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The effect of heat treatment on intrinsic and fortified selenium levels in cow's milk

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The effects of two heat processing methods (pasteurisation and spray drying) routinely used in the processing of cow's milk and the production of infant formula powder on the selenium (Se) content of liquid milk , milk fortified with sodium selenite and sodium selenate were studied. Pasteurisation reduced intrinsic Se and selenate levels by 7.9% and 6.2% at $p < 0.05$ level and selenite levels by 7.0% at $p > 0.05$ level. Se losses following spray drying were 44.8% ($p < 0.001$), 11.4% ($p < 0.01$) and 10.0% ($p < 0.01$) for intrinsic selenium, selenite and selenate fortified milk, respectively. Total Se losses from unprocessed milk following processing (pasteurisation and spray drying) were 49.2% ($p < 0.001$), 17.6% ($p < 0.001$) and 15.6% ($p < 0.001$) for intrinsic selenium, selenite and selenate fortified milk, respectively.

INTRODUCTION

The essential role of selenium (Se) for human health has been well established in recent years (NRC, 1989). Selenium forms an integral part of the enzyme glutathione peroxidase, which catalyses the reduction of peroxides, in conjunction with catalase, superoxide dismutase and vitamin E, to protect intracellular structures against oxidative damage (Rotruck *et al.*, 1973; Casey and Hambidge, 1985).

More recently Se was identified in type I iodothyronine 5-deiodinase, an enzyme involved in thyroid metabolism (Zachara, 1992). Se also has an active role in several metabolic pathways and has also been suggested as a modulator in inflammatory and immune responses (Neve, 1991). Low Se intakes have long been associated with Keshan disease (a juvenile cardiomyopathy) and Kaschin-Beck disease (osteoarthritis) (Levander, 1989). Previous data suggested that infants fed cow's milk based formula (1–4 μg Se intake per day) as opposed to breast milk (5–13 $\mu g\,day^{-1}$) as their sole source of nutrition may be at risk of selenium deficiency. Preterm infants may be particularly at risk of selenium inadequacy since these neonates have low hepatic stores and plasma Se concentrations, possibly making them vulnerable to haemolytic anaemia and cancer in later life (Foster and Sumar, 1996*a*).

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Manufacturers aim to produce cow's milk based formula products which closely resemble human milk as breast feeding is thought superior with respect to infant nutrition (Brady *et al.*, 1982). The intrinsic mineral content of cow's milk is variable; major influences being the trace element of feedstuffs, geographic location, breed of animal, season, elemental bioavailability, absorption and secretion from the mammary gland. (Zurera-Cosano *et al.*, 1994). In addition, processing conditions (pasteurisation, spray drying) and handling by manufacturers may induce further changes to the trace element content of milk. Consequently, manufacturers fortify infant formula with certain essential trace elements at levels higher than those occurring in human milk, to compensate for reduced bioavailability, losses incurred during processing and storage, thus ensuring optimum intake. Until recently, Se was not added as a specific component to commercial infant products. Within the last few years the USA, Canada and New Zealand have introduced the fortification of formula with inorganic forms of Se (sodium selenite and sodium selenate) (Smith *et al.*, 1995; Abbe *et al.*, 1996; Darlow *et al.*, 1995). In Europe and the UK, addition of selenium is not permitted though is under consideration (Goedhart and Bindels, 1994). A recent survey of UK infant formulae by the authors has shown the Se content is significantly lower than reported values for breast milk. Variability in levels between batches and different manufacturers was also observed (Foster and Sumar, 1996*a*,*b*; Foster *et al.*, 1996). In addition, one third of UK infant formulae brands analysed met the UK reference

nutrient intake (RNI) value of 10 $\mu g\,day^{-1}$, although 8 of the 9 brands satisfied the UK lower reference nutrient intake (LRNI) value of 4 $\mu g\,day^{-1}$, suggesting the daily Se intake for UK bottle-fed infants may be inadequate (Foster *et al.*, 1996). The low Se levels associated with cow's milk may be further compromised due to sequential heating and drying procedures pertinent to the production of infant formula. Very few studies have been conducted to evaluate the effect of heat treatments on the trace element content of cow's milk and infant formula. Coni *et al.* (1995) found a positive correlation between environmental situation, manufacturing process, equipment and levels of certain elements in raw milk and cheese, although Se was not studied.

The purpose of this study was to investigate the effects of pasteurisation and spray drying on the intrinsic Se content of skimmed cow's milk and milk fortified with inorganic Se salts (sodium selenium, sodium selenate) with a view to establish whether such heat treatment processes associated with the manufacture of infant formula influence final Se levels of these products.

MATERIALS AND METHODS

Samples

Nine litres of fresh skimmed pasteurised milk were purchased at retail outlets across South London during the summer of 1996. All samples (refrigerated at 4°C) were processed on the same day of purchase. The milk was divided into three groups; (i) control (intrinsic Se only), (ii) selenite (fortified with $0.14 \pm 0.02\,\mu g$ $Na_2SeO_3\,g^{-1}$ dry wt, approx. 98%, Sigma, Poole, Dorset, UK), (iii) selenate (fortified with $0.16 \pm 0.02\,\mu g$ $Na_2SeO_4\,g^{-1}$ dry wt, anhydrous, Sigma, Poole, Dorset, UK). Levels of Se fortification were 3 μg Se per 100 kcal, as suggested in the recently amended EEC Directive on infant formulae (EC Commission, 1995). Prior to processing, liquid milk samples were thoroughly mixed by inversion and then poured back and forth into beakers. Respresentative aliquots were removed and used as unprocessed samples for Se analysis. Following heat processing, representative pasteurised and non heat treated liquid samples were stored at −20°C until analysis; representative spray dried samples were stored in the absence of light at ambient temperature.

Milk pasteurisation process

Preweighed samples (1000 ml) of retail skimmed cow's milk were heated batchwise at 71.7°C for 15 s followed by cooling to not more than 10°C using a laboratory pasteuriser (model FT43 A, Armfield Ltd, UK), in accordance with UK Milk Special Designation HTST pasteurisation regulations 1977 SI No. 1033, which had been previously rinsed in double distilled, deionised water (15–18 MΩ specific resistivity, Elgastat, UK).

Milk spray drying process

Preweighed samples (1000 ml) of previously pasteurised skimmed cow's milk were spray dried batchwise under the following conditions: Inlet temperature 280°C, outlet temperature 110°C, pressure 2 $kg\,cm^{-3}$, total run time 19 min, flow rate 3 litre h^{-1}, using a prewashed Niro Atomiser (model 1529, Copenhagen, Denmark). Powdered milk samples were collected after 5, 15 and 19 min during each run to achieve moisture contents comparable to commercially produced dried milks (4.0% max, Egan *et al.*, 1987).

Analysis

Representative unprocessed, pasteurised and spray dried cow's milk and National Institute of Standard and Technology non fat milk powder standard reference material 1549 (Laboratory of the Government Chemist, UK) samples (1.0 ml liquid or 0.25 g powder) were subjected to overnight wet acid ashing with a mixture of nitric and perchloric acids. Total Se was determined by hydride generation atomic absorption spectrophotometry (HGAAS) using a GBC model 502 atomic absorption spectrophotometer equipped with a GBC model HG 900 manual vapour hydride generation system (GBC Scientific Equipment Pty, Melbourne, Australia). The calibration graph peak area response was directly proportional to the Se (IV) concentration (form of Se analysed by HGAAS) over the range 0–0.2 $\mu g\,ml^{-1}$ (correlation coefficient = 0.995). The coefficient of variation was 3.56% ($n = 6$). No apparent matrix interferences were observed. This procedure has been fully described elsewhere by the authors (Foster and Sumar, 1996*a*,*b*). Results were processed using multivariate statistical analysis (Statworks and Minitab Statistical Graphics Systems).

RESULTS AND DISCUSSION

Accuracy and detection limit

The accuracy of the method was assessed by analysing non fat milk powder standard reference material (National Institute of Standards and Technology SRM 1549, Laboratory of the Government Chemist, Teddington, UK). The results obtained were $0.11 \pm 0.004\,\mu g\,g^{-1}$ (coefficient of variation of 6.49%; $n = 9$) which is in excellent agreement with the certified value ($0.11 \pm 0.01\,\mu g\,g^{-1}$). The accuracy was also determined indirectly, on the basis of the recovery of added Se using standard reference material or Se (IV) standard. No evidence of Se loss in the digestion step was found; mean recovery was 99.6 ± 7.1% for 15 determi-

nations. The detection limit for HGAAS was 0.49 ng ml^{-1} (coefficient of variation of 6%; $n=7$). The detection limit was established by studying the standard deviation of the reagent blanks through the entire procedure. Background analytical levels were assessed by running blank acid digestions. The levels detected were not significant at $p<0.05$ level.

Se content of heat processed cow's milk

The mean levels of Se determined in unprocessed, pasteurised and spray dried cow's milk and the Se retention of different milks during processing are shown in Table 1 and Fig. 1, respectively. The results, blank corrected, are expressed in μg g^{-1} (dry wt). The concentration of Se in all three milk groups studied (control, selenite, selenate) was highest in the raw milk, decreasing in pasteurised milk with the lowest levels occurring in spray dried milk. For each milk type and processing stage, any variation in Se concentration between replicate runs (×3) was not significant at $p<0.05$ level.

Initially, processing experiments were conducted using fresh, raw milk from a local dairy which was defatted by centrifugation (1500*g*, 30 min, 4°C) in accordance with Van Dael *et al.* (1991) prior to pasteurisation. The Se level of raw, defatted, unfortified milk was 0.24 ± 0.015 μg g^{-1} (dry wt). The results given in this paragraph confirm that for subsequent experiments (Table 1) it was more appropriate to use commercially pasteurised skimmed milk (control group Se level $=0.24\pm0.051$ μg g^{-1}, dry wt) to represent unprocessed milk instead of defatted raw milk as the supplies of raw milk were limited.

The control group (milk containing no added Se) showed the greatest loss in its Se content following spray drying (19 min); pasteurisation of unprocessed milk resulted in a 7.9% decrease in Se content (decrease was significant at $p<0.05$ level) and pasteurised milk spray dried for 19 min showed a 44.8% decrease in Se content (decrease was significant at $p<0.001$ level). In total, the loss of Se from the control group on processing was 49.2% (decrease was significant at $p<0.001$ level). The selenite group (milk + 0.1314 μg Na_2SeO_3 g^{-1}) showed a similar decrease in Se content following processing; pasteurisation of unprocessed milk resulted in a 7.0 % decrease in Se although this difference was not significant at $p<0.05$ level (wide standard error of the mean). Pasteurised milk spray dried for 19 min showed a 11.4% decrease in Se content (decrease was significant at $p<0.05$ level). In total, the loss of Se from the selenite fortified group on processing was 17.6 % (decrease was significant at $p<0.001$). The selenate group (milk + 0.1523 μg Na_2SeO_4 g^{-1}) appeared slightly more stable than the selenite fortified milk (though not statistically significant at $p<0.05$ level), and showed a similar decrease in Se content following processing; pasteurisation of unprocessed milk resulted in a 6.2% decrease in Se (decrease was significant at $p<0.05$ level) and pasteurised milk spray dried for 19 min showed a 10.0% decrease in Se content (decrease was significant at $p<0.01$ level). In total the loss of Se from selenate fortified milk was 15.6% (decrease was significant at $p<0.001$ level). These trace element losses incurred on processing are in similar agreement with those of other authors. Coni *et al.* (1995) reported levels of several elements (Se not studied) were initially found to increase via the release of metals from equipment following contact with milk, but subsequently decreased during pasteurisation. Statistically significant differences were observed ($p<0.001$) following spray drying. Hojo (1986) reported that hot air convection oven heating reduced the Se content of Japanese cow's milk with increasing temperature and time of heating. The resulting loss of Se was 11.1% at 210°C following heating for 25 min. No other comparative studies detailing the effects of spray drying on the Se content of milk during infant formula manufacture are available to date.

In order to produce a spray dried milk product comparable to commercial products (moisture content <4%), the drying conditions used in the present study were higher than those typically used in commercial dried milk manufacture. This temperature difference between the hot air and powder particles may have been responsible for the significant loss in intrinsic Se, and to a lesser extent, Se added as inorganic salts (Table 1).

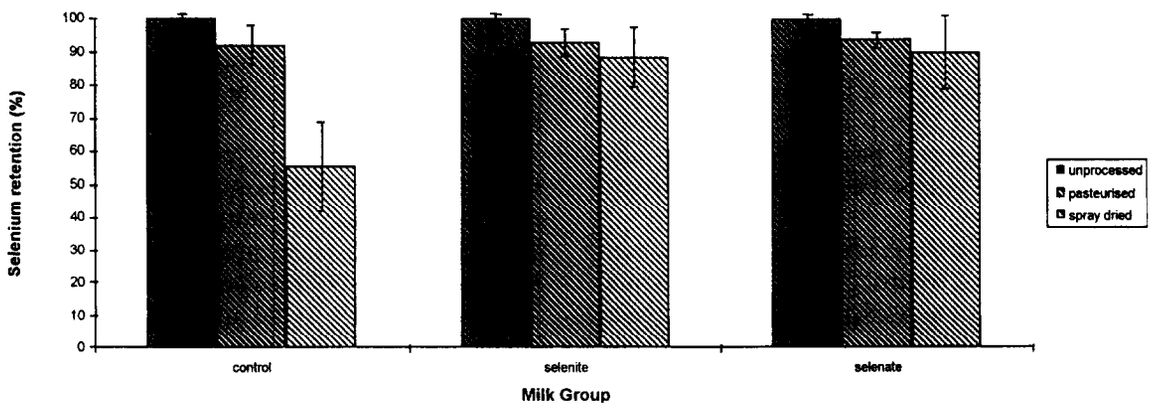

Fig. 1. Se retention (%) of different milks after processing. Error bar ± SEM.

Table 1. Se content ($\mu g\,g^{-1}$ dry wt) of heat processed skimmed cow's milk

Milk type		n^a	Water content (%)	Mean ± SEM[b]	F ratio[c]	p^d
Control (intrinsic Se)						
Unprocessed		18	90.0	0.24 ± 0.005		
Pasteurised		18	89.9	0.22 ± 0.008	4.43[e]	0.045[e]
Spray dried	(5 min)	18	1.34	0.14 ± 0.016		
	(15 min)	18	1.31	0.13 ± 0.008	150.9[f]	0.000[f]
	(19 min)	18	1.13	0.12 ± 0.019	294.5[g]	0.000[g]
Selenite (intrinsic Se + 0.1314 μg $Na_2SeO_3\,g^{-1}$)						
Unprocessed		18	91.1	0.41 ± 0.005		
Pasteurised		18	90.1	0.38 ± 0.011	2.06[e]	0.12[e]
Spray dried	(5 min)	18	1.84	0.30 ± 0.008		
	(15 min)	18	1.44	0.32 ± 0.008	10.18[f]	0.002[f]
	(19 min)	18	1.62	0.34 ± 0.001	22.6[g]	0.000[g]
Selenate (intrinsic Se + 0.1523 μg $Na_2SeO_4\,g^{-1}$)						
Unprocessed		18	90.9	0.42 ± 0.010		
Pasteurised		18	90.3	0.40 ± 0.009	52.28[e]	0.000[e]
Spray dried	(5 min)	18	1.97	0.34 ± 0.003		
	(15 min)	18	1.37	0.35 ± 0.004	8.61[f]	0.005[f]
	(19 min)	18	1.62	0.36 ± 0.002	76.23[g]	0.000[g]

[a] n = number of samples analysed.
[b] Mean with standard error of the mean (SEM).
[c] F ratio of variance.
[d] $p < 0.05$ is significant for differences between processing operations.
[e] Unprocessed vs pasteurised milk.
[f] Pasteurised vs spray dried milk.
[g] Total loss on heating.

Several reports in the literature suggested that glutathione peroxidase (whey protein) contributes significantly to the total intrinsic Se content of milk (17–30%) and that this enzyme is vulnerable to destruction by heat during processing (Hojo, 1982; Debski *et al.*, 1987; Van Dael *et al.*, 1991). Consequently, any losses in intrinsic Se during spray drying by volatilisation may have occurred following the degradation of this enzyme. Similarly the high heating conditions used in this study may have induced volatilisation of other forms of selenium (Davidek and Roton, 1995).

The control group (milk containing intrinsic Se only) spray dried pasteurised milk samples collected after 5, 15 and 19 min heating showed an apparent decline in Se content with time of heating , whereas the selenite and selenate fortified groups of spray dried pasteurised milk samples collected over similar conditions showed an apparent increase in Se content with time of heating (Table 1). However, for all three milk groups these differences in selenium content with time were not statistically significant at $p < 0.05$ level. Hojo (1986) reported hot air convection heating of pasteurised milk significantly reduced its Se content linearly with increasing time ($p < 0.01$).

Table 1 also shows there was no significant change in final moisture content of control (1.31–1.34%), selenite (1.44-1.84%) and selenate (1.37–1.97%) dried milk samples heated from 0–19 min. In all cases the moisture content was within the range specified for commercially produced skim milk powders (4.0% maximum, Egan *et al.*, 1987).

Although pasteurisation and spray drying considerably decreased the intrinsic Se content of powdered cow's milk ($0.122 \pm 0.02\,\mu g\,g^{-1}$) in this study (48.9% loss), this is insufficient to explain the ultimately low Se content of infant formulae available in the United Kingdom. Previous work by the present authors reported mean concentrations of Se in infant formulae on a dry wt basis, which ranged from 0.04 to $0.06\,\mu g\,g^{-1}$ (Foster *et al.*, 1996). Apart from possible losses during heat processing further losses in Se are likely via other unit operations involved in the manufacture of infant formula. For example, additional heat operations (evaporation, condensation) and physical separations (desalting, ion-exchange, dialysis) (Packard, 1982). Selenium is largly associated with the different whey and casein protein fractions of milk (Van Dael *et al.*, 1991). Therefore the application of electrodialysis, gel filtration, ion exchange and ultrafiltration in infant formula manufacture to separate various protein fractions and minerals may also separate the Se by the nature of its affinity for different milk proteins.

The presented effects of processing on the Se content of cow's milk are significant, particularly if one considers the vulnerability of bottle-fed preterm infants to Se deficiency and low Se intakes ($\mu g\,day^{-1}$) caused by consumption of unsupplemented UK infant formulae.

In conclusion, it would appear that the Se content of infant formula can be influenced by the processes used to manufacture it from cow's milk. In particular, pasteurisation and spray drying significantly lower the intrinsic Se content, and to a lesser extent, milk fortified

with inorganic Se salts. Further studies are needed to investigate whether the Se content of infant formula is affected by other aspects of its manufacture.

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