Production of calcium-reduced milks using an ion-exchange resin

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Ion exchange is used commercially in such processes as water softening, chemical purification, separation of ionic from non-ionic forms and analytical applications. Van Kreveld & van Minnen (1955) reported a method for the determination of Ca^{2+} and Mg^{2+} activity in milk using an ion-exchange resin. From this early work, it was established that with suitable resins exchange of salts was possible in milk, permeate and whey. This included the exchange of Ca and Mg from micelles by using Na^+ or K^+. Cationic resins of the Zerolit 236 type have been used successfully in the production of Ca-reduced single cream (Anderson *et al.* 1976). Such treatment prior to UHT processing was shown to increase physical stability when it was used as a coffee whitener. There is evidence that reduction of Ca^{2+} would also reduce the deposit formation that may occur during storage of UHT milk. Thus there is a need to establish commercial techniques to produce Ca-adjusted milks that could then be used as raw materials for industrial applications or product development work.

The present study investigated the use of ion exchange to reduce Ca in milk with particular reference to understanding the exchange of salts in Ca-reduced milk and the changes to some of its physical properties.

MATERIALS AND METHODS

Ion-exchange system

The resin chosen was Duolite C433 (The Permutit Co. Ltd, Isleworth TW7 4EL, UK), a weakly acidic cation-exchange polymer with a matrix of crosslinked polyacrylic acid. The functional group is COO^-, the total ion-exchange capacity 4·2 equiv./l and the particle size 0·3–1·1 mm diam. This resin can withstand a maximum operating temperature of 120 °C and is compatible with food applications. It can be regenerated with HCl, H_2SO_4 or HNO_3 into the H^+ form and then transformed into the Na^+ or K^+ form with NaOH or KOH solutions respectively.

A batch system using a stainless steel tank as the reaction vessel containing the resin in a stainless mesh basket was used for ion exchange. A centrifugal pump (Puma, APV Baker Ltd, Crawley RH10 2QB, UK) was connected to the bottom outlet of the tank for recirculating the regenerants.

Steam was injected into the resin in water at 50 kPa for 1 h to remove any volatile flavour components left after the manufacturing process. It was then acid regenerated with five bed volumes (375 l) of HNO_3 (100 ml/l), with agitation at 20

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min intervals. After 90 min the resin was rinsed with deionized water to neutral pH. One batch of resin was treated with NaOH (100 g/l; Tank 1) and a second with KOH (100 g/l; Tank 2). The end of conversion to the Na^+ or K^+ form was indicated by the pH of liquid draining from each tank changing from neutral to alkaline. The resins were then rinsed with deionized water to pH 6·7–7·1. Each resin was allowed to drain for at least 6 h before use, to reduce the water retained.

Ion exchange

Raw milk was obtained from Cliffords Dairy (Bracknell RG12 1QA, UK), separated at 45 °C to obtain skim milk and held at 4 °C overnight. A control portion (500 ml) of the skim milk was kept for compositional analysis and the remainder subjected to ion exchange at 10–15 °C. The K^+ form of the resin ($\sim$ 500 ml) was added to 5 l skim milk in a stainless steel vessel and the mixture stirred continuously at 22 rev./min by a variable speed mixer (Stir-Pak; Cole-Palmer Instrument Co., London W7 2QA, UK).

The progress of the ion-exchange process was monitored by withdrawing 10 ml samples and analysing for total Ca, using EDTA titration with Solochrome black dye as indicator as described by Schwarzenbach *et al.* (1946). When the Ca had been reduced by 20 % of its original value, a sample (200 ml) was transferred to a sterile container and stored at 4 °C. The ion-exchange process was continued and further samples were taken when approximate reductions of 35 and 60 % had been achieved. Samples were kept at 4 °C until analysed for protein, lactose and salts.

A second series of experiments was carried out to reduce the Ca by $>$ 60 % using either the K^+ form alone or a mixture of the K^+ and Na^+ forms.

Compositional changes were evaluated $\sim$ 2 h after the ion-exchange treatment to allow the ions to equilibrate before measurements were taken.

Analyses

The colour of the skim milk and the Ca-reduced skim milk was determined in CIELAB coordinates (McLaren & Rigg, 1976) using a Minolta CR310 Chromameter with a 50 mm tube (Minolta UK, Rooksley MK13 8HF, UK). Each milk sample (150 ml in a glass beaker) was placed on the analyser tube, when the instrument measured the reflection spectrum and produced a print of the CIELAB values. Milk pH was measured using a laboratory pH meter (model SC/CG840B; Camlab, Cambridge CB4 1TH, UK).

Ca-reduced milk samples were tasted and compared with milk prior to ion-exchange treatment. Taste tests were conducted by three experienced panellists, and each panellist was allowed to express the taste attributes found.

Lactose was analysed according to British Standards Institution (1989) by measuring the optical rotation of the clarified sample solution. Protein was analysed using the British Standards Institution (1990) micro-Kjeldahl method. Citrate was measured using the pyridine–acetic anhydride method of White & Davies (1963) using a Novospec 4049 photometer (Pharmacia LKB Biotech, St Albans LU7 8TN, UK). Phosphate was analysed by the colorimetric method of Pearson (Egan *et al.* 1988) with absorbance readings taken at 405 nm (model 4101 photometer; Clinicon International GmbH, Boehringer Mannheim, Lewes BN7 1LG, UK). Na, K, Ca and Mg were measured by the atomic absorption method of Murthy & Rhea (1967) with a known quantity of milk (10 g) or permeate obtained after ultrafiltration (Mini Ultraset; Flowgen Instruments Ltd, Sittingbourne ME9 8AQ, UK). Cl^- was measured with a chloride analyser (Corning model 926; Chiron Diagnostics Ltd,

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Halstead CO9 2DX, UK). The analytical results were statistically analysed using ANOVA.

RESULTS

Evaluation of the ion-exchange system

Skim milk was used in these experiments as preliminary results indicated that the fat in full cream milk interfered with the exchange process. Ion exchange of skim milk was successfully carried out using the batch method. After regeneration and the final rinse, it was found that the resin needed to be left to drain for at least 6 h before commencing ion exchange, so that dilution of skim milk was minimized. The difference in total solids determined before and after ion exchange indicated that the dilution corresponded to $< 1\,\%$ added water.

The progress of the removal of Ca^{2+} and other divalent ions was assessed by analysing for Ca using the EDTA method. The approximate times required for Ca reductions are given in Table 1. Residual Ca left in the skim milk after complete ion exchange was < 4 mM, from a starting value of ~ 30 mM.

Effect of ion exchange on colour and composition of milk

During the ion-exchange process, there was a steady loss in luminosity as Ca was removed (Table 1). Once the luminosity (L*) dropped below ~ 50 the perceived colour changed from white to pale green. The milk also became more acid. The concentrations of protein and lactose were not significantly affected by the ion-exchange process (Table 2). Ca reduction was accompanied by significant reductions in P and Mg ($P < 0{\cdot}001$), but citrate and Cl^- were not significantly affected. Since removal of Ca^{2+} and Mg^{2+} was achieved by exchanging them with K^+, the concentration of this ion increased as Ca^{2+} fell. The ratio of colloidal Ca to total Ca decreased as Ca was removed, following the relationship

$$[\text{colloidal Ca}]/[\text{total Ca}] = 5{\cdot}734/[\text{diffusible Ca}], \qquad (r = 0{\cdot}99)$$

where total Ca − colloidal Ca = diffusible Ca.

Effect of form of resin used in ion exchange

Further experiments to remove almost all divalent cations indicated that when the K^+ form resin was used, an off flavour described as 'bicarbonate type' was detected in the Ca-reduced milk. When a 1:1 mixture of Na^+ and K^+ resins was used, Na^+ was exchanged for divalent cations in preference to K^+ (Table 3). An off flavour described as 'soapy/salty' appeared in this product owing to excessive exchange with Na^+. The best combination for overcoming these off flavour problems was found to be a 1:2 molar mixture of Na^+ and K^+ resins. When this mixture was used, the molar ratio of Na:K in the final product was 1:2·8.

Effect of ion exchange on ionic balance in milk

The concentrations of the ionic constituents analysed are given in Table 4. Removal of Ca^{2+} and Mg^{2+} was not balanced by an increase in K^+ ions to maintain a constant level of positively charged ions in the skim milk. Indeed, the total positive charge decreased as Ca was reduced, suggesting that some K^+ was retained by the resin (see Tables 2 and 3). The difference in total positive charge in the control sample and 60 % Ca-reduced skim milk was $\sim 14\,\%$ (Table 4).

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Table 1. *Times taken to reduce calcium in skim milk using a batch ion-exchange process with a resin : milk ratio of 1 : 10, with changes in pH and luminosity*

(Values are means for $n = 3$)

Calcium reduction, %	Time, min	pH	L* value†
0	—	6·72	84·1 ± 0·5
10	4	6·70	81·1 ± 0·1
20	8	6·65	78·1 ± 0·1
35	20	6·61	66·2 ± 2·9
50	35	6·45	55·3 ± 2·9
60	45	6·43	47·1 ± 2·1
80	60	6·38	43·5 ± 1·2

† A measure of luminosity (0 = black, 100 = white); means ± SD.

Table 2. *Composition of raw skim milk before and after calcium reduction using a batch ion-exchange process with a K^+ resin*

(Values are means ± SD for $n = 3$)

		Approximate Ca reduction, %			
Constituent	Control	20	35	60	Significance of treatment
Protein, g/kg	34·3 ± 0·8	34·3 ± 0·7	34·1 ± 0·7	33·8 ± 0·5	NS
Lactose, g/kg	48·8 ± 0·3	48·0 ± 0·1	48·5 ± 0·1	49·1 ± 0·2	NS
Calcium, mM	30·6 ± 0·1	23·9 ± 1·0	20·3 ± 0·75	12·6 ± 0·8	***
Magnesium, mM	4·4 ± 0·2	3·5 ± 0·2	3·3 ± 0·2	2·0 ± 0·3	***
Sodium, mM	18·3 ± 0·9	20·0 ± 0·6	18·7 ± 0·5	22·0 ± 0·2	***
Potassium, mM	43·6 ± 0·7	46·7 ± 0·5	59·4 ± 1·9	62·3 ± 0·8	***
Citrate, mM	9·7 ± 0·50	9·7 ± 0·4	9·5 ± 0·4	9·6 ± 0·5	NS
Phosphorus, mM	25·0 ± 0·80	21·3 ± 0·9	16·8 ± 0·5	12·9 ± 0·4	***
Chloride, mM	29·6 ± 0·80	28·2 ± 0·7	29·1 ± 0·7	29·1 ± 1·3	NS

***, $P < 0·001$; NS, not significant.

Table 3. *Changes in ion concentrations when calcium was reduced in skim milks by a batch ion-exchange process using either K^+ resin or a mixture of K^+ and Na^+ resins*

(Values are means ± SD for $n = 3$)

		Approximate Ca reduction, %†			
Constituent	Control	50	70	80	Significance of treatment
Calcium, mM	30·9 ± 0·3	15·4 ± 0·7	12·5 ± 0·7	5·4 ± 0·6	***
Magnesium, mM	4·3 ± 0·2	3·5 ± 0·3	2·9 ± 0·1	2·1 ± 1·0	***
Sodium, mM	19·6 ± 0·4	19·0 ± 0·6	19·0 ± 0·3	50·9 ± 1·8	***
Potassium, mM	43·2 ± 1·0	50·8 ± 2·0	68·0 ± 1·0	45·0 ± 1·0	***
Phosphorus, mM	24·8 ± 0·2	15·1 ± 0·2	11·3 ± 0·7	5·0 ± 0·2	***

† Values for 50 and 70 % calcium reduction were for K^+ regenerated resin alone; for 80 % calcium reduction a 50 : 50 (v/v) mixture of K^+ and Na^+ resins was used.
*** $P < 0·001$.

There was also a decrease in the total negative charge of ~ 20 %, although anions were not involved in the exchange process. For P, valency was estimated using the Henderson–Hasselbach equation (Mc Meekin & Groves, 1965).

The ion-exchange process did not affect the Cl^- and citrate concentrations (Table 2) but did reduce the phosphate (Tables 2 and 3). Thus, as Ca was reduced, both positively and negatively charged species were reduced in skim milk, but more negatively charged species were lost owing to loss of phosphate.

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Table 4. *Estimation of charged species† in skim milks after calcium reduction using a batch ion-exchange process compared with values for an unprocessed control*

			Approximate Ca reduction, %				
Major ions	Expt‡	Control	10	20	30	50	60
Total positive	1	132	—	122	125	—	114
	2	122	116	114	112	105	105
Total negative	1	90	—	84	79	—	73
	2	88	87	80	82	74	70

† Values are mM × valency.
‡ 1, Values from Table 2; 2, values from a second experiment.

DISCUSSION

The batch method adapted for Ca removal from skim milk satisfactorily reduced the concentrations of Ca^{2+} and Mg^{2+} to achieve the different reduction levels required. This would be difficult using ion-exchange columns as the system would remove all the ions until the resin capacity was exhausted, resulting in micelle disruption in skim milk. The batch method was successful in preparing a range of Ca-reduced skim milks.

In this experiment, Ca and Mg reduction was accompanied by a fall in P. Ca removal was not balanced by exchange with an equal quantity of the exchanging ion, K^+, so as Ca was reduced there was a reduction in the total charged species, both positive and negative. One possible explanation is that $CaHPO_4$ precipitated in the resin when the HPO_4^{2-} in the aqueous phase formed bonds with the Ca^{2+} already on the resin surface (Dr C. Holt, pers. comm.). To balance the electrical charge, the K^+ would also be removed from the skim milk and retained by the resin.

During the ion-exchange process, the skim milk gradually changed in colour from white to pale green. This was noticeable at $\sim 60\,\%$ Ca reduction, although changes in luminosity were detected at 50 % reduction. This loss of light-scattering properties was probably due to the disintegration of the casein micelle structure resulting from the removal of Ca from the colloidal calcium phosphate, which eventually affected the framework of the micelle. Lin *et al.* (1972) reported that when Ca^{2+} activity in casein micelle suspensions in simulated milk ultrafiltrate was reduced by complexing it with EDTA, the light-scattering intensity decreased when it reached a critical level. Their findings suggest that Ca^{2+} removal initially dissociates weakly bound proteins (β- and κ-casein) that form part of the Ca^{2+} bridges in the micellar framework.

Further evidence for disintegration of the micelle comes from the transfer of Ca from the micelle. This must have occurred since, in the absence of such transfer, the maximum removal of Ca would be $< 10\,\%$, since the concentration of Ca^{2+} in skim milk is ~ 2 mM. As Ca^{2+} is removed, Ca is transferred from both undissociated calcium phosphate (soluble Ca) and the casein micelle in order to restore the equilibrium. The ratio of colloidal Ca : total Ca is 0·67 (Holt, 1985) and a value $> 0{\cdot}67$ implies reduction in colloidal Ca resulting from the decrease in pH during Ca removal.

When the concentration of K^+ or Na^+ increased in milk, undesirable taste characteristics were found to be a problem. However, this could be minimized by manipulating the molar ratio $K^+ : Na^+$. Future work will focus on the stability of Ca-reduced milks.

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