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Fractionation of Proteins in Reconstituted Skimmed Milk

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ABSTRACT

The effect of adding calcium chloride to milk before spray-drying on the properties of milk powder has been investigated. Proteins in reconstituted milk were fractionated by Sephadex G 100 gel filtration and by gel electrophoresis and the profiles compared with those in low, medium and high heat-treated milk.

The elution profile of the proteins in all samples was pooled into four peaks in reconstituted condensed milk and three peaks in reconstituted dried milk; however, whey proteins isolated from both reconstituted milks revealed four fractions.

The nitrogen distribution in the resultant fractions differed according to the amount of preheating given to the raw milk and the addition of calcium chloride, which also had an effect on the distribution of electrophoresis zones.

The addition of calcium chloride before pasteurization improved the solubility and wettability of spray-dried milk powder as well as reducing the level of whey protein denaturation during heat treatment.

INTRODUCTION

The suitability of dried milk for commercial purposes is limited when it has received severe heat treatment during manufacture. The American Dry Milk Institute (ADMI) (1971), classifies non-fat dry milk, according to its heat treatment during drying, into three categories, which differ in undenatured whey protein index (WPNI); these are: low heat-treated milk (not less than 6 mg/g (WPNI)), medium heat-treated milk (from 1·51 to 5·99 mg/g) and high heat-treated milk (not more than 1·5 mg/g).

Voss (1972) reported that these WPNIs resulted from heating at 70 °C for 15 s, 85 °C to 124 °C for 30 s and 135 °C for 30 s, respectively, while Bruel *et al.* (1972) found that 72 °C for 15 s, 95 °C for 30 s and 75 °C or 80 °C for 30 min gave the above levels of WPNI for the three categories.

The effect of heat treatment and calcium ion concentration on the denaturation of protein in fresh skim milk was studied by Śmietana *et al.* (1977), Śmietana (1979) and Omar (1983).

The present study was carried out to observe the denaturation of proteins in condensed and dried skimmed milk made from milk enriched with calcium chloride before pasteurization, using the American Dry Milk Institute classification.

MATERIALS AND METHODS

Fresh cow's milk was obtained from the farm of the University of Agriculture and Technology in Olsztyn, Poland. One batch of skimmed milk was used to prepare four samples of spray-dried skimmed milk processed under different heat treatments; these were 70–72 °C for 15 s (low heat-treated milk), 92–94 °C for 15 s (medium heat-treated milk) and 75 °C for 30 min (high heat-treated milk). In the control treatment 3·6 mM calcium chloride was added to the milk, followed by heating to 92 °C–94 °C for 15 s. The heated milks were concentrated to 47 % total solids in a double effect vacuum pan evaporator at 74 °C and 42 °C. The concentrate was spray-dried in an Anhydro spray-drier at inlet and outlet air temperatures of 170 °C and 84 °C, respectively.

Samples of condensed and dried milks were reconstituted to 10 % total solids. Whey proteins were separated from the reconstituted milk by adjusting the pH to 4·6 using 1M HCl. The whey was centrifuged at 13 000 *g* for 15 min to remove residual casein. Nitrogen contents of milk and whey were determined by Kjeldahl digestion and the distribution of nitrogen compounds was calculated.

The proteins in milk and whey were fractionated by Sephadex G 100 gel filtration and by polyacrylamide gel electrophoresis.

Gel filtration

Sephadex G 100 (Pharmacia Ltd, Sweden) was used to fractionate the proteins in reconstituted milk and in the corresponding whey samples. The column was equilibrated with the 0·033M sodium acetate buffer (pH 7·0) at a flow rate of 40 ml/h. Ten millilitres of sample were loaded onto the column (70 cm × 2·4 cm). Ten millilitre fractions were collected and the optical density at 280 nm measured in a Beckman spectrophotometer. The fractions were pooled according to the peak pattern. The aliquots were analysed for nitrogen content and expressed as a percentage of the total nitrogen of the original sample. The molecular weight of the fraction of each peak was estimated as the point equivalent to the maximum height of the individual peaks, as given by Determan (1968).

Electrophoresis

Gel electrophoresis was performed according to the method of Melachouris (1969) using 7·5% (w/v) acrylamide gels prepared in tris-citrate and borate buffer (pH 9·2) containing 4·5M urea at a constant voltage of 190 V for 12 h. Gels were stained with 0·7% (w/v) solution of Amido Black and destained with 1% (v/v) acetic acid.

Solubility index of dried milk

The solubility of the milk powders was measured by the ADMI (1971) method and according to the method described by Pijanowski (1971).

Wettability of dried milk

The wettability of the dried milk was measured by the method of Pijanowski (1971).

Undenatured whey protein (WPNI)

The level of WPNI, in milligrams per gram of powder, was determined according to the ADMI (1971) method.

Organoleptic scores

Quality control and grading of the milk powder was as described by Eisses (1973). The milk powder was scored on a 4-point scale for colour, particle size, presence of small clumps and/or hard particles according to this scale, 4 = good; 3 = satisfactory; 2 = fair and 1 = poor. Odour and flavour were assessed by dissolving one part of skimmed milk powder in ten parts of tap water at 50 °C to 60 °C and graded according to an 8-point scale where 8 = excellent; 7 = very good; 6 = good; 5 = satisfactory; 4 = fair; 3 = poor and 2 or 1 = very poor.

RESULTS AND DISCUSSION

Table 1 illustrates the elution profile (280 nm) of the proteins in condensed milk on a Sephadex G 100 superfine column with sodium acetate buffer. All samples gave similar elution profiles. Fraction I, containing the largest

TABLE 1
Fractions (From Sephadex G-100 Filtration) of Soluble Protein from Milk, and Their Nitrogen Percentages[a]

	Total elution pattern area	*Molecular weight* ($\times 10^3$)	*Heat-treatment*			
			Control	*Low*	*Medium*	*High*
Condensed milk	Fraction I	> 100	0·481 87·5	0·425 79·8	0·408 81·7	0·434 83·2
	Fraction II	30–40	0·016 2·93	0·038 7·27	0·021 4·29	0·019 3·65
	Fraction III	20–30	0·016 2·93	0·034 6·45	0·026 5·28	0·015 2·83
	Fraction IV	< 20	0·037 6·69	0·034 6·51	0·044 8·78	0·054 10·3
Dried milk	Fraction I	> 100	0·447 89·5	0·364 82·7	0·425 84·9	0·445 85·6
	Fractions II and III	20–40	0·010 2·00	0·033 7·54	0·022 4·36	0·013 2·48
	Fraction IV	< 20	0·043 8·55	0·043 9·74	0·054 10·7	0·062 11·9

[a] Percentages are expressed as per cent of total area under fractionation curve, i.e. absorbance at 280 nm against volume eluted.

particles, with molecular weights in excess of 100 000, was designated the casein micelle fraction. Fraction II was β-lactoglobulin, Fraction III was α-lactalbumin and Fraction IV contained non-protein nitrogen (Morr *et al.*, 1964).

Heating at 94 °C for 15 s caused a slight increase in the nitrogen content of the first fraction (void volume) and decreased that of the whey protein Fractions II and III.

Addition of calcium chloride to milk, before heating, resulted in an increase in nitrogen in Fraction I (87·5 % measured as grams of total area under absorbance peak at 280 nm) and a decrease of nitrogen in Fractions II and III (2·93 and 2·93 %). On the other hand, the content of nitrogen in Fraction IV increased when preheating time was prolonged. Forty per cent of whey protein nitrogen (Fractions II and III) was aggregated casein in the first fraction, with slight changes in Fraction IV as a result of calcium chloride addition to milk prior to the preheating process.

Increasing the severity of heat-treatment reduced the amount of β-lactoglobulin which resulted in an increase in the casein peak, presumably because of κ-casein-β-lactoglobulin interactions, the rate and the extent of this interaction being influenced by the concentration of calcium ions (Tessier *et al.*, 1968; Sawyer, 1969; Blanc *et al.*, 1977).

The increased formation of the whey–casein complex in this study may be explained by considerations of calcium binding. Addition of calcium ions formed a calcium-linked complex between whey protein and casein (Morr & Josephson, 1968), whilst denaturation was reduced because of the formation of the complex which is more resistant to heat-treatment (Poznanski *et al.*, 1972; Śmietana *et al.*, 1977).

The elution profile of the dried milk protein consisted of three peaks. The main fraction (Fraction I) was casein with a nitrogen content similar to that of condensed milk protein. Fractions II and III were associated together in the void volume (20 000 to 40 000 molecular weight) while more non-protein nitrogen appeared in Fraction IV. This may be attributed to the dissociation of β-lactoglobulin into the monomer form (Georges & Guinand, 1960; Webb *et al.*, 1978) and to the denaturation process during drying of the condensed milk which is dependent on the prior heating condition of the milk and condensed milk (O'Sullivan, 1971).

The elution patterns of the isolated proteins from reconstituted condensed and dried milks at pH 4·6 on a Sephadex G 100 column (Table

TABLE 2
Fractions (From Sephadex G-100 Filtration) of Soluble Protein from Isolated Whey at pH 4·6, and Their Nitrogen Percentages[a]

	Total elution pattern area	*Molecular weight* ($\times 10^3$)	*Heat-treatment*			
			Control	*Low*	*Medium*	*High*
Condensed milk whey	Fraction I	> 100	0·012 8·85	0·007 8·54	0·014 9·48	0·014 8·79
	Fraction II	30–40	0·075 58·0	0·064 53·3	0·082 54·8	0·091 56·8
	Fraction III	10–30	0·034 26·1	0·033 29·1	0·041 27·4	0·039 24·4
	Fraction IV	< 10	0·009 7·05	0·008 8·77	0·012 8·31	0·016 10·2
Dried milk whey	Fraction I	> 100	0·013 10·9	0·011 10·2	0·014 13·0	0·015 11·7
	Fraction II	30–40	0·067 55·9	0·058 52·5	0·055 51·3	0·060 50·0
	Fraction III	10–30	0·030 25·3	0·032 28·9	0·032 26·8	0·031 25·0
	Fraction IV	< 10	0·009 7·90	0·009 8·41	0·008 7·35	0·016 13·3

[a] Percentages are expressed as per cent of total area under fractionation curve, i.e. absorbance at 280 nm against volume eluted.

2) were pooled into four peaks: I, immunoglobulin fraction; II, β-lactoglobulin; III, α-lactalbumin and IV, non-protein components with traces of α-lactalbumin and proteose-peptones (Dimick, 1976). More than 50% of the nitrogen content was eluted in Fraction II, for both condensed and dried milks. High heat treatment released more non-protein and proteose-peptone nitrogen Fraction IV. Similar results were reported by Śmietana (1979).

Electrophoresis of protein from condensed milk (Fig. 1), showed four bands due to α-casein, β-lactoglobulin, β-casein and κ-casein (Morr, 1973).

The mobility of protein fractions depended on calcium chloride content. There was more of the complex formed between whey protein and casein in samples that had received more heat treatment, and zones of milk treated with calcium chloride were more clear. This may be due to

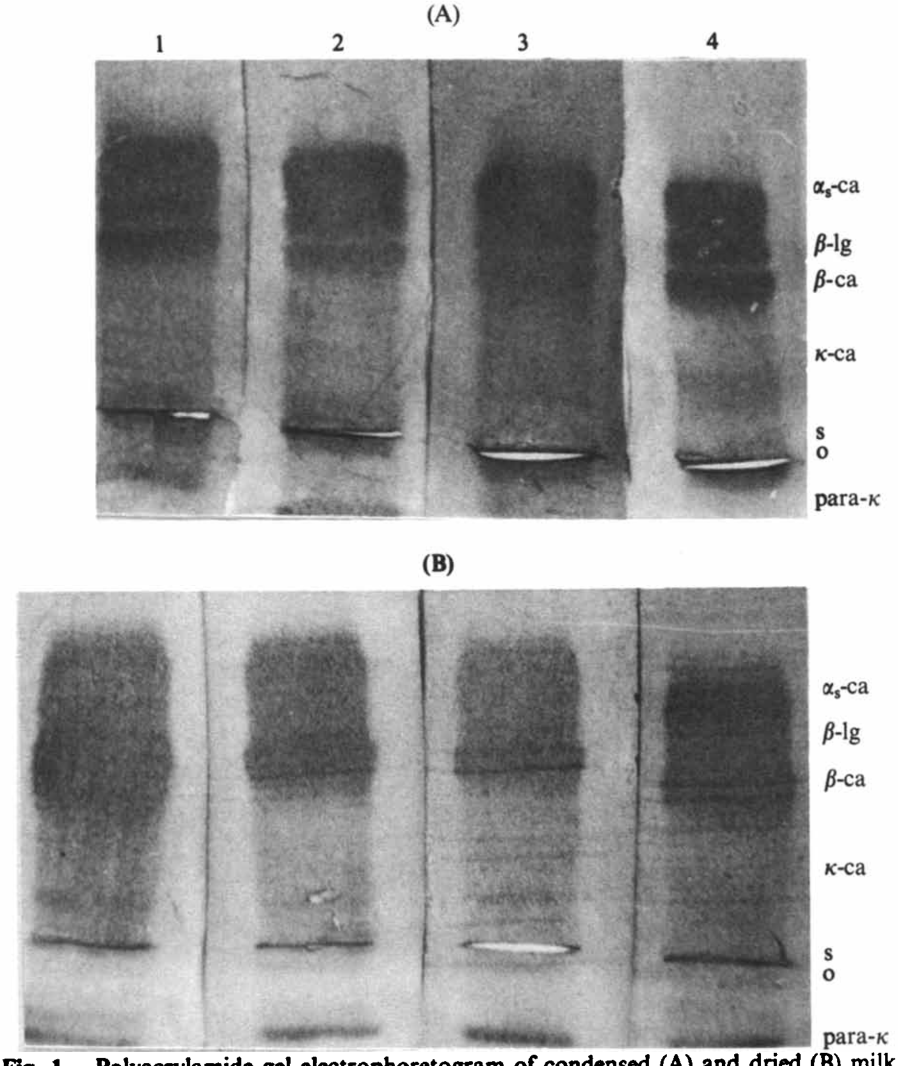

Fig. 1. **Polyacrylamide gel electrophoretogram of condensed (A) and dried (B) milk** prepared from skimmed milk. 1, Low heat-treated milk; 2, Medium heat-treated milk; 3, High heat-treated milk; 4, Control. ca; Casein. lg; Lactoglobulin.

the effect of calcium ions on the size of complexes formed between whey protein and casein (Tessier *et al.*, 1968), which can be increased by calcium salts (El-Negoumy, 1974).

Solubility, wettability and undenatured whey protein indices were higher for the control dried milk than for other treatments (Table 3).

TABLE 3
Solubility, Wetability and Undenatured Whey Protein Index (WPNI) of Dried Skimmed Milk. (Average of Three Replicates)

		Heat-treatment			
		Control	Low	Medium	High
Solubility (%)	Mean	98·2	98·0	94·1	90·3
	Standard deviation	0·17	0·14	1·05	2·23
Solubility (ml)	Mean	0·536	0·473	0·803	1·391
	Standard deviation	0·012	0·018	1·002	1·103
Wettability (g/ml)	Mean	18·8	16·0	13·5	9·67
	Standard deviation	1·16	0·98	0·85	1·18
WPNI (mg/g)	Mean	9·73	7·64	4·82	1·58
	Standard deviation	0·82	0·76	0·69	1·12

TABLE 4
Retention of Protein from Reconstituted Skimmed Milk

		Index	Heat-treatment			
			Control	Low	Medium	High
Milk	Total nitrogen (%)	Mean	0·550	0·531	0·498	0·522
		Standard deviation	0·042	0·008	0·016	0·005
	Non-protein N (%)	Mean	0·022	0·025	0·036	0·052
		Standard deviation	0·007	0·002	0·015	0·002
	Protein N (%)	Mean	0·528	0·506	0·459	0·470
		Standard deviation	0·037	0·010	0·005	0·006
Whey	Total nitrogen (%)	Mean	0·104	0·116	0·130	0·160
		Standard deviation	0·015	0·016	0·005	0·002
	Non-protein N (%)	Mean	0·014	0·028	0·032	0·047
		Standard deviation	0·006	0·008	0·002	0·005
	Protein N (%)	Mean	0·090	0·088	0·098	0·113
		Standard deviation	0·005	0·006	0·008	0·009
Total nitrogen retention (%)		Mean	81·1	78·2	74·6	69·4
		Standard deviation	0·909	0·407	0·788	0·673
Protein N retention (%)		Mean	83·0	82·6	78·2	74·0
		Standard deviation	0·064	0·414	0·614	0·614

TABLE 5
Organoleptic Properties of Dried and Reconstituted Skimmed Milk

Milk	*No.*	*Heat-treatment*			
		Control scores	*Low scores*	*Medium scores*	*High scores*
Dried	1	4, Good	3, Satisfactory	4, Good	2, Fair
	2	3, Satisfactory	4, Good	4, Good	3, Satisfactory
	3	4, Good	4, Good	3, Satisfactory	2, Fair
(max. 4)	Mean	3·67	3·67	3·67	2·33
Reconsti-tuted	1	8, Excellent	6, Good	7, Very good	4, Fair
	2	7, Very good	7, Very good	6, Good	5, Satisfactory
	3	8, Excellent	8, Excellent	6, Good	4, Fair
(Max. 8)	Mean	7·37	7·00	6·33	4·33

These indices have an effect on the reconstitutability of the dried milk and were improved by adding calcium chloride to the milk before preheating. Protein retention (Table 4) and organoleptic scores (Table 5) increased according to this treatment. Giles & Lawrence (1982) reported that, alternatively, the calcium chloride can be added to the skimmed milk concentrate prior to drying.

These experiments indicate that addition of calcium chloride to milk before it is heated at 92 °C to 94 °C for 15 s not only produced a dried milk conforming to the low heat category of the American Dry Milk Institute classification, but also improved its reconstitutability.

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