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Stability of vitamin D_3 during spray-drying of milk

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High-performance liquid chromatography was used to evaluate the stability of vitamin D_3 in milk when subjected to spray-drying. Measured losses through the pasteurisation, high-pressure evaporation and drying processes were demonstrated to be statistically insignificant ($P = 0.05$). The common practice of overage addition during manufacture of supplemented milk powder formulations is therefore a questionable risk factor to regulatory compliance. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The preservation of milk achieved through spray-drying provides significant nutritional benefits to many populations by facilitating the distribution and accessibility of a major food commodity in the human diet. While the potential for nutrient impairment during the dehydration process is generally recognised, the protective attributes against spoilage, gained through enzyme inactivation and moisture reduction, are considered to be of greater significance (Rolls, 1982; De Ritter, 1982).

Vitamins are commonly used to supplement milk products, either to restore production losses, or to further enhance their availability to potentially at-risk groups. These fortification strategies are controlled by legislation (e.g. Codex, 1982). Micronutrients share a general lability when exposed to thermal stress (Scott *et al.*, 1984; Finglas, 1993; Ryley & Kajda, 1994), although modern spray-drying techniques have been demonstrated to result in lower vitamin losses when compared to roller-drying or preservation of milk in the liquid state by higher temperature (UHT) processing (Renner, 1983; Oaman *et al.*, 1989). Such potential losses during production are to be distinguished from those further accruing under post-production storage conditions. Overall nutrient losses are therefore generally compensated by the addition of an 'overage' during manufacture, designed to be vitamin and product specific (Mays, 1982).

Losses of vitamins in heat-processed milk have been studied most commonly with respect to the water-soluble group, while, of the fat-soluble group, vitamin A stability in supplemented milk has received world-wide attention due to the importance of this nutrient in preventing deficiency diseases (De Man *et al.*, 1986; McCarthy *et al.*, 1986; Woollard & Fairweather, 1985; Woollard & Indyk, 1986).

Vitamin D is widely added to both milk and humanised infant formulas to furnish the special dietary needs of high-risk groups (Ball, 1988; Holick *et al.*, 1992). This practice recognises both the utility of milk as a major community health commodity and its low endogenous antirachitic content (Kurmann & Indyk, 1994). Cholecalciferol is the principal form in which this nutrient is supplied in foods, usually as water-dispersible beadlets. The complexities of fat-soluble vitamin addition have been reported (Packard, 1982), the favoured method being wet-blending into fluid milk in order to ensure adequate homogeneity.

Although there have been several reported surveys of vitamin D enriched consumer milks and infant formulas (Scott *et al.*, 1984; Rao & Mathur, 1988; Tanner *et al.*, 1988; Holick *et al.*, 1992), few data exist regarding its stability during milk processing and conversion to dried product. It has been variously described as thermally labile, leading to potential oxidative losses and isomerisation to the biologically inactive pyrocalciferols. When used to enrich milks, it has therefore been common practice through overage addition to allow for up to 30% destruction of supplemental vitamin D during the drying process (Bender, 1979). Recovery of vitamin D in dairy products has also been demonstrated to be positively correlated to the protective attributes of fat content (Tanner *et al.*, 1988). A recent study concluded that vitamin D in aerated solution was subject to losses at moderate temperatures (21°C), while degradation in milk was greater during exposure to light (Renken & Warthesen, 1993).

The aim of the present study was to evaluate the extent of loss of vitamin D_3 during the commercial spray drying of supplemented whole milk. This opportunity was made possible through the use of modern multidimensional high-performance liquid chromatographic (HPLC) techniques.

MATERIALS AND METHODS

Apparatus

Semi-preparative HPLC
The equipment used was a Model 510 pump, U6K injector, (Waters, Milford, MA, USA) and a PU4025 variable wavelength ultraviolet (UV) detector (Pye Unicam, Cambridge, UK). Outputs at 265 nm were recorded on a Model 745 data module (Waters). The column used was a Resolve silica (5 μm) cartridge fitted within an 8×10 radial compression module (RCM), silica insert in a Guard-Pak module with a mobile phase of hexane–isopropyl alcohol (99:1, v/v) at a flow rate of 2 ml min^{-1}.

Analytical HPLC
Analytical separation was achieved with two Resolve octadecylsilane (5 μm) columns configured in tandem within an extended 8×10 RCM, preceded by a Guard-Pak insert of similar material. The chromatograph was an integrated LC10 series automated HPLC system supported by PC-based Class software (Shimadzu, Tokyo, Japan). Dual-wavelength data were acquired at 265 and 280 nm. The mobile phase was methanol (100%) at a flow rate of 1.5 ml min^{-1}.

Sample collection

Spray-dried whole milk powder was manufactured by a semi-continuous process. Briefly, incoming raw milk was pasteurised, separated into chilled skim and cream fractions and batch recombined (120 000 litres, 10°C) to achieve standardised fat and protein levels. Sufficient predissolved vitamins A and D_3 were added with agitation in order to achieve consistent target levels in the finished product (3500 iu and 475 iu per 100 g total solids, respectively). Subsequent thermal processing parameters were typical of a medium heat protocol and included low pressure preheating, direct steam injection (95°C), five-stage evaporation and spray-drying (149°C) followed by a fluid-bed finish (107°C).

Samples were taken under steady-state production conditions over 3 consecutive days. In each case, liquid milk (approx. 1 litre) was withdrawn from the milk silo subsequent to vitamin addition, and the dried whole-milk powder (approx. 200 g) directly from the packing hopper. The flow characteristics of the plant facilitated sample traceability, based on the well-defined residence time within the closed system (50 min).

Each daily fluid milk sample was tempered at 35°C and triplicate sub-samples (approx. 85 g weighed accurately) stored separately in Duran flasks at −18°C until analysed. The corresponding powder samples were also sub-sampled into triplicates (approx. 10 g weighed accurately) in separate actinic Erlenmeyer flasks and stored at 4°C until analysed.

Analytical scheme

Compositional analysis
All collected samples were analysed, in duplicate, for total solids, moisture and fat as appropriate, by International Dairy Federation standard methods (oven-drying IDF 21B:1987, Karl Fischer titration IDF 23A:1988, and Rose-Gottlieb IDF IC:1987, respectively). This was necessary to facilitate expression of vitamin D content on a normalised basis.

Vitamin D analysis
The analysis of each of the paired liquid and powder samples was performed on different days in triplicate. Each analysis set included a reference control sample in order to monitor method performance. Pre-weighed samples were brought to ambient conditions and the liquids tempered at 35°C. Vitamin D analysis was achieved essentially according to the procedure of Kurmann & Indyk (1994) developed for the estimation of endogenous cholecalciferol in milk. Slight modifications were made to account for the higher level of analyte in supplemented milks, thereby avoiding the requirement for a solid-phase extraction stage during fractionation. Briefly, after addition of vitamin D_2 as internal standard, samples were saponified overnight at ambient temperature, extracted twice with hexane–diethyl ether (90:10, v/v) and the pooled extracts washed to neutrality with water. Extracts were dried over anhydrous sodium sulphate, evaporated to dryness (<40°C) and the residues dissolved in hexane (100 μl). The crude extracts were fractionated on the described normal-phase HPLC system and, following evaporation and reconstitution in methanol (1.0 ml), subjected to analytical reversed-phase HPLC with dual-wavelength UV detection.

Quantification was achieved by the internal standard technique. Identity and purity of both the internal standard and target analyte were based on the equivalence of retention time and absorbance ratio (265:280 nm) against authentic standards.

RESULTS

Figure 1 illustrates the analytical reversed-phase chromatogram of a typical milk sample fraction obtained after semi-preparative fractionation.

Acceptance of chromatographic data was based on retention time equivalence and the integrity of spectral absorbance ratios (1.37, range 1.33–1.43) compared to authentic vitamin D (1.37, range 1.35–1.40). Compliance of the reference control sample (mean 364 iu per 100 g; SD = 11.6; $n = 27$) within QC performance limits

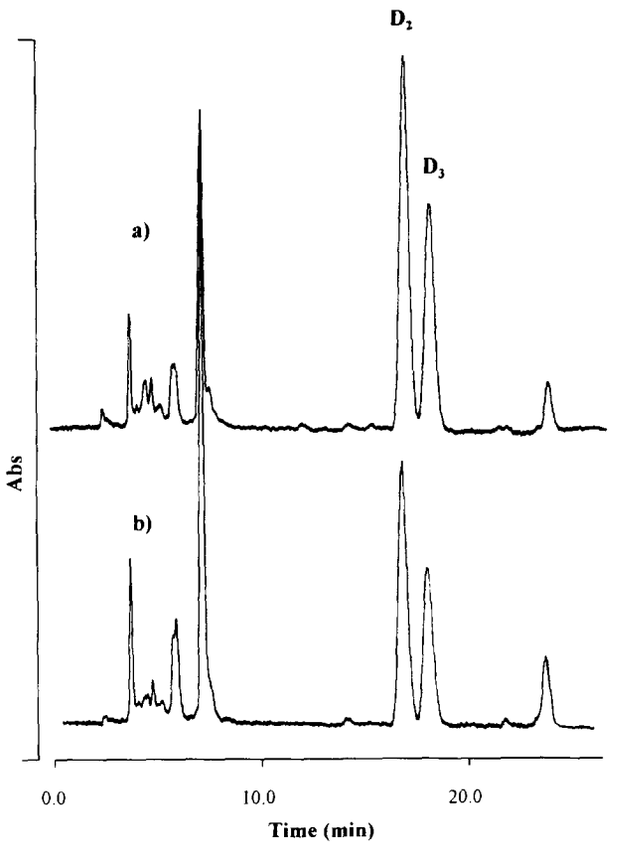

Fig. 1. Analytical chromatography of a vitamin D fortified milk sample subsequent to semi-preparative LC fractionation. Conditions as described in text; flow rate, 1.5 ml min^{-1}; injection volume, 50 µl. Detection: (a) 265 nm; (b) 280 nm.

(± 1 SD) confirmed that the assay was under statistical control. Further, chromatographic interferences were absent as demonstrated with a reagent blank and unfortified milk, while critical validation parameters of the overall method were as reported previously (Kurmann & Indyk, 1994) with respect to linearity, sensitivity, recovery and precision.

Table 1 collates the estimated quantitative data, inclusive of proximate analyses, individual assay determinations and overall means. Data for vitamin D are expressed (per 100 g) on an 'as-is', total solids and fat basis.

To estimate the magnitude of any potential loss of vitamin D during the production process, experimental data, expressed on a normalised total solids basis, was compared directly between liquid and powdered milks. Data from the three paired samples were analysed on a non-parametric basis, with each correlated sample yielding dependent triplicate means. Computation of these values yielded an apparent change in vitamin D_3 of −7 iu per 100 g total solids between fluid and powdered milk (SD = 20; SEM = 11), with a confidence interval of −56 to +42 ($P = 0.05$). An alternative analysis as nine independent data sets of a uniform product may be considered, based on the steady-state conditions existing over the 3-day production period. With this less conservative test (SD = 24; SEM = 8), the confidence interval is reduced to −20 to +6 ($P = 0.05$).

Either of these statistical treatments demonstrate that the estimated vitamin loss lies within the standard error

Table 1. Experimental data from vitamin D supplemented milk and milk powder

Sample	Total solids (%)	Fat (%)	Moisture (%)	Determination[a]	Vitamin D_3 (iu per 100 g)		
					'As-is'	Total solids	Fat
Liquid							
Day 1	12.37	3.73	87.63	1	61.4	496	16.5
				2	61.0	493	16.4
				3	57.7	466	15.5
				x	60.0	485	16.1
Day 2	12.27	3.98	87.83	1	60.5	493	15.2
				2	58.0	473	14.6
				3	58.7	478	14.7
				x	59.1	481	14.8
Day 3	12.38	3.71	87.62	1	59.9	484	16.1
				2	63.4	512	17.1
				3	61.9	500	16.7
				x	61.7	499	16.6
Powder							
Day 1	97.44	29.99	2.56	1	479	492	16.0
				2	471	483	15.7
				3	488	501	16.3
				x	479	492	16.0
Day 2	97.28	29.78	2.72	1	471	484	15.8
				2	474	487	15.9
				3	467	480	15.7
				x	471	484	15.8
Day 3	97.21	29.48	2.79	1	460	473	15.6
				2	446	459	15.1
				3	463	476	15.7
				x	456	469	15.5

[a] *x*, mean of triplicate determinations.

of the analytical mean. The data are therefore consistent with there being no statistically significant loss of vitamin D_3 during conversion from liquid to powdered milk at the 95% confidence level.

DISCUSSION

The common practice of fortifying milk with vitamin D_3 remains a somewhat controversial issue, with some having questioned both the efficacy of dietary absorption (Packard, 1982) and the possible preference for 25-hydroxyvitamin D_3 in the supplementation of infant formulas (Kunz *et al.*, 1984). A further concern has related to the reported incidences of intoxication or deficiency attributed to inconsistent fortification protocols (Holick *et al.*, 1992). Nevertheless, the practice of providing bovine milk with elevated levels of vitamin D, in both fluid and dried form, seems unlikely to change, in view of the substantial community benefits accruing from their consumption.

Where surveys of milks and formulas have indicated variability in vitamin D content, it has been unresolved as to whether this is due to analytical variability, inconsistencies during supplementation, or to instability of the vitamin during thermal processing. While such processing factors have generally been reported to be less detrimental to vitamin retention when compared to storage (Renner, 1983; Rao & Mathur, 1988; Oaman *et al.*, 1989), available information specific to vitamin D is currently regarded as incomplete (Finglas, 1993).

The present study has attempted to quantify the impact of thermal stresses associated with the spray-drying of milk on the retention of supplemental vitamin D. It has been established that any losses are statistically insignificant ($P=0.05$) under the process conditions described. Since similar thermal protocols are commonly employed during production of other dried whole milks and infant formulas, such conclusions regarding vitamin D retention may be generalised with confidence. However, each manufacturing plant should ideally evaluate its own specific process before implementing an arbitrary overage during the fortification stage.

It may be concluded that global regulatory and compliance concerns governing the manufacture of these nutritionally important products may be better served through moderating the ubiquitous practice of vitamin D overage addition. This generally arbitrary practice increases the risks associated with the potential toxicity of this vitamin and has, in this study, been demonstrated to be unjustified.

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