

Title	Effect of heat treatment during skim milk powder manufacture on the compositional and processing characteristics of reconstituted skim milk and concentrate
Authors	Lin, Yingchen;Kelly, Alan L.;O'Mahony, James A.;Guinee, Timothy P.
Publication date	2017-11-06
Original Citation	Lin, Y., Kelly, A. L., O'Mahony, J. A. and Guinee, T. P. (2017) 'Effect of heat treatment during skim milk powder manufacture on the compositional and processing characteristics of reconstituted skim milk and concentrate', International Dairy Journal, 78, pp. 53-64. doi:10.1016/j.idairyj.2017.10.007
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.idairyj.2017.10.007
Rights	© 2017, Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http:// creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2025-08-18 06:34:52
Item downloaded from	https://hdl.handle.net/10468/5059



University College Cork, Ireland Coláiste na hOllscoile Corcaigh

# Accepted Manuscript

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

Yingchen Lin, Alan L. Kelly, James A. O'Mahony, Timothy P. Guinee

PII: S0958-6946(17)30221-2

DOI: 10.1016/j.idairyj.2017.10.007

Reference: INDA 4230

To appear in: International Dairy Journal

Received Date: 24 May 2017

Revised Date: 18 October 2017

Accepted Date: 19 October 2017

Please cite this article as: Lin, Y., Kelly, A.L., O'Mahony, J.A., Guinee, T.P., Effect of heat treatment during skim milk powder manufacture on the compositional and processing characteristics of reconstituted skim milk and concentrate, *International Dairy Journal* (2017), doi: 10.1016/j.idairyj.2017.10.007.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



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

Yingchen Lin<sup>a</sup>, Alan L. Kelly<sup>b</sup>, James A. O'Mahony<sup>b</sup>, Timothy P. Guinee<sup>a,\*</sup>

<sup>a</sup> Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

<sup>b</sup> School of Food and Nutritional Sciences, University College Cork, Cork, Ireland

\*Corresponding author. Tel.: + 353 25 42204

*E-mail address*: tim.guinee@teagasc.ie (T.P. Guinee).

#### ABSTRACT

4	The effects of key manufacturing steps (heat treatment, evaporation and spray drying) during
5	the manufacture of low- and high-heat skim milk powders (SMP) on the physico-chemical
6	and processing characteristics of milk, and concentrates of varying total solids (TS) levels
7	prepared by reconstituting the milk powders, were evaluated. Milk heat treatment had the
8	most pronounced effect, with an increase in severity of heat treatment from 72 $^{\circ}\text{C} \times 15$ s to
9	120 °C $\times$ 120 s, prior to evaporation resulting in higher heat coagulation time (HCT) at pH
10	6.3-6.6 and ethanol stability (ES) at pH 6.2-6.6, and a marked deterioration of rennet-
11	induced coagulability. Increasing TS of the milk on reconstitution from 9.4 to 25% reduced
12	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
13	recovery of rennet-coagulability. The results highlight how heat treatment may be used to
14	customise the functionality of SMP to different applications.
15	

#### 16 **1.** Introduction

17

18 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 19 milk with different levels of total solids (e.g., 9-30%), for applications such as milk-based 20 beverages, condensed milks, and recombined milks for cheese or yoghurt manufacture (Gilles 21 & Lawrence, 1982; IDF, 19; Lagrange, Whitsett, & Burris, 201599). SMP is classified as 22 low, medium- or high-heat SMP according to the heat treatment applied to skim milk prior to 23 24 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, 25 O'Connell, & Fox, 2003). High-heat SMP is used as an ingredient in bakery, sweetened 26 condensed milk, and confectionery products such as UHT recombined concentrated milk, 27 toffee, caramel, fudge and milk chocolate (Aitken, Agustin, & Clarke, 1999; Stewart et al., 28 2017). Low-heat powder is also used extensively in food formulation, including applications 29 such as recombined milk for cheese manufacture, milk solids standardisation in products such 30 as cheese milk, yoghurt and fermented milk products (Patel, Anema, Holroyd, Singh, & 31 Creamer, 2007). 32

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

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

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.

62

- 63 2. Materials and methods
- 64

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

67	Skim milk powder was manufactured at Moorepark Technology Limited (Cork,
68	Ireland). Milk was separated at 55 $^{\circ}$ C (Westfalia Model MM1254 Separator; Westfalia,
69	Germany) and the skim milk (≤0.1% fat) was pasteurised using a plate heat-exchanger (APV
70	Pasilac SSP pilot plant, APV DK 8600, Silkeborg, Denmark) at 72 $^{\circ}C \times 15$ s (low heat, LH)
71	or using a MicroThermics <sup>®</sup> 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

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).

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 (Dalgleish & 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 <sup>™</sup> FT+ (N. Foss Electric
102	A/S, Hillerød, Denmark) and ionic calcium [Ca <sup>2+</sup> ], 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 case in ( $\kappa$ -, $\beta$ -, $\alpha_s$ -case ins)
117	and denatured whey protein, assumed to be complexed with $\kappa$ -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 case n and denatured whey protein/ $\kappa$ -case 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 case n and denatured whey protein/ $\kappa$ -case aggregates) and the serum case in
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	True protein in serum (%, w/w) = total protein (%, w/w) – (NPN × 6.38) (%, w/w) (1)
127	
128	Serum casein (%, w/w) = true protein (%, w/w) × casein as % of true protein (2)
129	
130	Denatured whey protein complexed with dissociated $\kappa$ -casein (%, w/w) = Total protein
131	(%, w/w) – serum casein $(%, w/w)$ – pH 4.6 soluble protein $(%, w/w)$ (3)
132	
133	Denatured whey protein complexed with $\kappa$ -casein on the casein micelle (%, w/w) =
134	Total denatured whey protein (%, w/w) – denatured whey protein complexed with
135	dissociated $\kappa$ -casein (%, w/w) (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 $[Ca^{2+}]$ of the pH-adjusted concentrates was
156	immediately measured, as described in section 2.3.
157	
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 <sup>®</sup> plus, 200 IMCU mL <sup>-1</sup> ; Chr. Hansen,
162	Hørsholm, Denmark), which had been diluted 20-fold with distilled water, at a level of 0.103
163	mL g <sup>-1</sup> 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 $\text{CSL}_{500}^2$ , 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 $\geq 0.2$ 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 $\geq 120$ °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 bathes 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.
202	
203	3. Results
204	
205	3.1. Gross composition of skim milk samples
206	
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 °C × 15 s to 80% at 120 °C ×
212	120 s (Table 2).
213	The concentration of ionic Ca, $[Ca^{2+}]$ , in the unheated skim milk from trials 1 (2.1

 $m_{M}$  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,

216	1982; White & Davies, 1958). Hence, the value of $[Ca^{2+}]$ was normalised to 100 for the skim
217	milk in both trials 1 and 2, to facilitate statistical analysis. HH treatment led to a significant
218	reduction in $[Ca^{2+}]$ , but low heat treatment had no effect, as reflected by the similar $[Ca^{2+}]$ in
219	the SM and LHSM samples (Table 2). During the manufacture of both LHP and HHP,
220	evaporation led to a reduction in $[Ca^{2+}]$ , while drying resulted in restoration to a level equal
221	to that of the LHSM and HHSM samples, respectively. The mean $[Ca^{2+}]$ value of the HHSM,
222	HHE-SM and HHP-SM were significantly lower than those of the corresponding samples of
223	LHSM, LHE-SM and LHP-SM (Table 2).
224	
225	3.2. Physico-chemical properties of skim milk samples
226	
227	All skim milk samples showed a mono-modal particle size/number distribution.
228	Casein micelle size increased significantly during the manufacture of both LHP and HHP,
229	with the increase occurring during evaporation for the former, and increased during milk heat
230	treatment and evaporation for the latter (Table 2). Particle sizes for the LHSM, LHE-SM and
231	LHP-SM were significantly lower than those of the corresponding HHSM, HHE-SM and
232	HHP-SM. The zeta potential and hydration of all skim milk samples ranged from -20.6 to -
233	2.9 mV and from 3.02 to 3.19 g water $g^{-1}$ casein, respectively, and were not significantly
234	affected by heat treatment, evaporation or drying.
235	
236	3.3. Composition of the sera from skim milk samples

237

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

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  $(120 \ ^{\circ}C \times 120 \ ^{\circ})$ , with no further reduction during evaporation and drying. In the manufacture of LHP, serum Ca and P were not affected by heat treatment (72  $\ ^{\circ}C \times 15 \ ^{\circ})$ , 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

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' <sub>60</sub> 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 $G'_{60}$ = total solids <sup><i>n</i></sup> , and the exponent <i>n</i>

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 ~8.5 Pa $g^{-1}$ 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

The HCT/pH curves for SM and the LH- and HH skim milk samples are shown in 301 Fig. 6A–D. All curves displayed the typical type A profile, with a maximum (HCT<sub>max</sub>) and a 302 minimum (HCT<sub>min</sub>). The processing steps during the manufacture of LHP had little, or no, 303 effect, as seen from the similar profiles of the SM, LHSM, LHE-SM and LHP-SM samples. 304 In contrast, HH treatment during the manufacture of HHP reduced the pH of HCT<sub>max</sub> by 0.1 305 and broadened the pH region of HCT<sub>min</sub>, as observed by comparing the SM and HHSM 306 samples. Otherwise, evaporation and drying during the manufacture of HHP had little impact 307 on the heat stability characteristics of skim milk, as seen by the similarity of the HCT/pH 308 profiles for the HHSM, HHE-SM and HHP-SM samples. High-solids recombined milks, 309 which generally have relatively low pH compared with skim milk, are frequently subjected to 310 heating (e.g., pasteurisation and sterilisation); consequently, the HCT/pH profile of 311 reconstituted skim milk with varying TS is of interest. 312 The HCT/pH profiles of milk samples with TS of 9.4 to 25% at 120 °C are shown in 313

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 <sup>2+</sup> ]
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 $\geq$ 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

333

332

### 334 3.7. ES of skim milk concentrates

of recombined milks.

335

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

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	influenced 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, &						

Boerrigter, 1987). 363

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

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 $\times$ 30 s or 120 °C $\times$ 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 $\kappa$ -casein/ $\beta$ -Lg aggregates (Kethireddipalli, Hill, & Dalgeleish 2010; Vasbinder,
395	Rollema, & de Kruif, 2003b) and reduction in [Ca <sup>2+</sup> ] in the HHSM (Sandra, Ho, Alexander,
396	& Corredig, 2012; Singh, Shalabi, Fox, Flynn, & Barry, 1988). Though the κ-casein in the κ-
397	case in/ $\beta$ -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 $\Box$ -
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 <sup>2+</sup> ] 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

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

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

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 highsolids concentrates. Nevertheless, the results of the current study showed that the  $[Ca^{2+}]$ /casein ratio decreased with increasing pH.

444

### 445 **5.** Conclusion

446

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

462

#### 463 Acknowledgement

TTT	A A A A	TTTC		TD/T
/ I H I )	N/  A		$\mathbf{K}$	
	TATT TT		$\mathcal{S}\mathcal{O}\mathcal{I}$	

464	
465	The authors gratefully acknowledge the financial assistance of the Dairy Levy Trust
466	Co-Operative Society Limited (Dublin, Ireland).
467	
468	References
469	
470	Aitken, B., Agustin, M. A. and Clarke, P. T., (1999). Recombined milk and milk products:
471	New special milk powders for recombined and reconstituted evaporated milk. Special
472	issue of the International Dairy Federation SI-9902 (pp.87-89). Brussels, Belgium:
473	International Dairy Federation.
474	Anema, S. G., & Li, Y. (2015). Reassociation of dissociated casein upon acidification of
475	heated pH-adjusted skim milk. Food Chemistry, 174, 339-347.
476	Chandrapala, J., McKinnon, I., Augstin, A. M., & Udabage, P. (2010). The influence of milk
477	composition on pH and calcium activity measured in situ during heat treatment of
478	reconstituted skim milk. Journal of Dairy Research, 77, 257–264.
479	Dalgleish, D. G., & Law, A. J. R. (1988). pH-induced dissociation of bovine casein micelles.
480	I. Analysis of liberated caseins. Journal of Dairy Research, 55, 529–538.
481	Donato, L., & Guyomarc'h, F. (2009). Formation and properties of the whey protein/ $\kappa$ -casein
482	complexes in heated skim milk - A review. Dairy Science and Technology, 89, 3–29.
483	Gilles, J., & Lawrence, R. C. (1982). The manufacture of cheese and other fermented
484	products from recombined milk. Proceedings of International Dairy Federation seminar
485	on Recombination of Milk and Milk Products (pp. 111–117). Singapore.
486	Guinee, T. P., Gorry, C. B., O'Callaghan, D, J., O'Kennedy, B. T., O'Brien, N., & Fenelon,
487	M. A. (1997). The effects of composition and some processing treatments on the rennet
488	coagulation properties of milk. International Journal of Dairy Technology, 50, 99–106.

- 489 Horne, D. S. & Parker, T. G. (1981). Factors affecting the ethanol stability of bovine milk:
- 490 IV. Effect of forwarming. *Journal of Dairy Research*, 48, 405–415.
- 491 Horne, D. S. & Parker, T. G. (1983). Factors affecting the ethanol stability of bovine milk:
- 492 VI. Effect of concentration. *Journal of Dairy Research*, *50*, 425–432.
- 493 IDF (1999). 3<sup>rd</sup> International Symposium on Recombined Milk and Milk Products. Brussels,
- 494 Belgium: International Dairy federation.
- Kelly, A. L., O'Connell, J. E., & Fox, P. F. (2003). Manufacture and properties of milk
- 496 powders. In P. F. Fox & P. L. H. McSweeney (Eds.) *Advanced dairy chemistry. Vol 1.*
- 497 *Proteins* (3<sup>rd</sup> edn, Part B, pp. 1027–1061). New York, NY, USA: Kluwer
- 498 Academic/Plenum Publishers.
- Kelly, P. M., Keogh, M. K., O'Keeffe, A. M. & Phelan J. A. (1982). Studies of milk
- composition and its relationship to some processing criteria II. Seasonal variation in the
  mineral levels of milk. *Irish Journal of Food Science and Technology*, *6*, 13–27.
- 502 Kethireddipalli, P., Hill, A. R., & Dalgleish, D. G. (2010). Protein interactions in heat-treated
- milk and effect on rennet coagulation. *International Dairy Journal*, 20, 838–843.
- Lagrange, V., Whitsett, D., & Burris, C. (2015). Global market for dairy proteins. *Journal of Food Science*, 80, 23–27.
- Lin, Y., Kelly, A. K., O'Mahony, J. A., & Guinee, T. P. (2017). Addition of sodium caseinate
   to skim milk increases non-sedimentable casein and causes significant changes rennet induced gelation, heat stability and ethanol stability. *Journal of Dairy Science*, *100*,
- 509 908–918.
- Martin, G. J. O., Williams, R. P. W., & Dunstan, D. E. (2007). Comparison of casein micelles
  in raw and reconstituted skim milk. *Journal of Dairy Science*, *90*, 4543–4551.
- 512 Ménard, O., Camier, B., & Guyomarc'h, F. (2005). Effect of heat treatment at alkaline pH on
- the rennet coagulation properties of skim milk. *Lait*, 85, 515–526.

514	Mohammed, K., & Fox, P. F. (1986). Heat and alcohol-induced coagulation of casein

515 micelles. *Irish Journal of Food Science and Technology*, *10*, 47–55.

- 516 Mollé, D., Jean, K., & Guyomarc'h, F. (2006). Chymosin sensitivity of the heat-induced
- serum protein aggregates isolated from skim milk. *International Dairy Journal*, *16*,
  1435–1441.
- 519 O'Connell, J. E., & Fox, P. F. (2003). Heat-induced coagulation of milk. In P. F. Fox &
- 520 P.L.H. McSweeney (Eds.) Advanced dairy chemistry. Vol 1. Proteins (3<sup>rd</sup> edn, Part B,

521 pp. 879–946). New York, NY, USA: Kluwer Academic/Plenum Publishers.

522 Patel, H. A., Anema, S. G., Holroyd, S. E., Singh, H., & Creamer, L. K. (2007). Methods to

determine denaturation and aggregation of proteins in low-, medium- and high-heat
skim milk powders. *Lait*, 87, 251–268.

525 Pomprasirt, V., Singh H., & Lucey, J. A. (1998). Effect of heat treatment on the rennet

coagulation properties of recombined high total solids milk made from milk protein
 concentrate powder. *International Journal of Dairy Technology*, *51*, 65–71.

527 concentrate powder. *International Journal of Dairy Technology*, *51*, 65–71.

- 528 Rynne, N. M., Beresford, T. P., Kelly, A. L. & Guinee, T. P. (2004). Effect of milk
- 529 pasteurization temperature and in situ whey protein denaturation on the composition,
- 530 texture and heat-induced functionality of half-fat Cheddar cheese. *International Dairy*
- *Journal*, *14*, 989–1001.
- Sandra, S., Ho, M., Alexander, M., & Corredig, M. (2012). Effect of soluble calcium on the
  renneting properties of casein micelles as measured by rheology and diffusing wave
  spectroscopy. *Journal of Dairy Science*, 95, 75–82.
- SAS Institute. (2011). SAS user's guide: Statistics. Version 9.3 edn. Cary, NC, USA: SAS
  Institute Inc.

······································	537	Sievanen,	K., Huppertz,	T., Kelly,	, A., & Fox.	P. F. (2008	). Influence of added calc
--	-----	-----------	---------------	------------	--------------	-------------	----------------------------

chloride on the heat stability of unconcentrated and concentrated bovine milk.

539 International Journal of Dairy Technology, 61, 151–155.

- 540 Singh, H., & Creamer, L. K. (1991). Denaturation, aggregation and heat stability of milk
- protein during the manufacture of skim milk powder. *Journal of Dairy Research*, 58,
  269–283.
- Singh, H., Roberts, M.S., Munro, P.A. & Teo, C. T. (1996). Acid-induced dissociation of
  casein micelles in milk: effects of heat treatment. *Journal of Dairy Science*, *79*, 1340–
  1346.
- 546 Singh, H., Shalabi, S.I., Fox, P. F., Flynn, A., & Barry, A. (1988). Rennet coagulation of
- heated milk: Influence of pH adjustment before or after heating. *Journal of Dairy Research*, 55, 205–215.
- 549 Stewart, A., Grandison, A. S., Ryan, A., Festring, D., Methven, L., & Parker, J. K. (2017).
- 550 Impact of the skim milk powder manufacturing processing on the flavour of model 551 white chocolate. *Journal of Agricultural and Food Chemistry*, *65*, 1186–1195.
- van Hooydonk, A. C. M., de Koster, P. G., & Boerrigter, I. J. (1987). The renneting

properties of heated milk. *Netherlands Milk and Dairy Journal*, *41*, 3–18.

- Vasbinder, A. J., Alting, A. C., & de Kruif, K. G. (2003a). Quantification of het-induced
- casein-whey protein interactions in milk and its relation to gelation kinetics. *Colloids and Surfcaes B: Biointerfaces, 31*, 115–123.
- Vasbinder, A. J., Rollema, H. S. & de Kruif, C. G. (2003b). Impaired rennetability of heated
  milk; study of enzymatic hydrolysis and gelation kinetics. *Journal of Dairy Science*, *86*,
  1548–1555.
- White, J. C. D. & Davies, D. T., (1958). The relation between the chemical composition of
  milk and the stability of the caseinate complex. I. General introduction, description of

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

563 236–255.

## 1 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 × g at 25 °C: $\alpha_{S1} + \alpha_{S2}$ -casein ( $\bigcirc$ ), $\beta$ -casein ( $\blacktriangle$ ) and $\kappa$ -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, $[Ca^{2+}]$ , and $[Ca^{2+}]$ :casein ratio as a function
20	of pH for skim milk concentrates with total solids content of 9.4% ( $\bigcirc$ , $\bigcirc$ ) 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

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'<sub>60</sub>, 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'<sub>60</sub> in both E and F is from trials 1 and 2.

38

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).

45

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).

<sup>31</sup> 

51	<b>Fig. 8.</b> 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 ( $\bigcirc$ ), 6.4( $\bigcirc$ ), 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.<sup>a</sup>

Samples	Codes
Samples taken during manufacture of skim mi	lk 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

<sup>a</sup> 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.<sup>a</sup>

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

<sup>a</sup> 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<sup>2+</sup>], ionic calcium;  $\alpha$ -lac,  $\alpha$ -lactalbumin;  $\beta$ -Lg A,  $\beta$ -lactoglobulin;  $\beta$ -Lg B,  $\beta$ -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.



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. 3



Fig. 4

AND AND







Fig. 6





