °C × 120 s.

Rennet gelation properties deteriorated significantly with HH treatment of the skim milk, and only partially recovered on increasing the TS of the reconstituted HHP to 25%. The adverse effect of heat treatment is likely to ensue from the associated increase in the level of serum soluble κ-casein/β-Lg aggregates (Kethireddipalli, Hill, & Dalgeleish 2010; Vasbinder, Rollema, & de Kruif, 2003b) and reduction in [Ca^{2+}] in the HHSM (Sandra, Ho, Alexander, & Corredig, 2012; Singh, Shalabi, Fox, Flynn, & Barry, 1988). Though the κ-casein in the κ-casein/β-Lg aggregates is hydrolysed by rennet, the aggregates, nevertheless, remain soluble following rennet-treatment and may impede the knitting of the *para*-casein micelles into a gel network continuum (Mollé et al, 2006). Various studies have shown that the hydrolysis of □-casein in milk is unaffected by increasing treatment from 70 to 90 °C for 10 min (Kethireddipalli et al., 2010; Mollé et al, 2006; Vasbinder et al., 2003b). Rennet coagulability further deteriorated during evaporation of the low-heat treated skim milk, as demonstrated by the significantly lower G'_{60} and GFR_{max} of the milks prepared by dilution of the LH evaporated milk (LHE-SM) compared with the LHSM. This was associated with a reduction in the serum concentration of [Ca^{2+}] (Table 2), probably because the time between concentrate dilution and measurement of rennet gelation (30–45 min) was insufficient to allow restoration of equilibrium between insoluble and soluble calcium (Chandrapala, McKinnon, Augstin, & Udabage, 2010). This is corroborated by the similar [Ca^{2+}] and the rennet-gelation behaviour of the LHSM and the LHP-SM (Table 2); following reconstitution of the powder, the LHP-SM was held at 4 °C for ~22 h.

HH treatment of skim milk before evaporation reduced the pH of HCT_{max}, broadened the HCT_{min} region, and increased HCT at pH values 6.3 to 6.6; this effect became more pronounced in skim milk concentrates as the TS was increased from 9.4 to 25%. These

414 effects in the HH milk were paralleled by an increase in the proportion of denatured whey
415 protein (86%) interacted with the casein micelle and a reduction in [Ca^{2+}]. It has been
416 suggested that the interaction of denatured whey protein with κ-casein on the surface of the
417 casein micelle limits the dissociation of κ-casein during HCT measurement (Singh &
418 Creamer, 1991). The role of ionic calcium has been corroborated by Sievanen et al. (2008),
419 who reported that the addition of 5 mM $CaCl_2$ to milk, before or after preheating (90 °C for 10
420 min) significantly reduced the HCT. The HCT of both LHP-SMC and HHP-SMC decreased
421 markedly on increasing TS from 9.4 to 25%. This trend, which concurs with results of Singh
422 and Creamer (1991), has been attributed to the increases in volume fraction of casein and
423 heat-induced acidification, associated with the thermal degradation of lactose to organic
424 acids, dephosphorylation of casein, and to the precipitation of calcium phosphate (O'Connell
425 & Fox, 2003).

426 At all TS levels (9.4–25%), the ES of the HHP-SMC concentrates in the pH range
427 6.2–6.6 was higher than that of corresponding LHP-SMC concentrates, an effect most likely
428 due to the lower [Ca^{2+}] of the former (Horne & Parker, 1981; Mohammed & Fox, 1986). ES
429 as a function of TS of both the LHP-SMC and HHP-SMC concentrates increased at pH 6.2
430 and 6.4 but decreased at pH 6.6–7.0. The pH-dependence of ES on TS may be related to the
431 effect of pH on [Ca^{2+}] and, in particular, the [Ca^{2+}]/casein ratio. It is feasible that the
432 difference in [Ca^{2+}] between the low and high TS concentrates is sufficiently large to
433 influence ES in the pH region 6.2–6.4 but not at pH 6.6–7.0. As the relative contribution of
434 the lower [Ca^{2+}]/casein ratio to the ES diminishes with increasing pH, the full effect of
435 increasing the level of TS, and hence casein, becomes apparent at higher pH values.
436 Likewise, Horne and Parker (1983) found that the ES of concentrates from unpasteurised
437 skim milk at pH 6.7–7.0 deteriorated progressively on increasing TS from ~9–23%. Based on
438 model experiments, Horne and Parker (1983) concluded that the negative effect of increasing

TS on ES at pH >6.7 was due to the increase in chloride content, and hence ionic strength. It was hypothesised that higher ionic strength resulted in a shift in calcium-citrate equilibrium, which favoured a higher [Ca^{2+}] concentration, and hence lower ethanol stability, in high-solids concentrates. Nevertheless, the results of the current study showed that the [Ca^{2+}]/casein ratio decreased with increasing pH.

5. Conclusion

The changes in the partition of milk components (minerals and proteins), between the casein micelle and serum, and processing characteristics of milk at the different stages of manufacture of low-heat and high-heat skim milk powder were investigated. Increasing heat treatment of skim milk from 72 °C × 15 s to 120 °C × 120 s resulted in higher levels of whey protein denaturation, serum casein, serum κ-casein as a proportion of total □-casein, and casein micelle size, and in lower concentrations of ionic calcium and of serum calcium and phosphorous in skim milk and reconstituted skim milk powder. These changes were paralleled by marked deterioration in rennet coagulability, and increases in HCT at pH 6.3–6.6 and ES at pH 6.2 and 6.4. Increasing the TS level from 9.4 to 25% in skim milk concentrates, prepared by reconstitution of the skim milk powder, coincided with lower HCT at pH 6.3–7.0, lower ES at pH 6.6–7.0, higher ES at pH 6.2 and 6.4, and a partial recovery of rennet coagulability (at TS ≥20%). The findings indicate how the intensity of heat treatment during the manufacture of skim milk powder can be altered to modulate the functionality of the reconstituted powder and its suitability in different applications, e.g., recombined milk cheese or UHT-based milk beverages

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Figure legends

Fig. 1. Flow chart (A) showing the separation of high-heated skim milk samples (high-heat treated skim milk, HHSM; skim milk prepared by dilution of evaporated high-heat treated skim milk, HHE-SM; and skim milk by reconstitution of high-heat skim milk powder, HHP-SM) into different nitrogen (N)/protein fractions, and analysis (B) undertaken on the different fractions. Abbreviations: N, nitrogen; NPN, non-protein nitrogen; NCN, non-casein nitrogen; TN, total nitrogen.

Fig. 2. Concentration of caseins in serum prepared by ultracentrifugation of skim milk samples at 100,000 × *g* at 25 °C: α_{S1} + α_{S2}-casein (○), β-casein (▲) and κ-casein (△). Samples, as defined in Table 1, include unheated skim milk (SM), low-heat treated skim milk (LHSM), skim milk prepared by dilution of evaporated low-heat treated skim milk (LHE-SM) or by reconstitution of low-heat skim milk powder (LHP-SM); the corresponding samples from high-heat treated skim milk, i.e., HHSM, HHE-SM, and HHP-SM. Data presented are the means of duplicate batches of each treatment; error bars represent the standard deviation of the mean.

Fig. 3. Changes in concentration ionic calcium, [Ca^{2+}], and [Ca^{2+}]:casein ratio as a function of pH for skim milk concentrates with total solids content of 9.4% (○,●) or 25% (△,▲), prepared by reconstituting low-heat skim milk powder (A, C) or high-heat skim milk powder (B, D).

Fig. 4. Development of storage modulus, G', in rennet-treated skim milk samples from duplicate batches: Trial 1 (A) and Trial 2 (B). Samples, as defined in Table 1, include

unheated skim milk (∗), low heat-treated skim milk (●), and skim milk prepared by dilution of evaporated low-heat treated skim milk (■) or by reconstitution of low-heat skim milk powder (□); high-heat treated skim milk (△), and skim milk prepared by dilution of evaporated high-heat treated skim milk (▲) or by reconstitution of high-heat skim milk powder (◇).

Fig. 5. Development of storage modulus, G', in rennet-treated skim milk concentrates with 9.4% (△), 15% (▲), 20% (○) or 25% (●) total solids. The concentrates were prepared by reconstituting low-heat (A, C) or high-heat (B, D) skim milk powder from duplicate batches: Trial 1 (A, B) and Trial 2 (C, D). Storage modulus at 60 min, $G'_{60,}$ as a function of total solids level for concentrates prepared from low-heat (E) or high-heat (F) skim milk powder; presented data for G'_{60} in both E and F is from trials 1 and 2.

Fig. 6. Heat coagulation time, HCT, at 140 °C as a function of pH for skim milk samples, as defined in Table 1: unheated skim milk (∗); low-heat treated skim milk (△); high-heat treated skim milk (▲); skim milk prepared by dilution of evaporated low-heat treated skim milk (○) or high-heat treated skim milk (●); and skim milk prepared by reconstitution of low-heat skim milk powder (□) or high-heat skim milk powder (■). Samples were obtained from duplicate batches, trial 1 (A, B) and Trial 2 (C, D).

Fig. 7. Heat coagulation time, HCT, at 120 °C as a function of pH for skim milk concentrates with 9.4% (△), 15% (▲), 20% (○) or 25% (●)total solids. The concentrates were prepared by reconstitution of low-heat (A, C) or high-heat (B, D) skim milk powder. Samples were obtained from duplicate batches of skim milk powder, trial 1 (A, B) and Trial 2 (C, D).

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Fig. 8. Ethanol stability as a function of (A–D) pH for skim milk concentrates [prepared by reconstituting low-heat (△) or high-heat (▲) skim milk powder] with 9.4% (A), 15% (B), 20% (C) or 25% (D) total solids level and ethanol stability of concentrates [prepared by reconstituting low-heat (E) or high-heat (F) skim milk powder] as a function of (E–F) total solids at pH 6.2 (○), 6.4(●), 6.6 (△), 6.8(▲) and 7.0 (□). Data are the means of duplicate batches of each treatment; error bars represent the standard deviation of the mean.

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Table 1

Samples collected and analysed during manufacture of skim milk powder. [a]

Samples	Codes
Samples taken during manufacture of skim milk powder	
Skim milk (unheated)	SM
Low-heat treated skim milk	LHSM
High-heat treated skim milk	HHSM
Low-heat evaporated skim milk	LHE
High-heat evaporated skim milk	HHE
Low-heat skim milk powder	LHP
High-heat skim milk powder	HHP
Skim milk samples analysed	
SM	SM
LHSM	LHSM
HHSM	HHSM
Diluted LHE	LHE-SM
Diluted HHE	HHE-SM
Reconstituted LHP	LHP-SM
Reconstituted HHP	HHP-SM
Skim milk concentrates analysed	
Reconstituted LHP	LHP-SMC
Reconstituted HHP	HHP-SMC

[a] Skim milk was subjected to low-heat treatment (LH, 72 °C × 15 s) or high-heat treatment (HH, 120 °C × 120 s); the total solids content of skim milk samples was 9.4%, and that of skim milk concentrates was 9.4, 15, 20 or 25%.

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Table 2
Composition of skim milk and serum. [a]

Composition	Heat treatment						
	None	Low-heat (LH)			High-heat (HH)		
	SM	LHSM	LHE-SM	LHP-SM	HHSM	HHE-SM	HHP-SM
Skim milk							
Total solids (%, w/w)	9.39[a]	9.40[a]	9.30[a]	9.43[a]	9.38[a]	9.48[a]	9.50[a]
Lactose (%, w/w)	4.60[a]	4.57[a]	4.58[a]	4.59[a]	4.56[a]	4.58[a]	4.53[a]
Total protein (%, w/w)	3.91[a]	3.90[a]	3.90[a]	3.92[a]	3.90[a]	3.89[a]	4.06[a]
Casein (%, w/w)	3.09[a]	3.09[a]	3.09[a]	3.09[a]	3.09[a]	3.09[a]	3.09[a]
WP (%, w/w)	0.62[a]	0.62[a]	0.62[a]	0.62[a]	0.62[a]	0.62[a]	0.62[a]
DWP (% total WP)	0[c]	4.78[b]	4.18[b]	5.44[b]	82.46[a]	81.70[a]	80.75[a]
DWP associated with CN micelle (% total DWP)		ND	ND	ND	92.0[a]	85.6[a]	86.7[a]
NPN (% TN)	5.60[a]	5.97[a]	6.06[a]	5.85[a]	5.77[a]	6.07[a]	5.97[a]
$[Ca^{2+}]$ (normalised, % $[Ca^{2+}]$ in SM)	100.0[a]	99.5[ab]	94.0[c]	97.3[b]	90.7[d]	81.2[e]	88.7[d]
Total calcium (mg 100 g^{-1})	124[a]	123[a]	122[a]	122[a]	124[a]	122[a]	126[a]
Total phosphorus (mg 100 g^{-1})	102[a]	100[a]	103[a]	105[a]	100[a]	103[a]	103[a]
pH	6.68[a]	6.68[a]	6.68[a]	6.69[a]	6.66[a]	6.69[a]	6.70[a]
Casein hydration (g water g^{-1} casein)	3.05[a]	3.09[a]	3.10[a]	3.02[a]	3.19[a]	3.05[a]	3.02[a]
Particle size (nm)	166[d]	167[cd]	176[bc]	179[b]	186[b]	209[a]	213[a]
Zeta potential (mV)	–22.4[a]	–22.9[a]	–20.6[a]	–24.0[a]	–22.8[a]	–22.8[a]	–22.3[a]
Skim milk serum							
Protein (%, w/w)	1.10[a]	1.11[a]	1.02[a]	1.09[a]	0.70[b]	0.71[b]	0.70[b]
Protein (% milk protein)	27.9[a]	28.3[a]	26.0[a]	27.4[a]	17.8[b]	18.2[b]	17.8[b]
Casein (%, w/w)	0.21[b]	0.25[b]	0.21[ab]	0.22[b]	0.42[a]	0.44[a]	0.42[a]
Casein (% milk casein)	6.79[b]	8.01[b]	6.93[ab]	6.95[b]	13.58[a]	14.16[a]	13.68[a]
Whey protein							
α-lactalbumin (% α-Lac in SM)	100.0[a]	98.9[a]	98.9[a]	95.5[a]	38.6[b]	24.1[b]	29.5[b]
β-lactoglobulin A (% β-Lg A in SM)	100.0[a]	100.0[a]	94.8[a]	96.4[a]	18.7[b]	21.6[b]	20.6[b]
β-lactoglobulin B (% β-Lg B in SM)	100.0[a]	100.0[a]	96.3[a]	97.2[a]	13.9[b]	16.1[b]	15.6[b]
Ca (mg 100 g^{-1})	45[a]	45[a]	29[b]	45[a]	30[b]	29[b]	31[b]
Ca (% milk Ca)	35.9[a]	37.1[a]	23.9[b]	37.2[a]	24.3[b]	24.1[b]	24.9[b]
P (mg 100 g^{-1})	47[a]	50[a]	30[b]	50[a]	32[b]	34[b]	30[b]
P (% milk P)	46.2[a]	49.9[a]	29.0[b]	47.2[a]	31.6[b]	32.4[b]	29.1[b]

[a] Samples, as defined in Table 1 include: unheated skim milk, low heat-treated skim milk (LHSM), skim milk prepared by dilution of evaporated low-heat treated skim milk (LHE-SM) or by reconstitution of low-heat skim milk powder (LHP-SM); the corresponding samples from high-heat treated skim milk

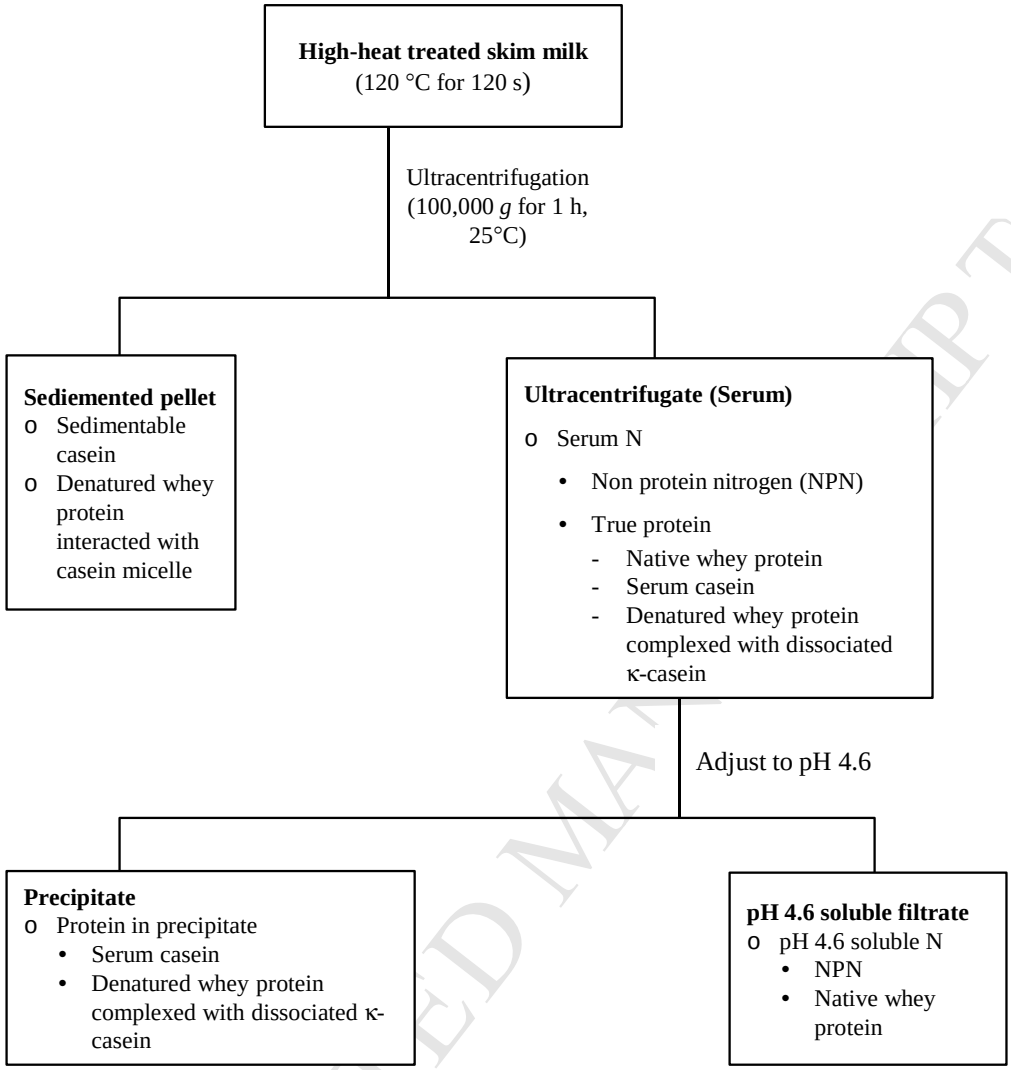
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include HHSM, HHE-SM, and HHP-SM. Skim milk serum was obtained by ultracentrifugation at 100,000 × *g* for 1 h at 25 °C. Abbreviations are: NPN, non-protein nitrogen; TP, total protein; TN, total nitrogen; WP, whey protein; DWP, denatured whey protein; CN, casein; [Ca^{2+}], ionic calcium; α-lac, α-lactalbumin; β-Lg A, β-lactoglobulin; β-Lg B, β-lactoglobulin B. Data are the mean values of duplicate trials (ND, not determined); values within a row not sharing a common lower-case superscript letter differ significantly ($P < 0.05$); the ionic Ca content of SM was set at 100, and the values for all other samples as a percentage of the value in SM.

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(A)



(B)

Sample	Measured parameter	Derived parameter
Skim milk samples: HHSM, HHE-SM, HHP-SM	Total protein, Non-casein N, NPN Protein profile	True protein Whey protein Casein
Serum	Total protein, NCN, NPN Protein profile	True protein (native whey protein, serum casein, serum-soluble denatured whey protein) Serum casein
pH4.6-soluble filtrate	TN	Native whey protein + NPN

Fig. 1.

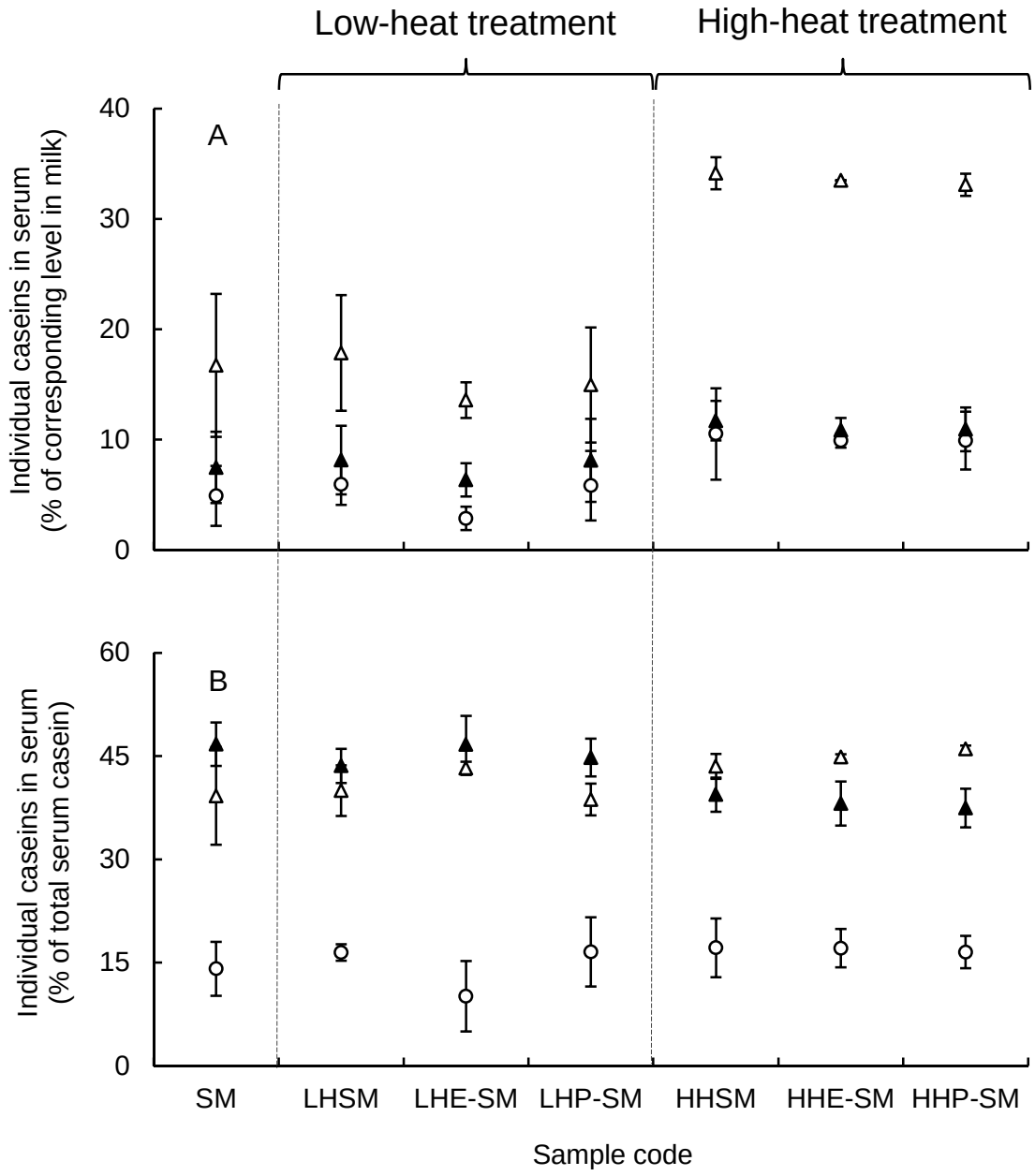

Low-heat treatment
High-heat treatment
A
B
Individual caseins in serum
(% of corresponding level in milk)
Individual caseins in serum
(% of total serum casein)
0
10
20
30
40
0
15
30
45
60
SM
LHSM
LHE-SM
LHP-SM
HHSM
HHE-SM
HHP-SM
Sample code

Fig. 2

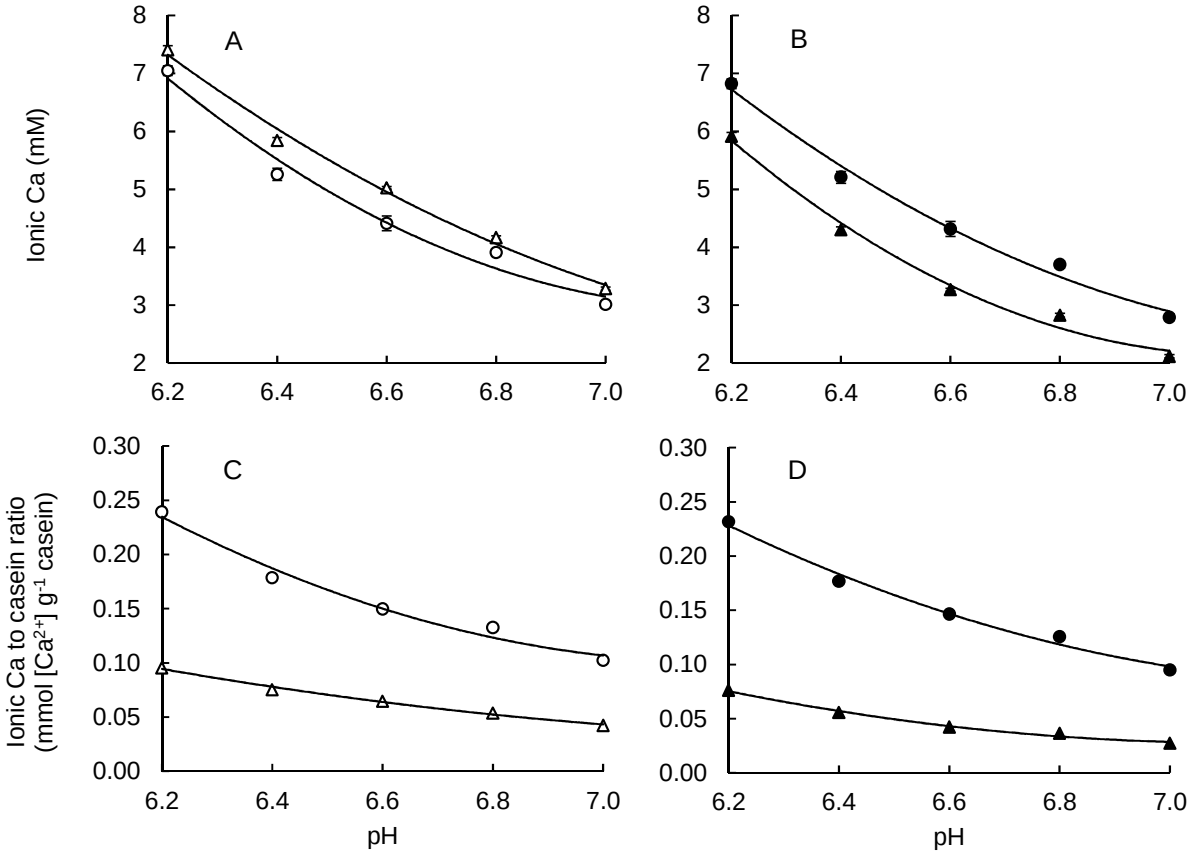


Fig. 3

ACCEPTED MANUSCRIPT

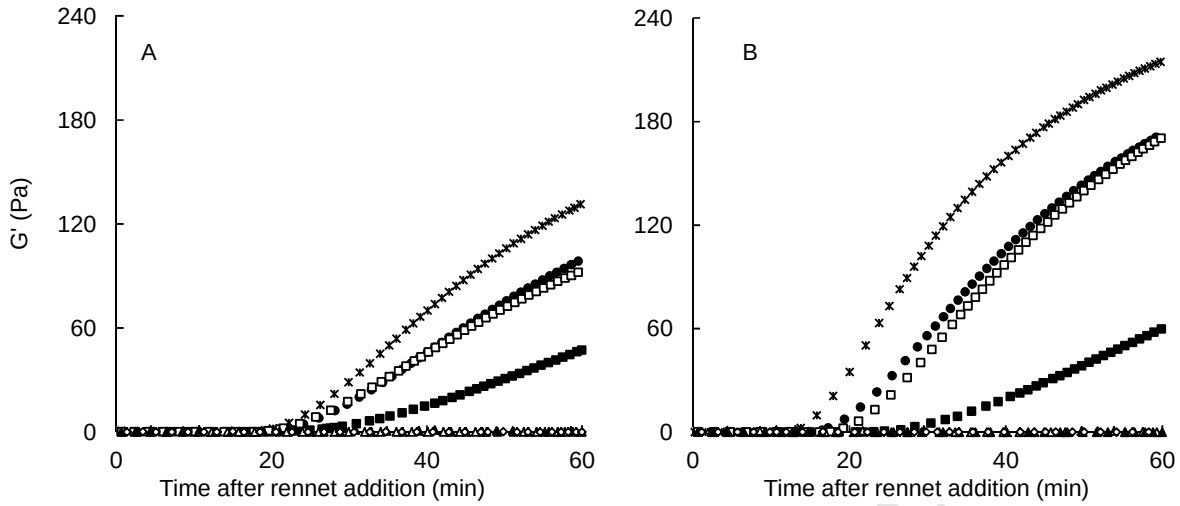


Fig. 4

ACCEPTED MANUSCRIPT

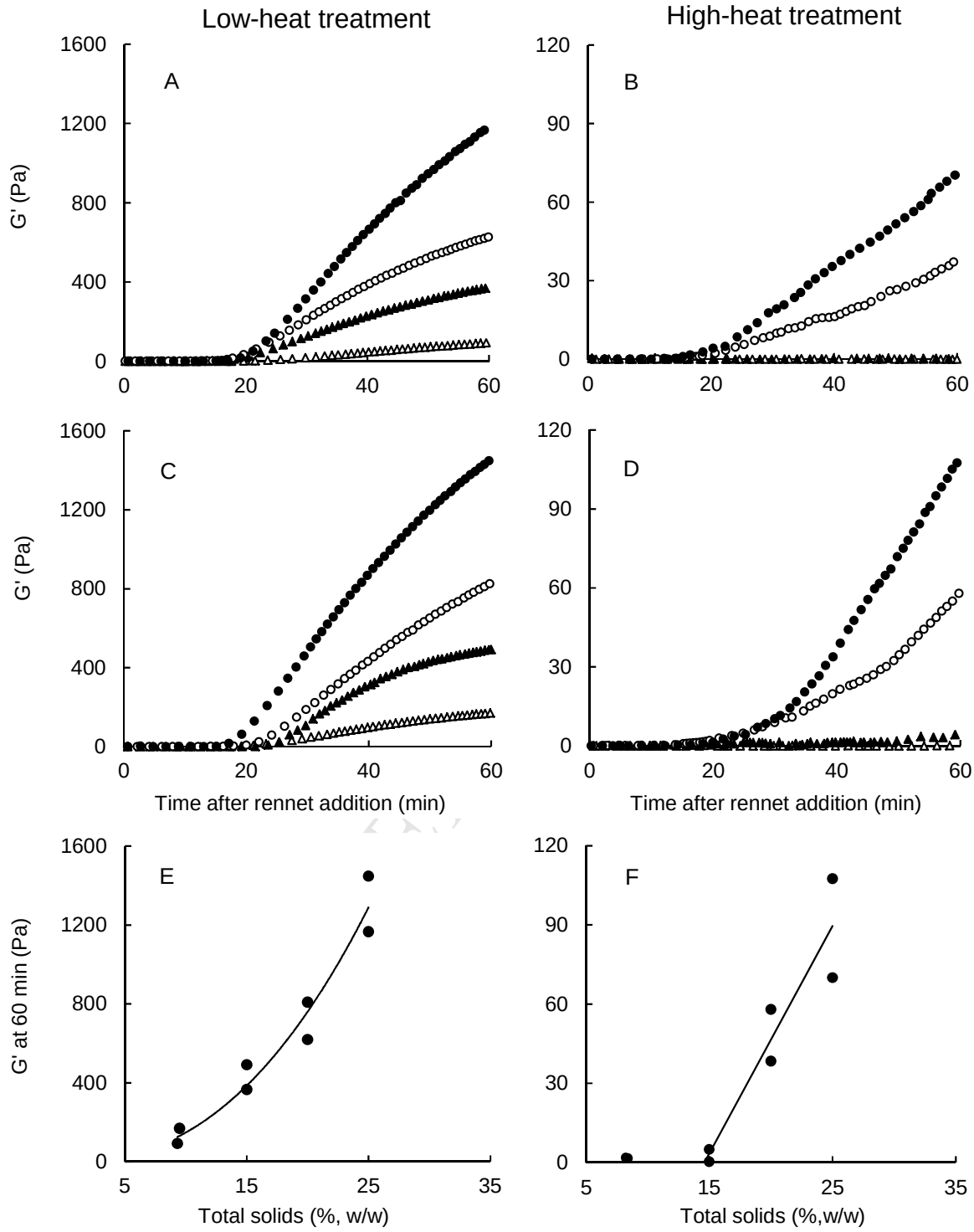

Low-heat treatment
High-heat treatment
A
B
C
D
E
F
G' (Pa)
G' at 60 min (Pa)
Time after rennet addition (min)
Total solids (%, w/w)
Total solids (%,w/w)

Fig. 5

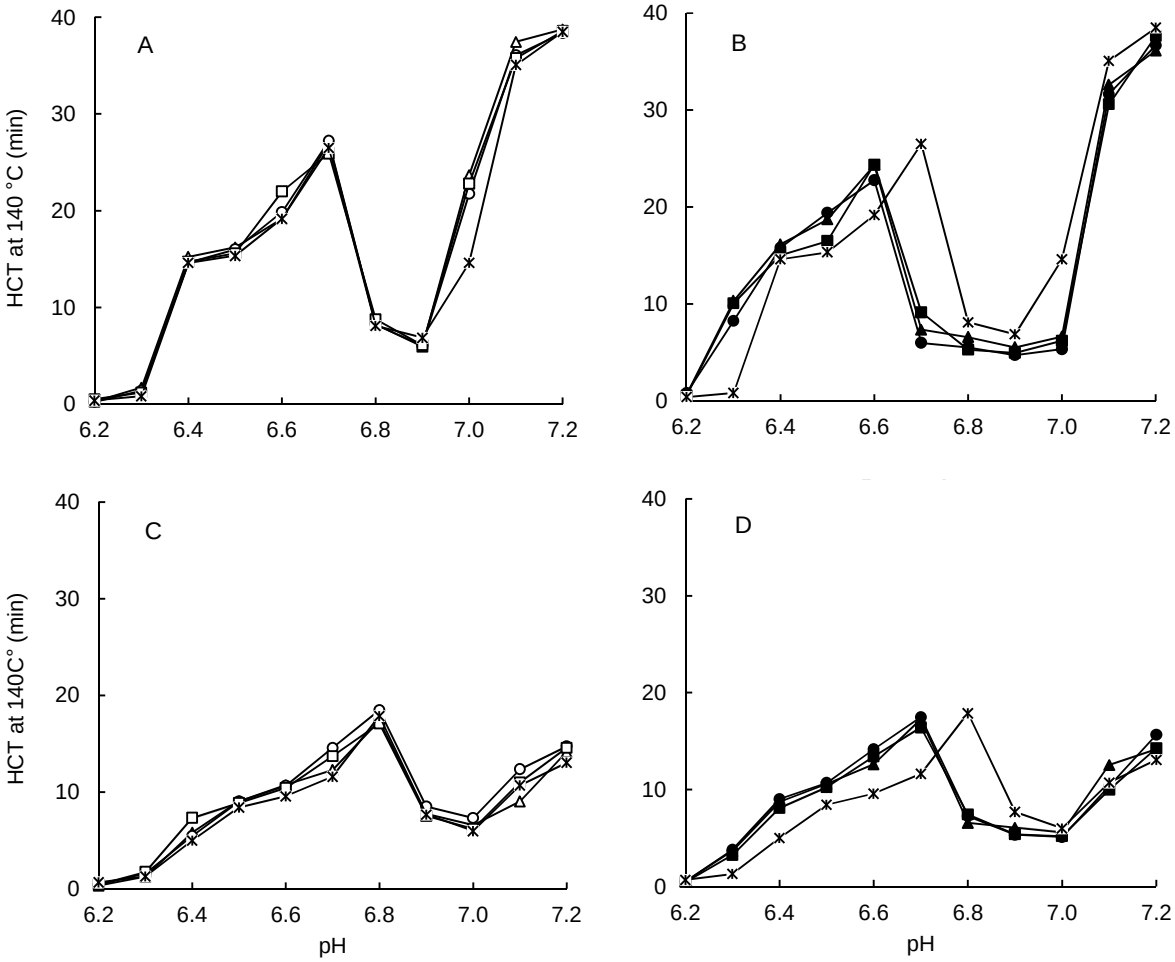


Fig. 6

ACCEPTED MANUSCRIPT

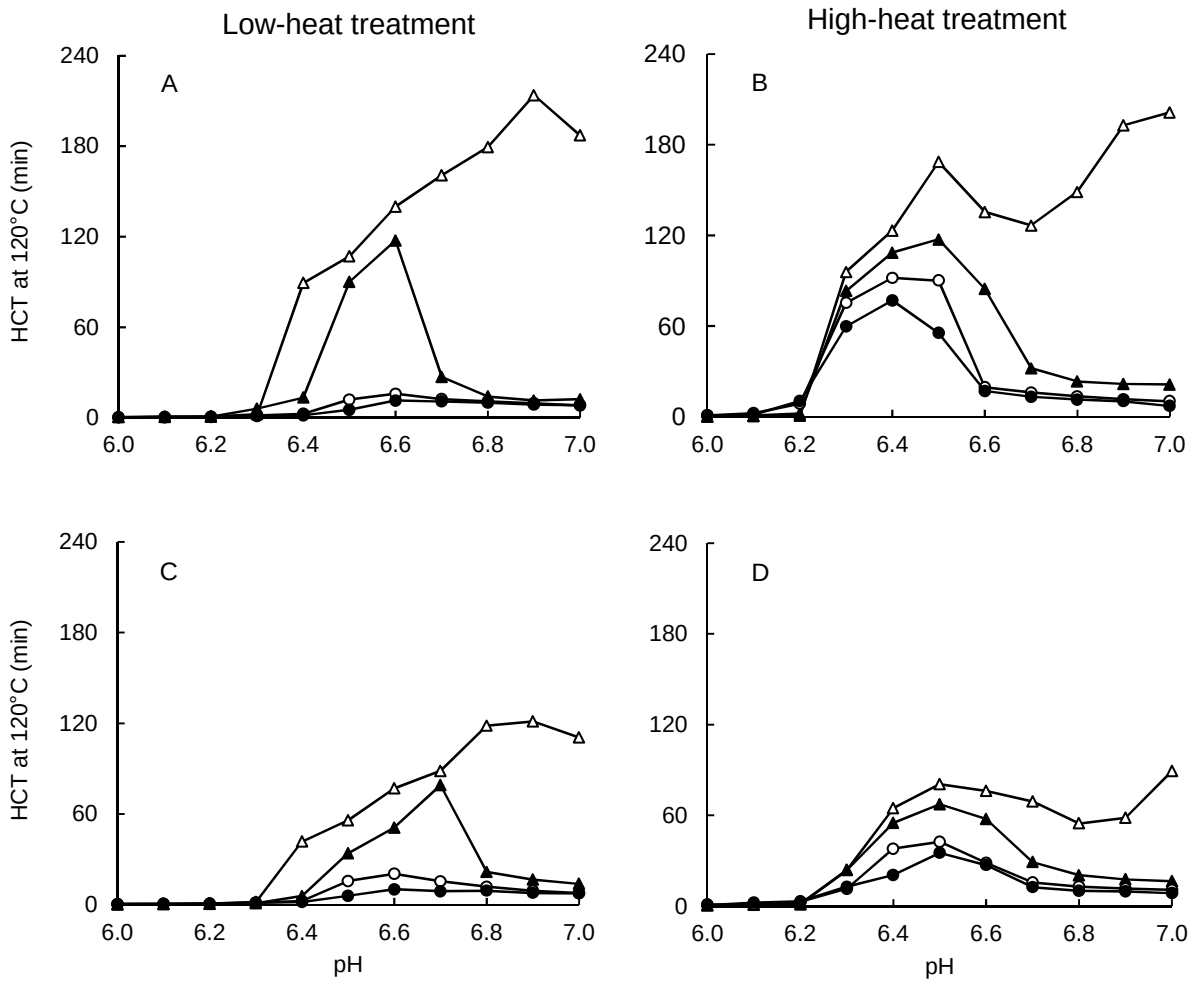


Fig. 7

ACCEPTED

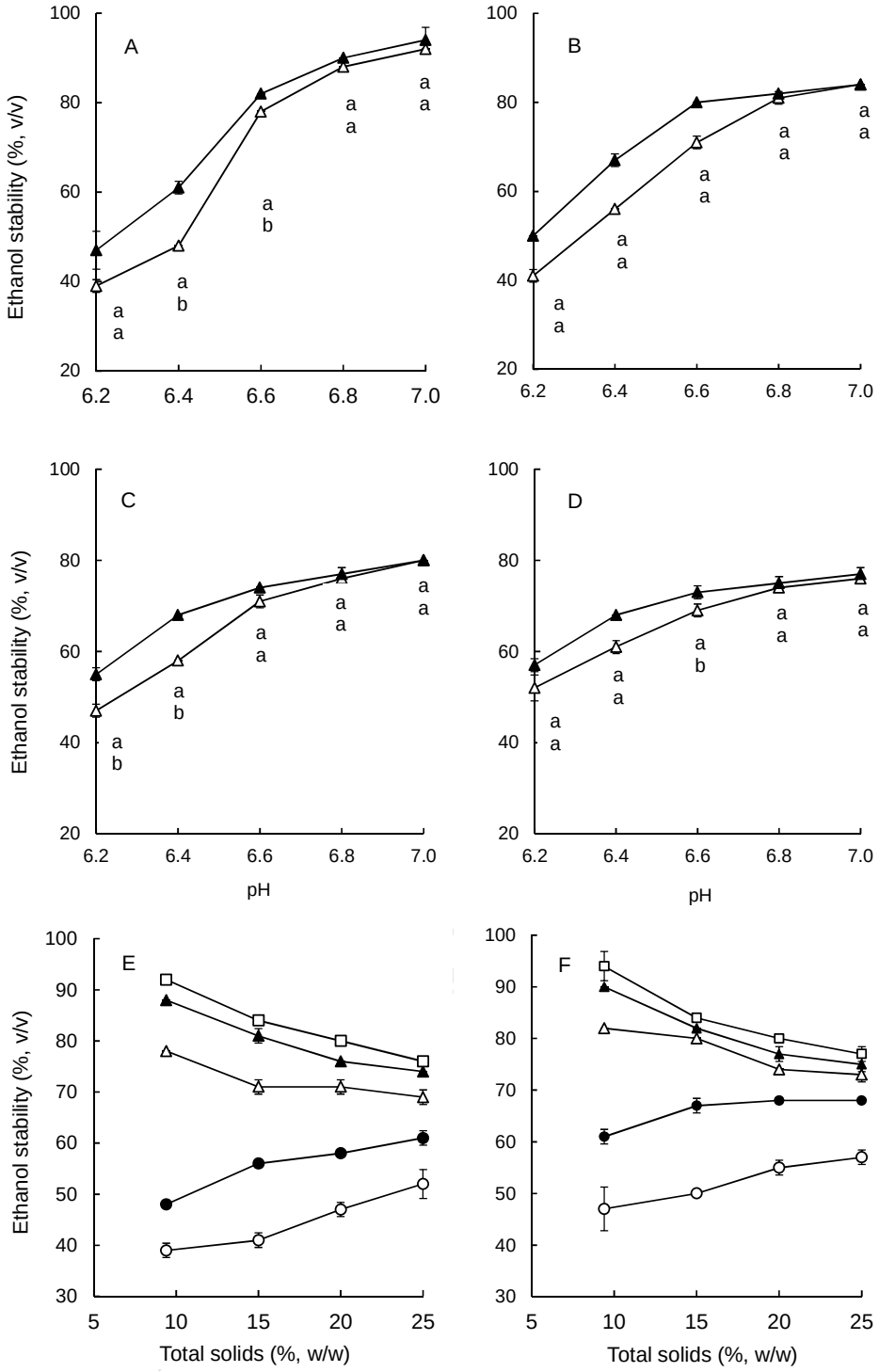


Fig. 8