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# Selenium concentrations in soya based milks and infant formulae available in the United Kingdom

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A simple hydride generation atomic absorption spectrometric method previously described for bovine infant formulae has been successfuly applied for the analysis of selenium in UK soya based milks and infant formulae. Levels reported for the first time ranged from 0.011 to 0.040 μg/g (wet wt) for soya milks and from 0.023 to 0.089 μg/g (dry wt) for soya isolate infant formulae. No matrix interferences were encountered and the mean recovery was 100.1%. These values exhibit variation between batches, hence routine QC analysis and fortification by manufacturers is recommended. Copyright © 1996 Elsevier Science Ltd.

## INTRODUCTION

The importance of selenium (Se) in animal nutrition is well documented (Combs & Combs, 1986; Shamberger, 1987). Selenium deficiency has been associated with muscular dystrophy in ruminants, pancreatic degeneration and exudative diathesis in poultry and liver necrosis in rats (Mason & Weaver, 1986). However, it was not until the late 1950s that the element was thought to play a role in human metabolism (Schwartz & Foltz, 1957). Prior to that period, it had been recognized only for its toxic properties. The only currently known function of selenium in man is as an integral part of the enzyme glutathione peroxidase; one of the mechanisms whereby intracellular structures are protected against oxidative damage, in conjunction with catalase, superoxide dismutase and vitamin E (Casey & Hambidge, 1985).

Soya milk is increasingly being chosen by consumers as a nutritious alternative to cow's milk as it has a low fat content, no cholesterol and is generally lower in calories than ordinary cow's milk (Table 1). Recent trends in soya milk consumption, as reported by the Soya Milk Information Bureau (SMIB), have shown a rapidly expanding UK market, producing 10.5 million litres in 1991 (Anon., 1992). Infant formulae based on soya proteins represent almost 25% of the (American) infant formula market. These products tend to be used where there is suspicion or evidence of an allergy to cow's milk protein, for newborns and infants of families at risk. In addition, these formulae are sometimes chosen by consumers who, for various reasons, do not wish to give their children products based on animal proteins (Commission of the European Communities, 1993). Soya beans (*Glycine max*) are well suited for use in these restricted diets as they possess a high protein content (40%) that is generally less allergenic than most other vegetable proteins. Selenium is primarily associated with protein, and although the protein content of soya beans is high, whole soya beans exhibit significant variation in Se content, based on geographical differences in soil selenium content (Mason & Weaver, 1986).

Two diseases associated with severe nutritional selenium deficiency are now recognized in children: a juvenile cardiomyopathy known as Keshan disease and an osteoarthritis called Kaschin–Beck disease (Levander, 1989). Previous data suggest that term infants who are fed formula (2–4 μg Se intake/day) as opposed to breast milk (5–13 μg Se intake/day) (Lombeck *et al.*, 1978; Smith *et al.*, 1982) as their sole source of nutrition may be at risk of selenium deficiency. In particular, infants suffering from conditions such as galactosemia, primary lactase deficiency and secondary lactose intolerance complications (temporary loss of lactase activity), are vulnerable due to their total nutritional dependency on soya feeds and formulae for extended periods (Brady *et al.*, 1986).

Until recently, selenium was not added as a specific component to commercial infant formulae. Within the last 3 years the USA, and more recently, Europe, have introduced the fortification of formulae with inorganic forms of selenium. As yet, UK based manufacturers are not required to supplement or monitor the selenium content of formulae; levels present being those naturally occurring in milk or soya protein isolates. However, a

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**Table 1. Approximate composition of soya and cow's milk (per 100 ml)**

| Nutrient | Unsweetened soya[a] milk | Whole cow's milk[b] |
|---|---|---|
| Protein | 3.6 g | 3.3 g |
| Fat | 2.1 g | 4.0 g |
| (saturates) | 0.4 g | 2.6 g |
| Carbohydrates | 0.6 g | 4.7 g |
| Energy | 36 kcal | 68 kcal |
| Cholesterol | 0 g | 14 mg |
| Calcium | 15 mg | 120 mg |

[a]Product of Vandemoortele (UK) Ltd, Hounslow, Middlesex. Reproduced with kind permission.
[b]Holland *et al.* (1991)

recent study investigating the selenium content of infant formulae currently available in the UK has shown the selenium content is significantly lower (0.023–0.093 μg/g) (Foster & Sumar, 1996) than reported values for breast milk (Department of Health, 1991). In addition considerable variation in levels between batches was detected. Earlier studies conducted in the USA and Finland (Smith *et al.*, 1982; Kumpulainen *et al.*, 1987) indicated that infants fed formulae not fortified with selenium have intakes below the recommended dietary allowance of 10 μg Se/day for infants 0–3 months of age. Owing to lack of sufficient data, precise amounts recommended for selenium intakes for infants in Great Britiain (estimated average requirements) have not yet been defined.

No British data on soya milks or soya infant formulae has been reported with respect to selenium and very little information is available on the selenium content of similar soya products generally (Zabel *et al.*, 1978; Smith *et al.*, 1982; Lewis *et al.*, 1985). The purpose of this study is to assess the matrix versatility of a simple hydride generation atomic absorption spectrometric method (HGAAS), previously developed by the authors for cow's milk infant formulae analysis (Foster & Sumar, 1996), by analysing infant feeds of plant origin. This method will be applied to investigate the variation in selenium content of commercially produced soya milk and soya infant formulae products currently available for purchase in the United Kingdom.

## MATERIALS AND METHODS

### Samples

Nine leading brands of soya feeds (four infant formulae and five milks) were purchased at retail outlets across South London during the spring of 1995. Several quantities of each type, bearing different sell by dates and batch numbers were also acquired. All samples were stored in the absence of light at ambient temperature. On the day of analysis, the tin seal of each formula was broken and a representative sample obtained by mixing and quartering. Soya milks were thoroughly mixed by inversion, pouring back and forth into beakers. In total, 162 samples were analysed.

### Sample digestion

Representative soya milk samples (0.25 g powder; 1.0 ml liquid) were placed in Pyrex glass digestion tubes, specially designed, with ground glass B19 joints and stoppers, long neck with dimensions of 24×235 mm and a nominal capacity of 56 ml which had been previously soaked overnight in 10% v/v nitric acid (AnalaR, BDH-Merck Ltd, Poole, Dorset, UK), rinsed in double distilled, deionised water (15–18 MΩ specific resistivity, Elgastat, UK) and acetone (AnalaR, BDH-Merck Ltd) then dried with compresssed air. The water content of liquid samples was reduced by gently heating the tubes in a waterbath at 100°C.

Samples of 0.25 g (dry wt) non-fat milk powder, National Institute of Standards and Technology standard reference material 1549 (Laboratory of the Government Chemist, Teddington, UK), were also treated similarly throughout the entire digestion process. Aliquots of 10.0 ml nitric acid (S.G 1.43 g/ml, AnalaR, BDH-Merck Ltd) were added and the stoppered tubes left in a class A fume cupboard at ambient temperature for digestion overnight. The following day, the tubes were placed in an ultrasonic bath for 2 min then the following added in sucession; 2.0 ml perchloric acid (AnalaR, BDH-Merck), 3 drops 10% aqueous antifoam emulsion (Dow Corning 1510 silicone antifoam concentrate, BDH-Merck Ltd) and a few acid washed, rinsed antibumping granules (fused silica, BDH-Merck). The tube contents were heated to 150°C (without stoppers) in a Kjeldatherm digestion block (Gerhardt UK Ltd, Cheshire, UK) for approximately 30 min until the evolution of brown fumes of $NO_2$ had ceased, then gradually increased to 220°C for 45–60 min after the appearance of dense white fumes of perchloric acid.

Care was taken to ensure that the solution was not heated to complete dryness because of the explosive nature of metal perchorates (Analytical Methods Committee, 1979). In addition to the inclusion of an antifoaming agent, foaming was controlled by passing a jet of compressed air over the mouth of the tubes. A few drops of nitric acid were administered periodically to prevent charring. The digestion was completed when approximately 0.5 ml of colourless solution remained. On cooling, the tubes were made up to 5 ml volume with deionized water, 5.0 ml hydrochloric acid (S.G. 1.18 g/ml, AnalaR, BDH-Merck) added and heated at 100°C for 30 min to reduce Se(VI) to Se(IV).

### Determination

Selenium was determined by hydride generation atomic absorption spectrometry using a GBC model 502 atomic absorption spectrophotometer equipped with a GBC model HG 900 manual vapour hydride generation system (GBC Scientific Equipment Pty Ltd, Melbourne, Australia) as described previously (Foster & Sumar, 1996).

**Table 2. Selenium concentrations (μg/g dry wt) in UK available soya milk feeds**

| Soya infant feed | Origin of milk | $n$[a] | Mean ± SEM[b] |
|---|---|---|---|
| Soya formulae | | | |
| Cow & Gate Infasoy | Holland | 18 | 0.089 ± 0.0082 |
| Farleys Soya | Ireland | 18 | 0.046 ± 0.0041 |
| Milupa Prejomin | Germany | 18 | 0.033 ± 0.0041 |
| SMA Wysoy | Ireland | 18 | 0.023 ± 0.0041 |
| Soya milks | | | |
| Granose (unsweetened) | British Isles | 18 | 0.035 ± 0.0024[c] |
| Plamil (unsweetened) | British Isles | 18 | 0.011 ± 0.0012[c] |
| Provamel (unsweetened) | Belgium | 18 | 0.040 ± 0.0045[c] |
| Sunrise (unsweetened) | British Isles | 18 | 0.013 ± 0.041[c] |
| Unisoy Gold | British Isles | 18 | 0.020 ± 0.0008[c] |

[a]$n$ = number of samples analysed.
[b]Mean with standard error of the mean (SEM).
[c]Concentration expressed on a wet weight basis.

## RESULTS AND DISCUSSION

Losses of Se can occur during the destruction of a particular organic matrix by wet oxidation depending on the nature of the oxidant mixture used, i.e. binary acid mixtures (nitric–perchloric acid) or tertiary acid mixtures (nitric–perchloric–sulphuric acid) (Foster & Sumar, 1995). For maximum elemental recovery all native forms of selenium in the matrix must be converted to selenite–selenate; any selenate formed during oxidation should be reconverted to selenite and measures taken to prevent losses by volatilisation (Janghorbani *et al.*, 1982).

Experimentally, predigestion of the samples with nitric acid oxidized a large part of the available organic matter prior to heating, and consequently minimized any potential losses through risk of explosion (Bock, 1979). Losses through charring due to localised heating were prevented by the addition of anti bumping granules and periodic dropwise administration of small quantities of nitric acid. As previously described (Foster & Sumar, 1996), foaming was a prominent feature of the digestion process. This was probably caused by the high protein content associated with soya bean derived products (Byun *et al.*, 1995). However, this effect was more pronounced with the soya formulae (as shown by the higher standard error of the mean associated with samples of Dutch origin) due to differences in the matrix, i.e. the presence of emulsifiers (lecithin, mono- and diglycerides) which are added to formulae to hold the fat in homogenous dispersion. Sample losses from these foam-induced violent reactions were controlled by disruption of surfactant structure (sample ultrasonic treatment, compressed air), acid hydrolysis (sample predigestion) and the addition of an antifoaming agent. Losses can also occur during the reduction step with hydrochloric acid. The addition of pear-shaped stoppers prior to the reduction of Se(VI) to Se (IV) at 100°C was found to condense any gases present, minimizing losses through volatilization. The reduction temperature is important as any chlorides present will form nitrosyl chloride with the remaining nitric acid, and this compound is preferentially volatilized thus avoiding subsequent loss of Se as selenium chloride (Bellanger, 1995).

For the determination of selenium by hydride generation atomic absorption spectrometry the calibration graph peak area response was directly proportional to the selenium (IV) concentration over the range 0–0.01 μg/ml (correlation coefficient = 0.965). The coefficient of variation was 5.00% ($n$ = 6). No apparent matrix interferences were observed.

### 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 ± 0.006 μg/g (coefficient of variation of 5.86%; $n$ = 9) which is in excellent agreement with the certified value (0.11 ± 0.01 μg/g). The accuracy was also determined indirectly, on the basis of the recovery of added Se using standard reference material or selenium (IV) standard. No evidence of Se loss in the digestion step was found; mean recovery was 100.1% for 12 determinations. The detection limit for HGAAS was 0.37 ng/ml (coefficient of variation of 5.22%; $n$ = 7). The detection limit was established by studying the standard deviation of the reagent blanks through the entire procedure.

Background analytical levels of selenium were assessed by running blank acid digestions. The levels detected were insignificant.

### Selenium content of soya milks and soya formulae available in the United Kingdom

The levels of selenium found in nine leading brands of soya feeds purchased in 1995 are shown in Table 2. The results, blank corrected, are expressed in μg/g (dry weight) milk powder and μg/g (wet weight) for the liquid soya milks.

Concentrations of selenium in the soya milks ranged from 0.0045 to 0.044 μg/g (mean, 0.024 μg/g). Selenium values determined for the soya formulae ranged from 0.014 to 0.098 μg/g (mean, 0.048 μg/g). For all the brands of milks and formulae studied, a significant variation in selenium content between batches was observed (Figs 1 and 2).

Although the first British soya milk was introduced almost 30 years ago, there is very little data available relating to its micronutrient composition (Anon., 1992). However, the levels of selenium shown in Table 2 are in agreement with a series of studies conducted in the USA investigating the influence of geographic variation on the selenium content of soya beans. Reported Se levels were in the range 0.007–0.04 μg/g (mean, 0.021μg/g), based on 89.7% water content (Combs, 1988). As far as the authors are aware no comparative information for British soya milk is available.

Concentrations of selenium in soya formulae (Table 2), are in similar agreement with those of other authors. In the United States, Zabel *et al.* (1978) determined the selenium levels in a wide range of infant formulae by fluorimetric analysis. For soya formulae, levels of 0.048–0.097 μg/g (dry weight) were reported. Later studies, also conducted in the USA, reported levels (dry weight) in the range 0.048–0.063 μg/g (Smith *et al.*, 1982) and 0.029–0.1 μg/g (Lewis *et al.*, 1985), for commercially available soya formulae analysed by gas chromatography with electron capture detection. The wide variation in selenium content reported by these authors may be attributed to geographical differences in soil selenium content across the USA, aqueous solubility and subsequent availabilty of the element to the soya bean plant (Combs, 1988). Similarly, the average values obtained for Cow and Gate Infasoy (0.089 μg/g) in this study are generally higher than the other formulae analysed (0.023–0.046 μg/g). This is probably due to the fact that this product orginates from Holland, rather than Ireland, as is the case with most of the formulae analysed. Geographically speaking, it is interesting to note that the average Se levels obtained for the formula of German origin (0.033 μg/g), are more comparable with the products originating from Ireland not Holland. Over the last 15 years, German population studies (via analysis of breast milk samples) have identified a 50% reduction in selenium supply from the food chain as a result of soil leaching effects and alteration in the type and composition of forage crops (Brätter *et al.*, 1991).

As recently reported in a similar study conducted by the authors on cows' milk based formulae, some variation in selenium content between soya formulae batches was observed (Fig. 2). This finding is significant, particularly if one considers that these formulae may be used as a sole source of nourishment from birth, often for extended periods, and consequently provide the only source of selenium. A similar degree of variation was observed between different batches of soya milk (Fig. 1).

From this study it is evident that the selenium content of soya bean derived milk products is influenced by the processing operations used by different manufacturers. Because of the reported lack of uniformity between batches, routine monitoring of selenium levels by manufacturers is recommended. In particular, soya formulae could be fortified with sodium selenite to increase concentrations similar to those of mature human milk, as is current practice in USA and Europe (EC Commission, 1995). Further studies are needed to assess the interactive effects of Se on other components within the formula matrix.

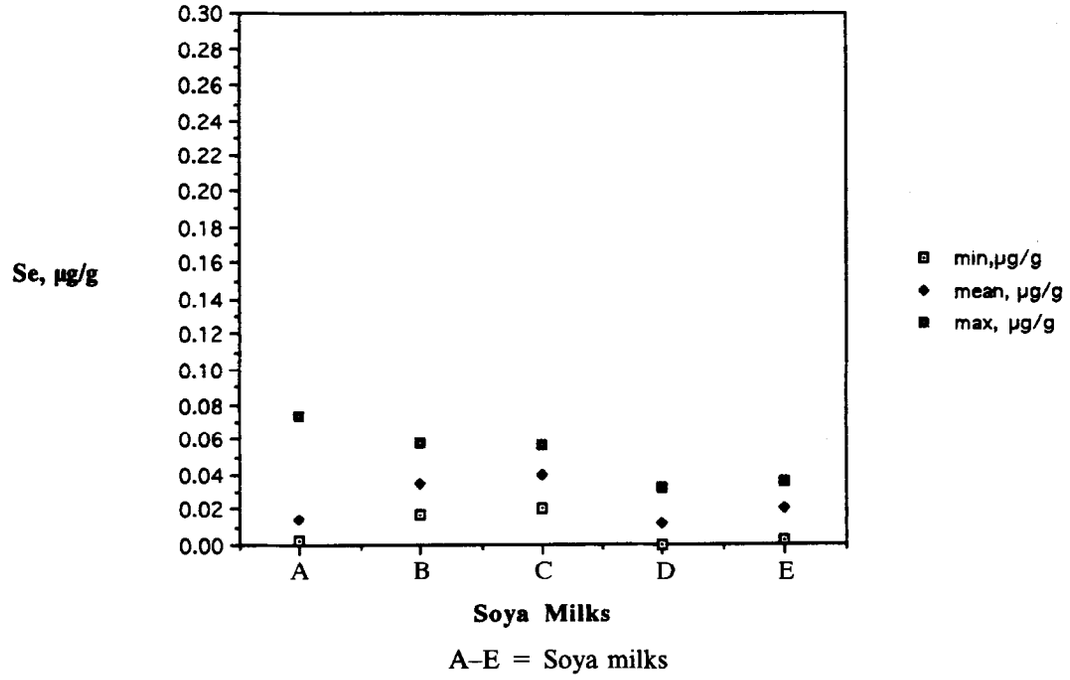


**Fig. 1.** Variation in Se concentration between batches of commercially available soya milks (UK).

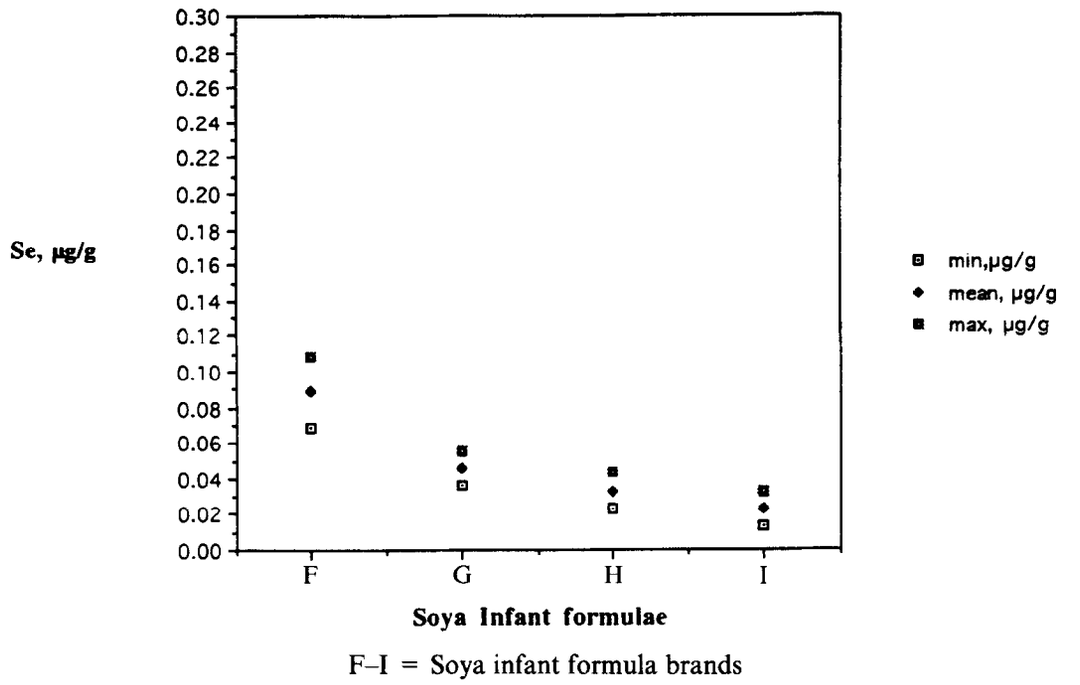


**Fig. 2.** Variation in Se concentration between batches of commercially available soya infant formulae (UK).

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