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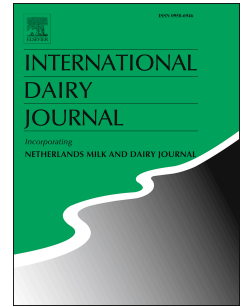
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Effect of heat treatment during skim milk powder manufacture on the compositional and processing characteristics of reconstituted skim milk and concentrate

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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 $72\text{ }^{\circ}\text{C} \times 15\text{ s}$ to $120\text{ }^{\circ}\text{C} \times 120\text{ s}$, 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.

16 **1. Introduction**

17

18 Apart from its use in formulated foods such as sauces, custards, ice-cream and
19 processed cheese products, skim milk powder (SMP) is extensively reconstituted to skim
20 milk with different levels of total solids (e.g., 9–30%), for applications such as milk-based
21 beverages, condensed milks, and recombined milks for cheese or yoghurt manufacture (Gilles
22 & Lawrence, 1982; IDF, 19; Lagrange, Whitsett, & Burris, 201599). SMP is classified as
23 low, medium- or high-heat SMP according to the heat treatment applied to skim milk prior to
24 evaporation and drying (Martin, Williams, & Dunstan, 2007). Typical heat treatments are 70–
25 72 °C for 15 s for low-heat SMP, and 120 °C for 60–120 s, or 90 °C for 300 s (Kelly,
26 O’Connell, & Fox, 2003). High-heat SMP is used as an ingredient in bakery, sweetened
27 condensed milk, and confectionery products such as UHT recombined concentrated milk,
28 toffee, caramel, fudge and milk chocolate (Aitken, Agustin, & Clarke, 1999; Stewart et al.,
29 2017). Low-heat powder is also used extensively in food formulation, including applications
30 such as recombined milk for cheese manufacture, milk solids standardisation in products such
31 as cheese milk, yoghurt and fermented milk products (Patel, Anema, Holroyd, Singh, &
32 Creamer, 2007).

33 For all types of SMP, the stages of manufacture include heat treatment of the milk,
34 evaporation to ~45–50% total solids (TS) and spray drying to ~97% TS. Heat treatment,
35 depending on the severity (temperature and time) and milk pH, affects the extent of whey
36 protein denaturation, the binding of denatured whey protein to the casein micelle and the
37 partitioning of components (salts, whey protein and caseins) between the serum and colloidal
38 phases of milk (Donato & Guyomarc’h, 2009). These changes affect milk processing
39 characteristics such as rennet gelation (Guinee et al., 1997; Pomprasirt, Singh, & Lucey,
40 1998), acid-induced gelation (Vasbinder, Alting, & de Kruif, 2003a), heat stability (Sievanen,

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

44 Studies on the impact of heat treatment on the ethanol stability (ES) of skim milk
45 concentrates are scarce, though the separate effects of heat treatment (Horne & Parker, 1981;
46 Mohammed & Fox, 1986) and concentration (Horne & Parker, 1983) have been investigated.
47 ES is of relevance in alcoholic milk-based beverages (e.g., cream liquor, eggnog and coquito)
48 as an indicator of the resistance of the milk protein to aggregation and, hence, emulsion
49 stability. Martin et al. (2007) reported that the casein micelle sizes in low-, medium- and
50 high-heat treated skim milk increased during evaporation to 45% TS, and remained high in
51 high-heat SMP on reconstitution. Singh and Creamer (1991) found that the heat coagulation
52 time (HCT) of concentrated milk (prepared by diluting evaporated milk to 20% TS) in the pH
53 region 6.3 to 6.6 increased significantly on increasing severity of heat treatment from 72 °C ×
54 15 s to 120 °C × 180 s. Similarly, an increase in heat treatment from 110 °C × 120 s to 120
55 °C × 180 s affected the heat stability of reconstituted milk (9.7% TS), as evidenced by a shift
56 in the HCT/pH curve to lower pH and the concomitant increase in HCT at pH 6.5–6.6 and
57 reduction at pH 6.8–7.1 (Singh & Creamer, 1991).

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

62

63 **2. Materials and methods**

64

65 *2.1. Manufacture of low heat and high heat skim milk powder*

66

67 Skim milk powder was manufactured at Moorepark Technology Limited (Cork,
68 Ireland). Milk was separated at 55 °C (Westfalia Model MM1254 Separator; Westfalia,
69 Germany) and the skim milk ($\leq 0.1\%$ fat) was pasteurised using a plate heat-exchanger (APV
70 Pasilac SSP pilot plant, APV DK 8600, Silkeborg, Denmark) at 72 °C \times 15 s (low heat, LH)
71 or using a MicroThermics[®] pilot-scale tubular heat-exchanger (MicroThermics, Raleigh, NC,
72 USA) at 120 °C for 120 s (high heat, HH). The pasteurised skim milk was cooled directly to
73 4 °C, held at 4 °C overnight, heated to 50 °C, stirred for 30 min, concentrated to 45% TS
74 (Anhydro Falling Film Evaporator Type F, SPX Flow Technology Danmark A/S, Soeborg,
75 DK-2860, Denmark) and spray-dried (Anhydro Spray Dryer, SPX Flow Technology
76 Danmark A/S) using centrifugal disc atomisation at inlet and outlet air temperatures of 180
77 and 85 °C, respectively. The resultant LH- and HH-skim milk powders were each produced
78 on two separate occasions (trials), with both powder types being produced from the same
79 milk on each occasion.

80

81 2.2. Preparation of skim milk samples

82

83 Samples taken during powder manufacture included: skim milk, heat-treated skim
84 milk, evaporated skim milk (45% TS) and powder. Samples of low heat-treated skim milk,
85 evaporated skim milk and powder are denoted as LHSM, LHE and LHP, respectively, and
86 the corresponding high heat-treated samples as HHSM, HHE and HHP, respectively (Table
87 1).

88

89 The LHE and HHE samples were diluted with distilled water at 25 °C and stirred
90 (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).

91 Skim milk samples (9.4% TS), denoted LHP-SM and HHP-SM, were also prepared by
92 reconstitution of the LHP and HHP in distilled water. The powder was dispersed in distilled
93 water (50 °C), held in a water bath (50 °C) while stirring at 750 rpm for 30 min and stored at
94 4 °C for 22 h to allow hydration of the protein; prior to analysis, the reconstituted skim milk
95 samples were warmed to 40 °C and held for 30 min to reverse the cold-aging, and then
96 cooled to 25 °C for analysis (Dalglish & Law, 1988).

97

98 2.3. *Compositional analysis of skim milk and serum*

99

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

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

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

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

$$126 \quad \text{True protein in serum (\%, w/w)} = \text{total protein (\%, w/w)} - (\text{NPN} \times 6.38) (\%, \text{ w/w}) \quad (1)$$

127

$$128 \quad \text{Serum casein (\%, w/w)} = \text{true protein (\%, w/w)} \times \text{casein as \% of true protein} \quad (2)$$

129

$$130 \quad \text{Denatured whey protein complexed with dissociated } \kappa\text{-casein (\%, w/w)} = \text{Total protein} \\ 131 \quad (\%, \text{ w/w}) - \text{serum casein (\%, w/w)} - \text{pH 4.6 soluble protein (\%, w/w)} \quad (3)$$

132

$$133 \quad \text{Denatured whey protein complexed with } \kappa\text{-casein on the casein micelle (\%, w/w)} = \\ 134 \quad \text{Total denatured whey protein (\%, w/w)} - \text{denatured whey protein complexed with} \\ 135 \quad \text{dissociated } \kappa\text{-casein (\%, w/w)} \quad (4)$$

136

137 2.4. Physico-chemical characteristics of skim milk samples

138

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

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

144

145 2.5. *Preparation of skim milk concentrates*

146

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

151

152 2.6. *Calcium ion concentration of skim milk concentrates*

153

154 The LHP-SMC and HHP-SMC samples, at 25% TS, were adjusted to pH values in the
155 range 6.2 to 7.0, at 0.2 pH unit intervals. The [Ca²⁺] of the pH-adjusted concentrates was
156 immediately measured, as described in section 2.3.

157

158 2.7. *Rennet gelation of skim milk and skim milk concentrates*

159

160 Samples of skim milk concentrates with 9.4–25% TS were adjusted to pH 6.55 and
161 inoculated with chymosin (single strength Chy-Max[®] plus, 200 IMCU mL⁻¹; Chr. Hansen,
162 Hørsholm, Denmark), which had been diluted 20-fold with distilled water, at a level of 0.103
163 mL g⁻¹ protein. Milk samples were tested for rennet gelation properties at 31 °C using
164 dynamic low-amplitude strain oscillation rheometry in a controlled-stress rheometer (Carri-
165 Med, type CSL²₅₀₀, TA instruments, New Castle, DE, USA) at a strain of 0.025 and a

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

170

171 2.8. *HCT of skim milk and skim milk concentrates*

172

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

181

182 2.9. *ES of skim milk concentrates*

183

184 Skim milk concentrates with 9.4–25% TS were prepared by reconstitution of SMP
185 and adjusted to pH values in the range 6.2 to 7.0 at 0.2 pH unit intervals. The ES was tested
186 by blending 1 mL of sample with aqueous ethanol solutions of different concentrations (30–
187 98%) while keeping the ethanol-to-protein ratio constant. The mixture of aqueous ethanol and
188 sample was mixed for 30 s before inspection for visible flocculation.

189

190 2.10. *Statistical analysis*

191 Data were analysed using a randomised complete block design, which incorporated
192 the skim milk samples (SM, LHSM, HHSM, LHE-SM, HHE-SM, LHP-SM, HHP-SM) or
193 skim milk concentrate (LHP-SMC and HHP-SMC) and 2 replicate blocks (samples from the
194 2 separate batches of SMP or evaporated milk made on different days). Analysis of variance
195 (ANOVA) was carried out using a general linear model (GLM) procedure of SAS 9.3 (SAS
196 Institute, 2011) and the effects of treatment (stage of manufacture: heat treatment,
197 evaporation and drying) and replicate on each response variable was determined. Tukey's
198 multiple-comparison test was used for paired comparison of treatment means and the level of
199 significance was determined at $P < 0.05$.

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

203 3. Results

205 3.1. Gross composition of skim milk samples

207 The composition of the skim milk samples (SM, LHSM, HHSM, LHE-SM, HHE-SM,
208 LHP-SM, HHP-SM; Table 1) is shown in Table 2. As expected, all samples had similar levels
209 of TS, lactose, total protein, casein, NPN (% total N), total Ca and P. Increasing the heat
210 treatment of the skim milk prior to evaporation led to a significant increase in whey protein
211 denaturation from ~5% of total whey protein on heating at $72\text{ }^{\circ}\text{C} \times 15\text{ s}$ to 80% at $120\text{ }^{\circ}\text{C} \times$
212 120 s (Table 2).

213 The concentration of ionic Ca, $[\text{Ca}^{2+}]$, in the unheated skim milk from trials 1 (2.1
214 mM) and 2 (~5.0 mM) differed markedly. The values, though very different, reflect the range
215 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

241 milk, SM; the corresponding levels in the LHSM, LHE-SM and LHP-SM samples were ~96–
242 99, 95–100 and 96–100%, respectively. This result is consistent with the increase in whey
243 protein denaturation on intensifying milk heat treatment (Table 2). Evaporation and drying
244 did not induce denaturation of whey proteins during the preparation of the SMP, as evidenced
245 by the similar levels of whey proteins in the serum (expressed as a % of the unheated SM) in
246 the heated skim milk, evaporate and reconstituted SMP for both the LH and HH treatments.

247 The concentration of serum caseins, α_S -, β - or κ -casein, expressed as % of the
248 corresponding casein in skim milk, was not affected by heat treatment ($72\text{ }^\circ\text{C} \times 15\text{ s}$),
249 evaporation or drying during the manufacture of LHP, as indicated by the similar values in
250 the SM, LHSM, LHE-SM and LHP-SM. In contrast, heat treatment ($120\text{ }^\circ\text{C} \times 120\text{ s}$) during
251 the manufacture of HHP resulted in significant increases in the levels of serum casein and κ -
252 casein (Table 2, Fig. 2A). For both the LH and HH milk samples, the level of serum κ -casein
253 (% κ -casein in milk) was higher than that of serum β - or α_S -casein (Fig. 2A). Nevertheless,
254 owing to the different concentrations of the individual caseins in milk, the proportions of
255 different serum caseins, expressed as % of total serum casein, were not significantly affected
256 by heat treatment, evaporation or drying during the manufacture of the LHP or HHP (Fig.
257 2B)

258 In contrast to serum casein, the concentrations of serum Ca and P decreased
259 significantly during the manufacture of HHP, as seen on comparing the SM and HHP-SM
260 skim milk samples (Table 2); the reduction was observed entirely during the heating step
261 ($120\text{ }^\circ\text{C} \times 120\text{ s}$), with no further reduction during evaporation and drying. In the
262 manufacture of LHP, serum Ca and P were not affected by heat treatment ($72\text{ }^\circ\text{C} \times 15\text{ s}$),
263 decreased during evaporation, and increased during drying. Nevertheless, the levels of serum
264 Ca and P in the LHP-SM and SM were similar, indicating no overall influence during the

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

267

268 3.4. Calcium ion content of skim milk concentrates

269

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

275

276 3.5. Rennet gelation of skim milk and skim milk concentrates

277

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

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

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

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

298

299 3.6. Heat stability of skim milk and skim milk concentrates

300

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

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

315 corresponding pH values, probably because of the slightly higher protein content and $[Ca^{2+}]$
316 of milk from trial 2. Nevertheless, the trend in HCT with TS was similar for both trials. At
317 9.4% TS, the HCT of HHP-SM showed a typical type A profile, with a distinct HCT_{max} at 6.5
318 and HCT_{min} at 6.7–6.8, whereas that of the LHP-SM increased continuously on increasing pH
319 to pH 6.9 and then decreased slightly as pH was further increased to 7.0. Compared with the
320 LHP-SM (9.4% TS), the HCT of the HHP-SM skim milk was 35 to 100 min higher than that
321 of the LHP-SM at pH 6.3–6.5 and ~20 to 34 min lower at pH 6.7–6.9.

322 The HCT of both the LHP-SMC and HHP-SMC samples at pH values ≥ 6.4 decreased
323 on increasing TS from 9.4 to 25% (Fig. 7A–D). A major difference between the LHP-SMC
324 and HHP-SMC samples was the higher HCT of HHP-SMC concentrates (20 and 25% TS) at
325 pH values 6.3–6.6. Hence, while the HCT of the LHP-SMC with 20–25% TS was very low
326 (<10 min) at all pH values, that of the corresponding HHP-SMC was quite high in the pH
327 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
328 (trial 2) at pH 6.5 (Fig. 7B). The results clearly indicate that increasing the severity of the
329 heat treatment of the skim milk prior to powder manufacture enhances the heat stability of
330 high-solids skim milk concentrates, or conversely enables reconstitution of skim milk powder
331 to higher TS while retaining adequate heat stability at pH 6.3–6.6 during thermal processing
332 of recombined milks.

333

334 3.7. *ES of skim milk concentrates*

335

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

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

345

346 **4. Discussion**

347

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

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

364 In contrast to the trend for serum whey protein, the concentration of serum casein
365 increased significantly with HH treatment, mainly as a consequence of an increase in the
366 concentration of serum κ -casein (% total κ -casein). This increase in serum κ -casein and
367 denatured whey protein complexed with the κ -casein confirms the results of previous studies
368 showing a significant increase in the extent of dissociation of κ -casein from the micelle into
369 the serum as the temperature during heat treatment was increased, e.g., from 60 to 120 °C
370 (Anema & Li, 2015; Ménard, Camier, & Guyomarc'h, 2005). It has been shown that the
371 dissociated κ -casein interacts with denatured whey protein in the serum to form serum-
372 soluble aggregates or particles (Donato & Guyomarc'h, 2009; Ménard et al., 2005; Mollé et
373 al, 2006). Using a combination of chymosin-induced precipitation and capillary
374 electrophoresis, Vasbinder et al. (2003a) determined the proportions of β -Lg and α -La that
375 complexed with dissociated κ -casein (in the serum) and non-dissociated κ -casein (on the
376 casein micelle) in milk as the pasteurisation temperature was increased from 70 to 90 °C (for
377 10 min) at native pH; the level of β -Lg denaturation increased from ~2 to 95% of total β -Lg,
378 and the level of serum casein increased from <5 to 10 % of total casein. Simultaneously, the
379 proportions of total β -Lg involved in the formation of serum-soluble aggregates or associated
380 with the casein micelle increased from ~2 to 25% or 1 to 65 % of total, respectively. Hence,
381 the proportions of denatured β -Lg that form serum-soluble aggregates or reacted with the
382 casein micelle were ~28 and 72% of total denatured whey protein. In the current study, the
383 proportion of denatured whey protein interacted with dissociated κ -casein was estimated at
384 ~14% of total denatured whey protein in the HH-SMP; this estimate was based on the
385 difference between the whey protein content of the HH-SMP serum and the filtrate obtained
386 on pH-adjustment of the serum to pH 4.6. The interaction of most of the denatured whey
387 protein (~86%) with casein micelle was supported by the significantly higher casein micelle
388 size in the HH skim milk samples. Likewise, Martin et al (2007) reported progressive

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

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

411 HH treatment of skim milk before evaporation reduced the pH of HCT_{max} , broadened
412 the HCT_{min} region, and increased HCT at pH values 6.3 to 6.6; this effect became more
413 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

439 TS on ES at pH >6.7 was due to the increase in chloride content, and hence ionic strength. It
440 was hypothesised that higher ionic strength resulted in a shift in calcium-citrate equilibrium,
441 which favoured a higher $[Ca^{2+}]$ concentration, and hence lower ethanol stability, in high-
442 solids concentrates. Nevertheless, the results of the current study showed that the
443 $[Ca^{2+}]$ /casein ratio decreased with increasing pH.

444

445 **5. Conclusion**

446

447 The changes in the partition of milk components (minerals and proteins), between the
448 casein micelle and serum, and processing characteristics of milk at the different stages of
449 manufacture of low-heat and high-heat skim milk powder were investigated. Increasing heat
450 treatment of skim milk from $72\text{ }^{\circ}\text{C} \times 15\text{ s}$ to $120\text{ }^{\circ}\text{C} \times 120\text{ s}$ resulted in higher levels of whey
451 protein denaturation, serum casein, serum κ -casein as a proportion of total κ -casein, and
452 casein micelle size, and in lower concentrations of ionic calcium and of serum calcium and
453 phosphorous in skim milk and reconstituted skim milk powder. These changes were
454 paralleled by marked deterioration in rennet coagulability, and increases in HCT at pH 6.3–
455 6.6 and ES at pH 6.2 and 6.4. Increasing the TS level from 9.4 to 25% in skim milk
456 concentrates, prepared by reconstitution of the skim milk powder, coincided with lower HCT
457 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
458 rennet coagulability (at $TS \geq 20\%$). The findings indicate how the intensity of heat treatment
459 during the manufacture of skim milk powder can be altered to modulate the functionality of
460 the reconstituted powder and its suitability in different applications, e.g., recombined milk
461 cheese or UHT-based milk beverages

462

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464

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467

468 **References**

469

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562 samples, methods and chemical composition of samples *Journal of Dairy Research*, 25,
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Figure legends

2

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

9

10 **Fig. 2.** Concentration of caseins in serum prepared by ultracentrifugation of skim milk
11 samples at $100,000 \times g$ at 25°C : $\alpha_{S1} + \alpha_{S2}$ -casein (\circ), β -casein (\blacktriangle) and κ -casein (\triangle).
12 Samples, as defined in Table 1, include unheated skim milk (SM), low-heat treated skim milk
13 (LHSM), skim milk prepared by dilution of evaporated low-heat treated skim milk (LHE-
14 SM) or by reconstitution of low-heat skim milk powder (LHP-SM); the corresponding
15 samples from high-heat treated skim milk, i.e., HHSM, HHE-SM, and HHP-SM. Data
16 presented are the means of duplicate batches of each treatment; error bars represent the
17 standard deviation of the mean.

18

19 **Fig. 3.** Changes in concentration ionic calcium, $[\text{Ca}^{2+}]$, and $[\text{Ca}^{2+}]$:casein ratio as a function
20 of pH for skim milk concentrates with total solids content of 9.4% (\circ, \bullet) or 25% ($\triangle, \blacktriangle$),
21 prepared by reconstituting low-heat skim milk powder (A, C) or high-heat skim milk powder
22 (B, D).

23

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

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

31

32 **Fig. 5.** Development of storage modulus, G' , in rennet-treated skim milk concentrates with
33 9.4% (△), 15% (▲), 20% (○) or 25% (●) total solids. The concentrates were prepared by
34 reconstituting low-heat (A, C) or high-heat (B, D) skim milk powder from duplicate batches:
35 Trial 1 (A, B) and Trial 2 (C, D). Storage modulus at 60 min, G'_{60} , as a function of total
36 solids level for concentrates prepared from low-heat (E) or high-heat (F) skim milk powder;
37 presented data for G'_{60} in both E and F is from trials 1 and 2.

38

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

45

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

50

51 **Fig. 8.** Ethanol stability as a function of (A–D) pH for skim milk concentrates [prepared by
52 reconstituting low-heat (\triangle) or high-heat (\blacktriangle) skim milk powder] with 9.4% (A), 15% (B),
53 20% (C) or 25% (D) total solids level and ethanol stability of concentrates [prepared by
54 reconstituting low-heat (E) or high-heat (F) skim milk powder] as a function of (E–F) total
55 solids at pH 6.2 (\circ), 6.4(\bullet), 6.6 (\triangle), 6.8(\blacktriangle) and 7.0 (\square). Data are the means of duplicate
56 batches of each treatment; error bars represent the standard deviation of the mean.

57

58

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%.

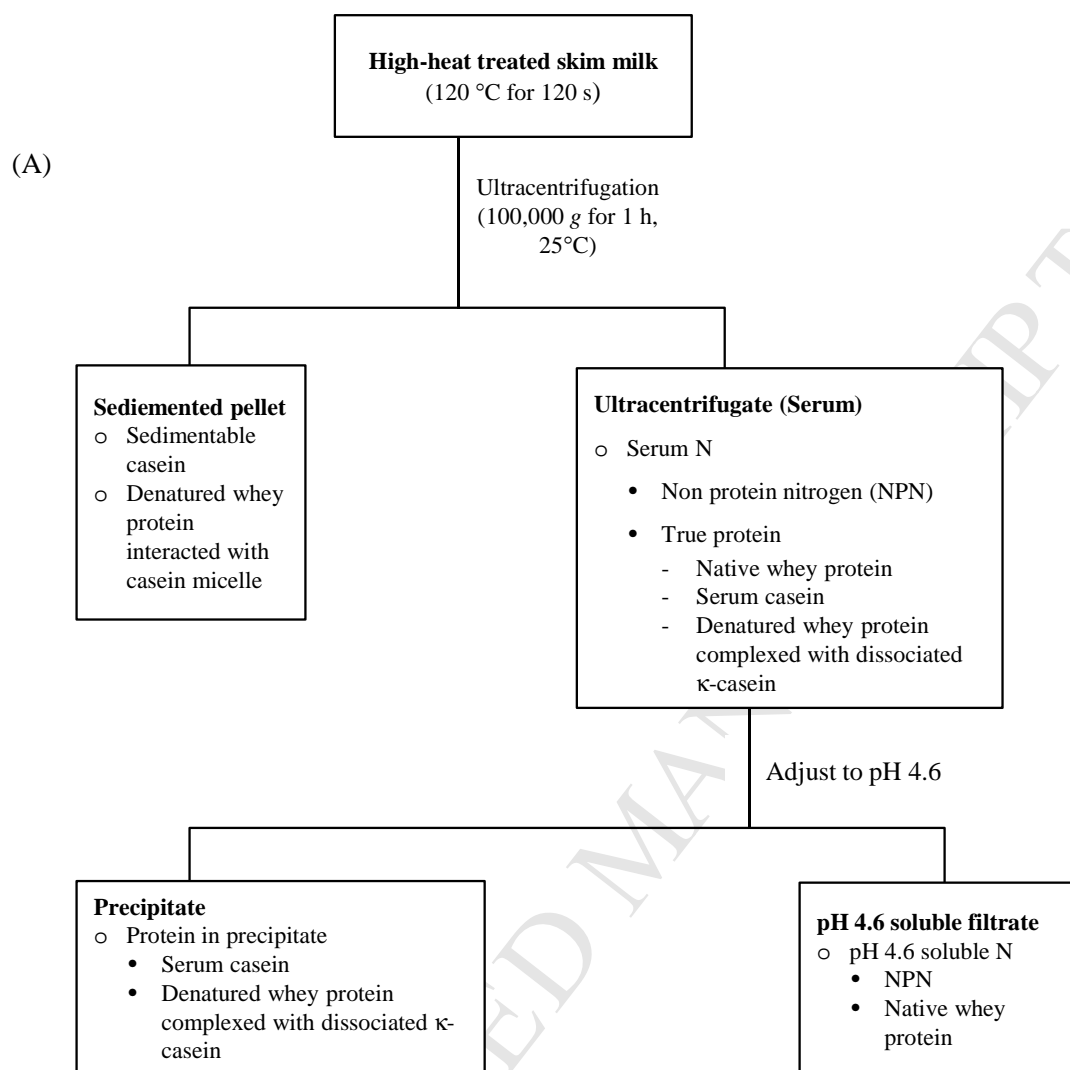
Table 2
Composition of skim milk and serum. ^a

Composition	Heat treatment						
	None SM	Low-heat (LH)			High-heat (HH)		
		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

include HHSM, HHE-SM, and HHP-SM. Skim milk serum was obtained by ultracentrifugation at $100,000 \times g$ 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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(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.

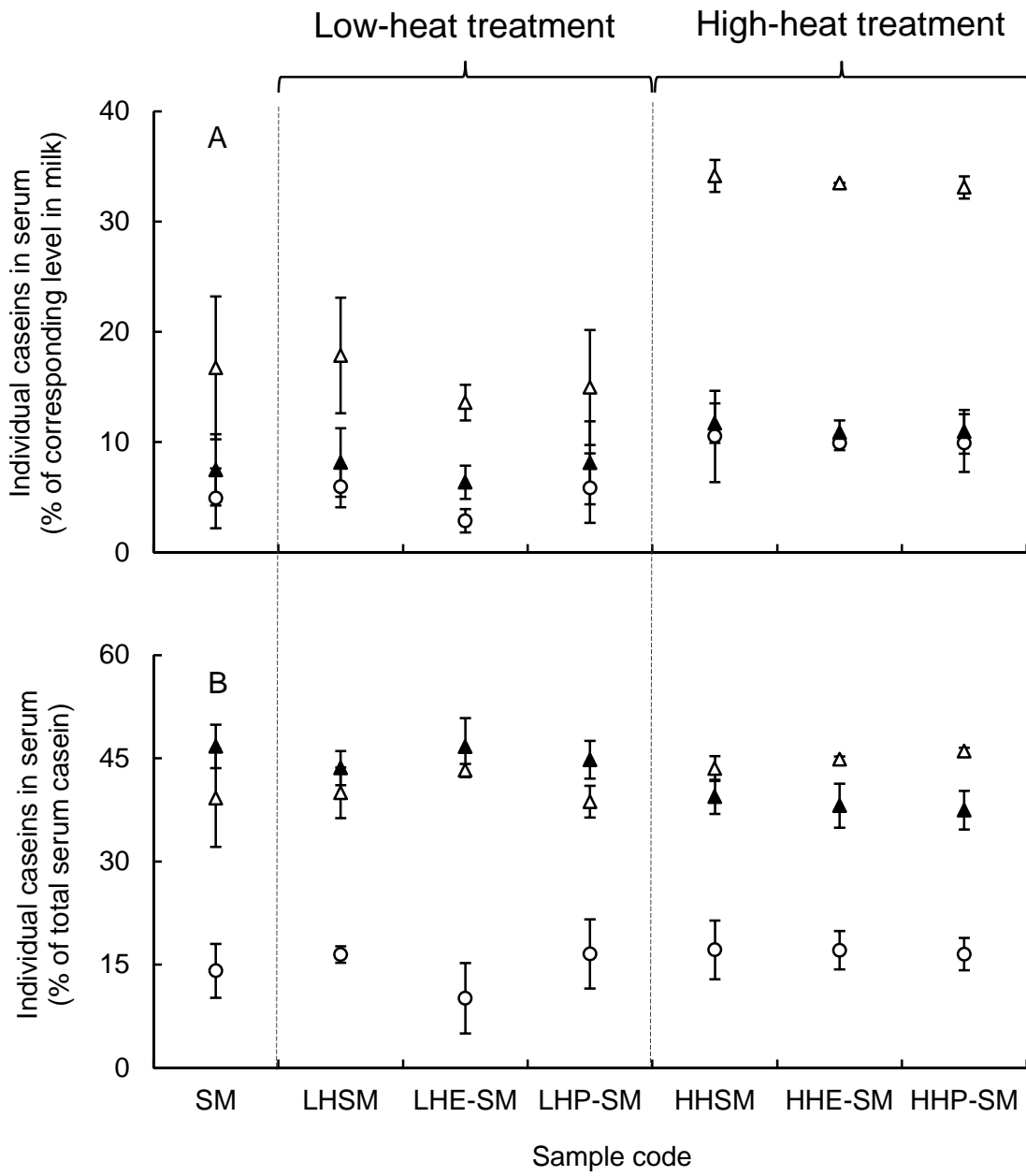


Fig. 2

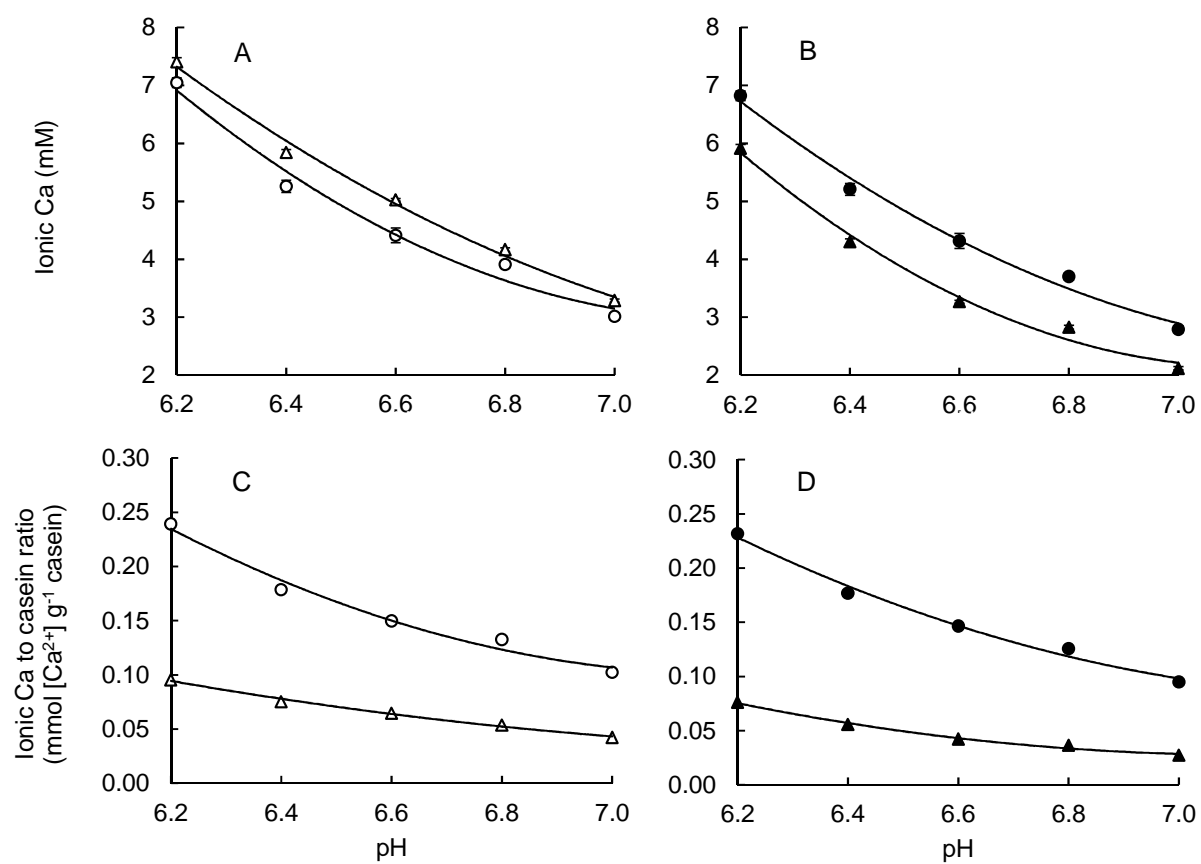


Fig. 3

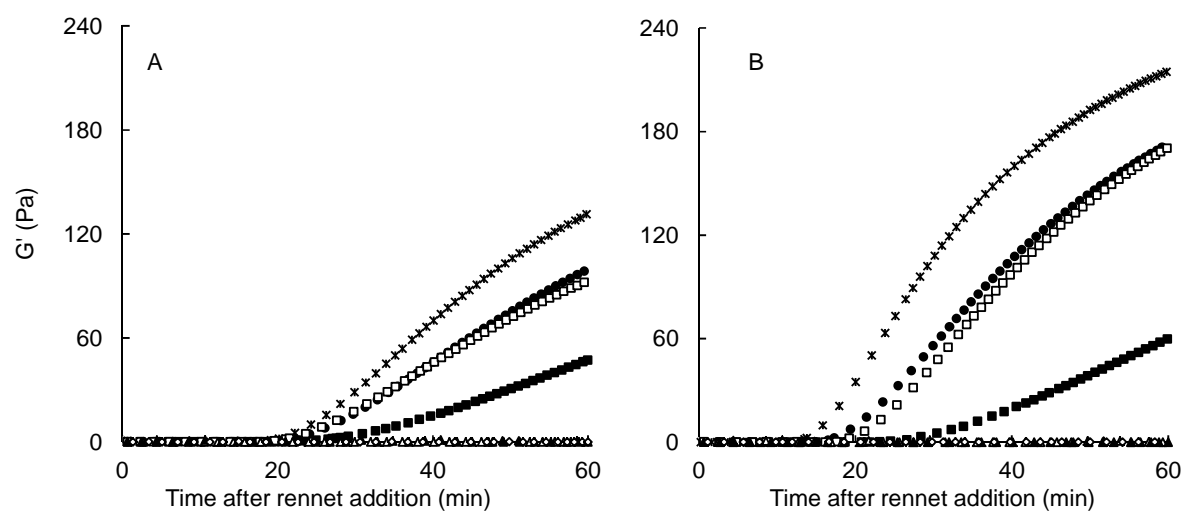


Fig. 4

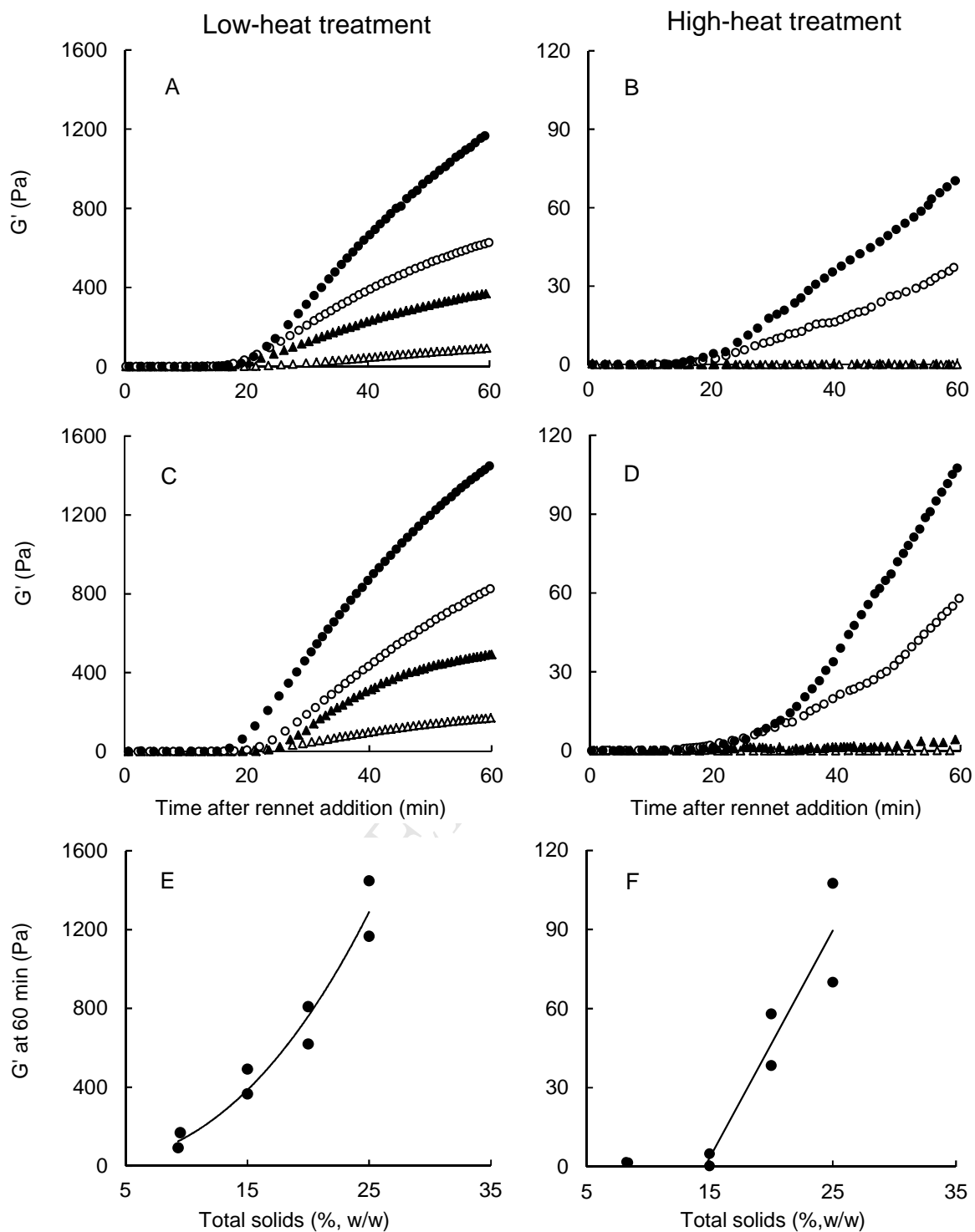


Fig. 5

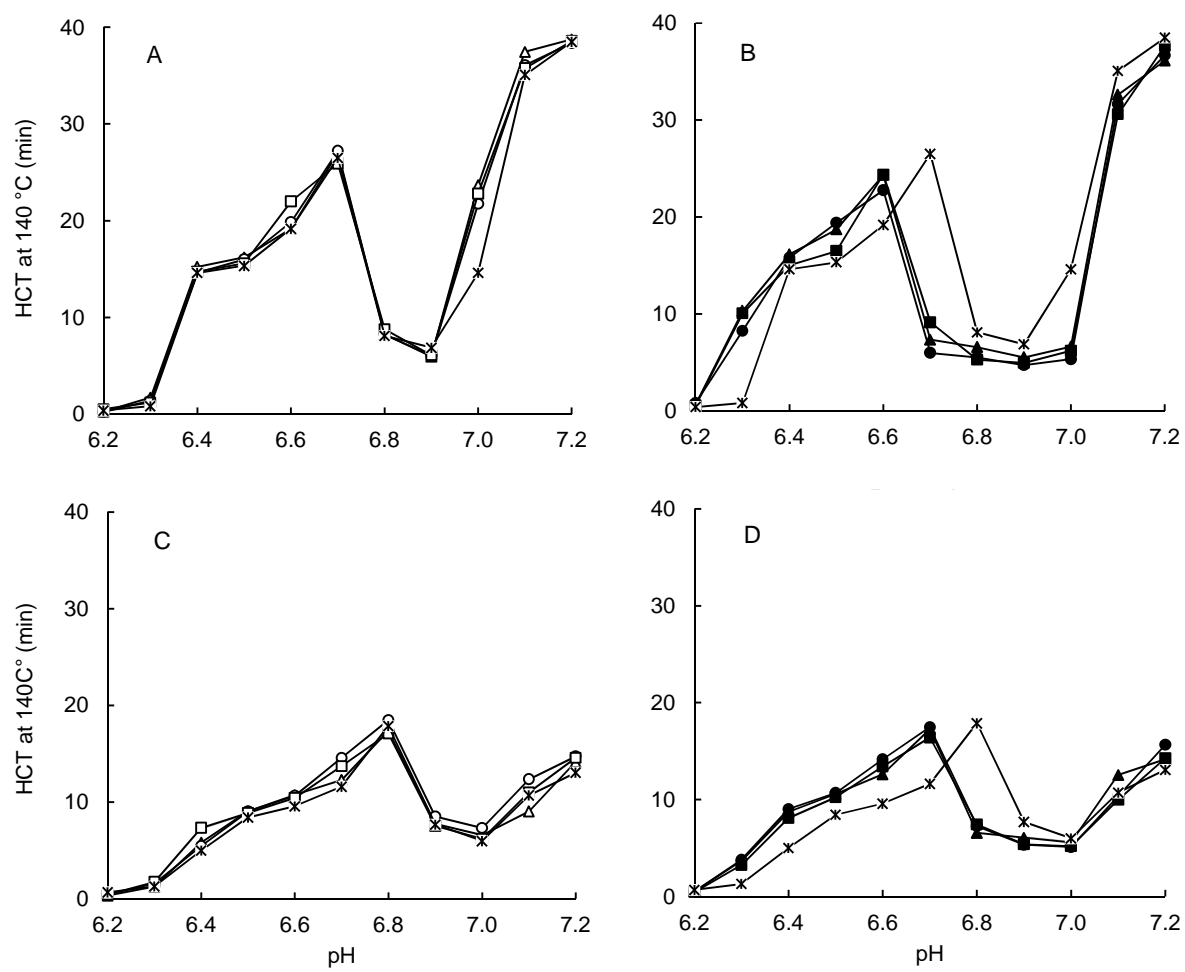


Fig. 6

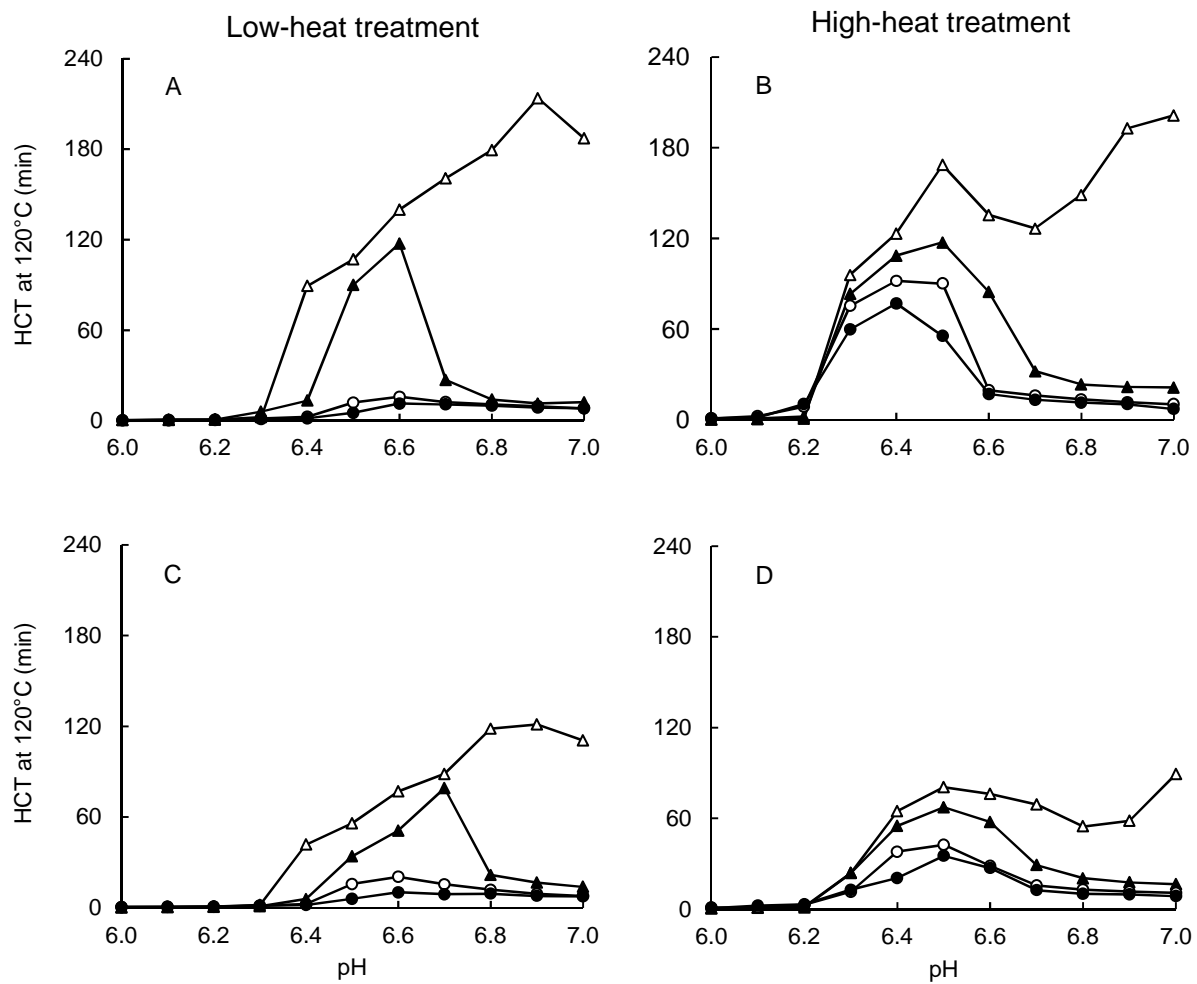


Fig. 7

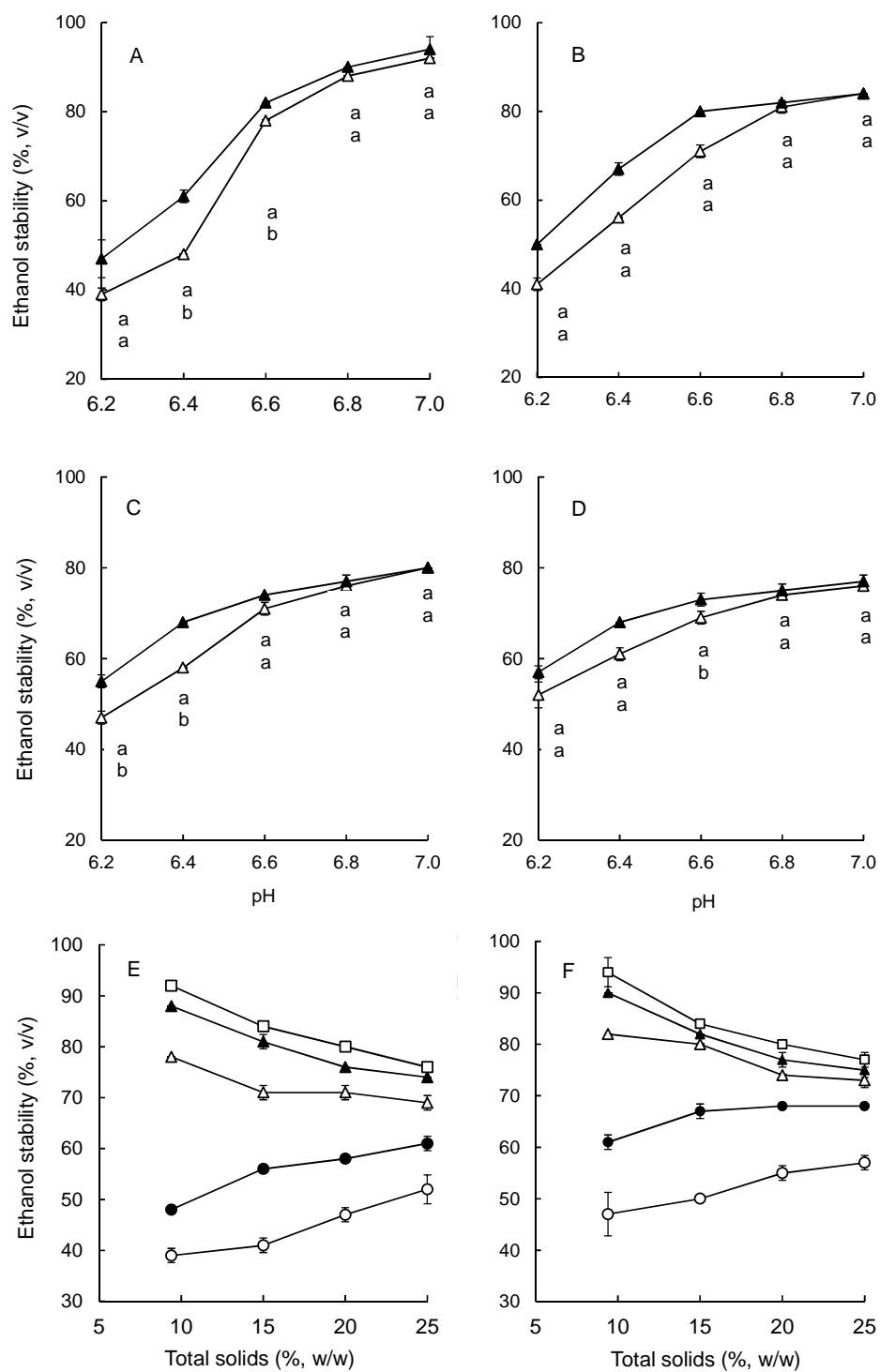


Fig. 8