Food Chemistry **46** (1993) 429–437
Analytical Methods Section

The determination and distribution of taurine in dairy products

D. C. Woollard

Lynfield Food Services Centre, Ministry of Agriculture and Fisheries, 131 Boundary Rd., Auckland, New Zealand

&

H. E. Indyk

Anchor Foods Ltd., Waitoa, New Zealand

(Received 20 April 1992; revised version received 20 July 1992; accepted 24 July 1992)

A method for the evaluation of taurine in milk has been developed and evaluated. The technique is based on dansylation of the free amino acids and separation of the derivatives using HPLC and UV detection. The procedure is suitable for rapid data collection at sample concentrations above 1 mg per 100 g and can be extended using fluorescence to assay products with lower taurine contents.

The technique was applied to investigate the temporal variation of taurine in milk from a single animal and from pooled herd milk. Further studies included seasonal and annual variations in whole milk and skim powders from selected Australian and New Zealand manufacturing plants. Early-season milk contained higher concentrations of taurine than late season, while the distribution of taurine in a wide selection of milk powders, taken during the summer months, revealed significant differences.

Other powdered dairy products, such as casein and whey protein, were also examined for their taurine contents. There were large variations between product types as a result of taurine migration during differing manufacturing procedures. Taurine-fortified infant formulae were also tested.

INTRODUCTION

Taurine, 2-aminoethanesulphonic acid, is a major component of the circulating free amino acid pool within animals, although it has not been found within any known protein structure. It is an important metabolite of the sulphur amino acids and its acceptance as an essential growth factor followed recognition of its role in bile acid synthesis and in the prevention of certain pathologies. Taurine is pervasive in most mammals and its accumulation in certain excitable tissues and organs supports the clinical observation that it is necessary for functional regulation of the eyes, heart, muscles, brain and central nervous system (Barbeau & Huxtable, 1978; Hayes & Sturman, 1981; Gaull, 1982*a*,*b*; Trautwein & Hayes, 1990).

The nutritional role of taurine is rapidly gaining interest because of the anatomical consequences of its deficiency in many species. In particular, it is an essential amino acid in cats, where ocular degeneration follows taurine deficiency. In humans, where taurine is regarded as conditionally essential, depletion is possible under certain circumstances requiring that the balance be sustained from exogenous sources to avoid potential disease syndromes (Hayes, 1988). Vegans are vulnerable to taurine depletion because of the limited presence of this amino acid in their diet (Rana & Sanders, 1986). Similarly, patients on parenteral nutrition are at risk unless taurine is included in the infusion solutions (Geggel *et al.*, 1985; Paauw & Davis, 1990).

The demand for taurine by infants often exceeds *de novo* metabolic supply due to their immature enzyme systems. When breast-milk substitutes are used, dietary intervention may become necessary in view of the lower taurine content of bovine compared to mother's milk (Sturman *et al.*, 1977; Gaull, 1982*a*). Commercial formulae are increasingly being supplemented with taurine to enable infants to maintain the same serum levels as observed in maternally fed counterparts (Gaull, 1982*b*; Rassin *et al.*, 1983; Chapman & Greenwood, 1988).

There have been relatively few studies of the taurine content of foods (Pasantes-Morales *et al.*, 1989; Laidlaw

Food Chemistry 0308-8146/93/$06.00

et al., 1990; Pasantes-Morales & Flores, 1991). Animal products, notably seafoods and meats, are taurine-rich with large variations between samples of the same food due to disparate tissue distributions. Dairy products supply a significant proportion of the predicted omnivorous and lacto-ovovegetarian dietary needs (Nishikawa *et al.*, 1984; Erbersdobler & Trautwein, 1984; Donovan & Lönnerdal, 1989; Saidi & Warthesen, 1990).

A method of analysis was implemented using HPLC of the dansyl (DNS) derivative, focusing on the evaluation of taurine without reference to other free amino acids and is similar to one published independently by Hischenhuber (1988). Following validation of the method, its robustness was evaluated during a two-year period through a quality-control monitoring program of supplemented infant formulae. The technique was then applied to the investigation of endogenous taurine contents in various dairy products.

The data-base for taurine in bovine milk and manufactured dairy products is not as extensive as for human milk and other physiological fluids. This current study therefore surveys seasonal, annual and geographical influences on taurine distributions in Australasian dairy products derived exclusively from pasture-fed dairy herds.

EXPERIMENTAL

Equipment

The isocratic HPLC system consisted of a model 510 pump fitted with a manual two-way solvent select valve, with a model 490E programmable UV detector (Waters Associates, Milford, MA, USA) and an F1000 fluorescence detector with xenon lamp (Hitachi, Tokyo, Japan) connected in series. The chromatographic column was a Waters C18 Resolve cartridge, 8 mm i.d. with 5 μm packing, fitted inside an '8 × 10' radial compression module and preceded by a Waters Guard-Pak protective column with C18 insert. Sample introduction was accomplished with a model 7125 6-port injector with 25 μl loop (Rheodyne Inc., Cotati, CA, USA). Chromatograms were recorded on an SE120 dual-pen chart recorder (Brown Boveri Corp., Nurnberg, Austria). In later studies the HPLC was automated for unattended operation with a Waters model 915 autosampler, an additional model 510 pump, a dual-channel Hitachi D-2500 integrator with IBM PC/XT data processor, equipped with Waters SIM interfacing and 'Maxima' software. Solvent clarification and degassing was performed using vacuum apparatus through 4·5 μm Nylon-66 membranes (Alltech Assoc. Inc., Deerfield, IL, USA).

Reagents

Pure water was generated by a Milli-RO reverse osmosis and Milli-Q ion-exchange system (Millipore, Bedford, MA, USA) to a nominal resistance of 18 MΩ. HPLC grade acetonitrile (J. T. Baker, Phillipsburg, NJ, USA), tetrahydrofuran (May and Baker, Dagenham, UK) and AR-grade acetic acid, hydrochloric acid (Ajax, Sydney, Australia), potassium hexacyanoferrate(II) trihydrate, zinc acetate dihydrate, sodium carbonate (BDH, Poole, UK), sodium acetate trihydrate, dansyl chloride (5-dimethylamino-1-naphthalenesulphonyl chloride, DNS-Cl), taurine (Sigma, St Louis, MO, USA) and methylamine hydrochloride (Aldrich, Milwaukee, WI, USA) were used as received.

Carrez solutions 1 and 2 were prepared according to International Dairy Federation Standard IDF 79A: 1989, by separately dissolving potassium hexacyanoferrate(II) trihydrate (3·6 g) and zinc acetate dihydrate (7·2 g) in water (100 ml). Sodium carbonate buffer was prepared as an 8·0% (m/v) solution, adjusted to pH 9·5 with hydrochloric acid. Methylamine hydrochloride solution was made by dissolving 2·0 g in water (100 ml). The dansyl chloride derivatising solution was prepared in acetonitrile to a strength of 1·5 mg ml^{-1}. Acetate buffer (0·1 M) was prepared by dissolving sodium acetate trihydrate (13·6 g) in water, adjusting the pH to 4·2 with glacial acetic acid and making to 1 litre.

Standard preparation

Approximately 10 mg of taurine was weighed accurately and dissolved in water (100 ml). Working standards were obtained by dilution in 100 ml volumetric flasks of 1, 5 and 10 ml, achieving concentrations of between 1 and 10 μg ml^{-1}.

Sample collection

Raw milk was collected from a 5-year-old Jersey cow (4th calving) and pooled herd milk from the refrigerated holding silo at the manufacturing site. Samples of dried dairy products were sealed immediately after production within laminated foil sachets and despatched to the analytical laboratory where they were stored desiccated at 4°C until assayed (within 14 days).

Sample preparation

Well-mixed samples of fluid milk (25 g) or powdered products (1–5 g) were weighed accurately into 100 ml volumetric flasks. Water (~50 ml) was added and the flasks incubated at 65°C for ~10 min with periodic shaking to aid dissolution. Some samples, particularly casein and caseinates, with solubility difficulties, required ultrasonic treatment at 65°C for 30–45 min to release the taurine. After cooling to room temperature, Carrez solutions 1 and 2 (5 ml of each) were added sequentially and the flasks allowed to stand for 30–60 min with occasional agitation. The flasks were made to volume with water and filtered through Whatman 542 paper, discarding the first 10–20 ml of filtrate.

In later studies the assays were scaled down ten-fold for analytical convenience. Appropriate weights of samples were placed in 10-ml volumetric flasks and

1 ml of precipitation reagents used. The strength of the Carrez 1 and 2 solutions was increased to 18 g per 100 ml and 36 g per 100 ml accordingly.

The method is also suitable for raw materials and premixes where taurine is presented in high concentration, ready for incorporation into infant formulae. The samples are simply diluted with water to the desired concentration (5–10 μg ml^{-1}) then analysed in the same way but without the need for protein/fat precipitation with Carrez solution.

Derivatisation

One millilitre of the sample filtrates and the standards were pipetted separately into 5-ml reaction vessels. Sodium carbonate buffer (1·0 ml) and dansyl chloride solution (1·0 ml) were added and following vortex mixing, the capped vials were allowed to stand for two hours in the dark at ambient temperature. The reactions were stopped by the addition of methylamine hydrochloride solution (100 μl) and the precipitates allowed to settle overnight in the absence of light. A portion of the supernatants was passed through 4·5 μm filters (DynaGard PP, Microgon Inc., Laguna Hills, CA, USA) prior to chromatographic analysis. Although this is analytically convenient, sample throughput can be increased by cooling the samples at 4°C for 2 hours to enhance the speed of the precipitation process.

Chromatography

Separation of the taurine derivatives was accomplished using a filtered ternary mobile phase consisting of 0·1 M acetate buffer/acetonitrile/tetrahydrofuran (81 : 17 : 2 (v/v/v)) at a flow rate of 2 ml min^{-1}. UV detection was at 254 nm and fluorescence was monitored at 330 nm (excitation) and 530 nm (emission). Following elution of the components of interest, the column was purged of retained artefacts using acetonitrile at 4 ml min^{-1} for 10 min, then initial conditions restored subsequent to the next assay. This wash cycle was performed manually or under automatic control.

Quantitation was by comparison of the peak sizes using external standardisation. Accurate standards calibration was accomplished by area integration and linear regression through the origin. At higher sample concentrations (>3 g per 100 ml) data were corrected for volume (V–P) adjustments caused by fat and protein precipitation using well-accepted parameters of the International Dairy Federation [IDF 79A:1989, where $P = 1{\cdot}1 \times$ fat (g) + $0{\cdot}73 \times$ protein (g)].

Compositional analysis

Fat, protein, total solids, moisture and ash were estimated by standard procedures (Röse-Gottlieb, Kjeldahl, oven drying, Karl Fischer and muffle furnace, respectively) as described by International Dairy Federation standards.

Collaborative studies

The dansylation method was compared against two other HPLC derivatisation procedures involving post-column reaction with ninhydrin (NIN) and a pre-column phenylisocyanate (PITC) procedure. A judgement of the current methodology was also undertaken using the protocols set out in International Dairy Federation Standard 135A:1988. Participants in these verification studies were the Australian Government Analytical Laboratory (Sydney), Dairy Technical Services (Melbourne), the Government Chemical Laboratory (Brisbane), the Central Animal Health Laboratory (Wellington), and the laboratories of the authors.

RESULTS

Chromatography

Figure 1 illustrates the chromatogram obtained for a typical liquid milk by UV and fluorescence detection. Taurine elutes after about 9 min using the described conditions while a sample blank showed the absence of interfering substances. Other amino acids or amines were essentially missing in the chromatogram with the exception of an unknown peak at 7 min. A blank placebo, without dansyl derivatisation, showed no spectral activity other than early solvent and reagent peaks, confirming freedom from interference.

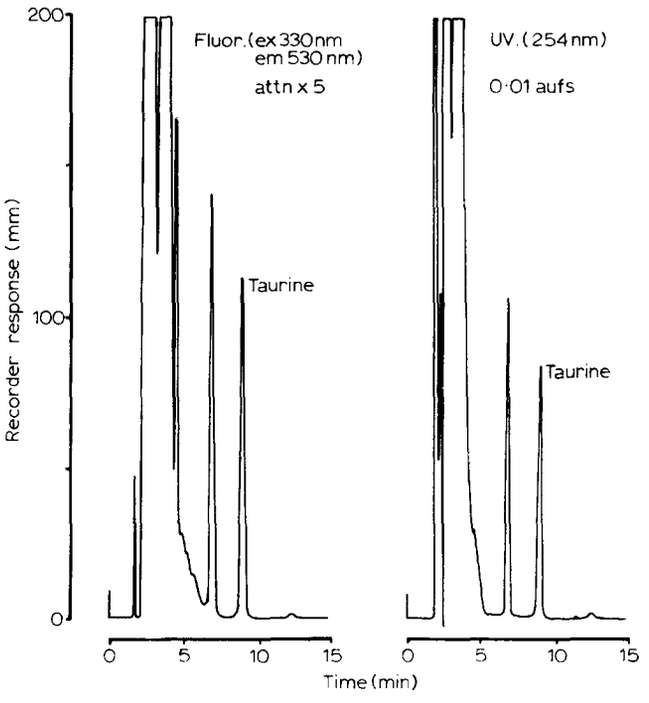

Fig. 1. Chromatogram of endogenous taurine in fluid bovine milk following derivatisation. Column, 5 μm C_{18} Rad-PAK; mobile phase, sodium acetate buffer (10 mM; pH 4·2): acetonitrile : tetrahydrofuran (81 : 17 : 2 (v/v/v)); flow rate, 2·0 ml min^{-1}; injection vol. 25 μl).

Method reliability

Linearity of detector response was established for taurine over the range of 0–40 μg ml^{-1}, corresponding to 0–320 ng injected on-column. This observation applied to UV and fluorescence detection, although the latter had a lower limit of detection (0·8 ng) compared to UV (5 ng). Both detectors possessed sufficient sensitivity to facilitate the assay of all samples at endogenous taurine concentrations and produced identical data, thereby increasing analytical confidence.

Method precision was monitored by between-run replicate analysis, by different analysts, of an early-season whole milk powder (mean = 10·9 mg per 100 g, RSD = 5·6%, n = 9) and a fortified infant formulation (mean = 54·6 mg per 100 g, RSD = 4·7%, n = 23). During survey studies these served as control samples to monitor analytical performance. These studies also demonstrated that taurine is stable in milk powders under normal storage conditions for over 12 months. Standard recovery studies were also performed on several powdered and liquid infant formulations with yields of 96–103% as detailed in Table 1.

The precision characteristics of the current method were also tested by six participating laboratories using ten supplemented milk-powder samples (five duplicates) according to International Dairy Federation guidelines, IDF135A:1988. Applying the described mathematical treatment, no significantly deviant data were recognised using the Cochran statistic and one outlier was confirmed by the double Grubbs test. This is confirmation of the robustness of the described procedure and yielded relative repeatability (RSD_r) and reproducibility (RSD_R) parameters of 4·9% and 6·8%, respectively, at the 95% confidence level.

Table 1. Taurine concentrations in fortified infant formulae: Recovery analysis

Sample	Label claim (mg per 100 g) (*mg per litre^{-1})	Added (% of label claim)	Found (mg per 100 g)	Recovery (%)
Powdered infant formula	40	0	46	—
		50	68	103·0
		100	85	98·8
Powdered infant formula	42	0	49	—
		50	70	100·0
		100	93	102·2
Powdered infant formula	45	0	51	—
		50	74	100·7
		100	94	97·9
Powdered infant formula	50	0	53	—
		50	75	96·2
		100	102	99·2
Ready-to-use UHT, milk-based	40*	0	52	—
		50	73	101·4
		100	90	97·8
Ready-to-use UHT, soy-based	43*	0	42	—
		50	63	99·2
		100	82	96·5
			Average recovery = 99·4%	

Table 2. Taurine concentrations in various milk powders: Comparison with other methods[a]

Sample	DNS[b]	NIN[c]	PITC[d]
Skim milk powder	8·1 (4·3%)	9·2 (8·1%)	8·0 (7·5%)
Infant formula	38·5 (4·9%)	36·4 (7·9%)	40·3 (9·0%)
Infant formula	49·6 (5·3%)	46·1 (9·7%)	49·8 (8·3%)

[a] Results in mg per 100 g. RSDs in parentheses, n = 5.
[b] Dansyl chloride, current method.
[c] Ninhydrin, method of Nicolas *et al.* (1990).
[d] Phenyl isothiocyanate, method of Fierabracci *et al.* (1991).

Table 2 compares the taurine information obtained by collaborative study using a conventional amino acid analyser with ion-exchange separation and post-column ninhydrin derivatisation, and by the PITC pre-column technique. The methods gave statistically equivalent answers using Friedman's ranked χ^2 test ($p < 0{\cdot}01$) for each of the three samples, lending further credibility to the current methodology. However, the dansylation method proved easier to perform, with no need for complex gradients, and was free of any resolution problems as shown by the better precision parameters. No suitable *o*-phthalaldehyde (OPA) method was available for comparison by an independent laboratory.

Applications

Taurine data were collected from milk of single cow *post partum* (colostrum milk 2·3–4·0 mg per 100 g;

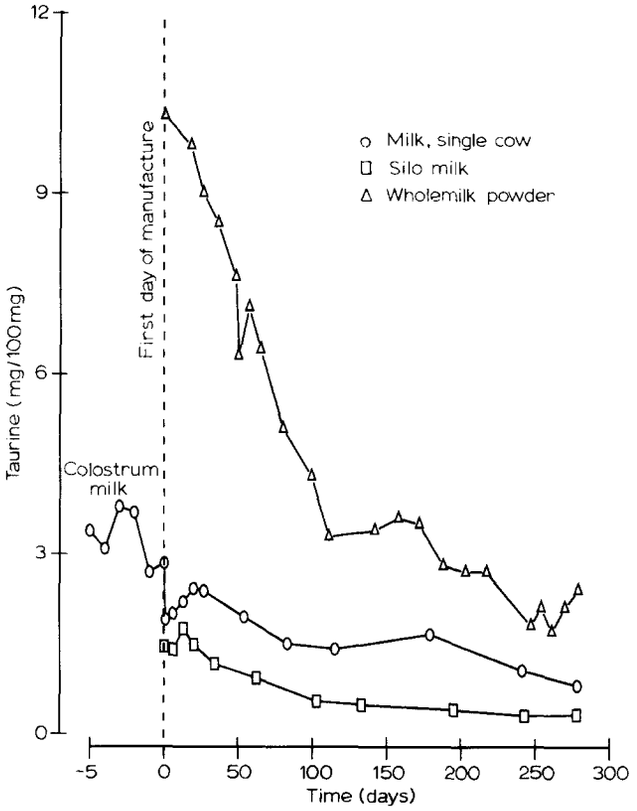

Fig. 2. Taurine concentrations in milk from a single cow, pooled silo milk and derived wholemilk powder.

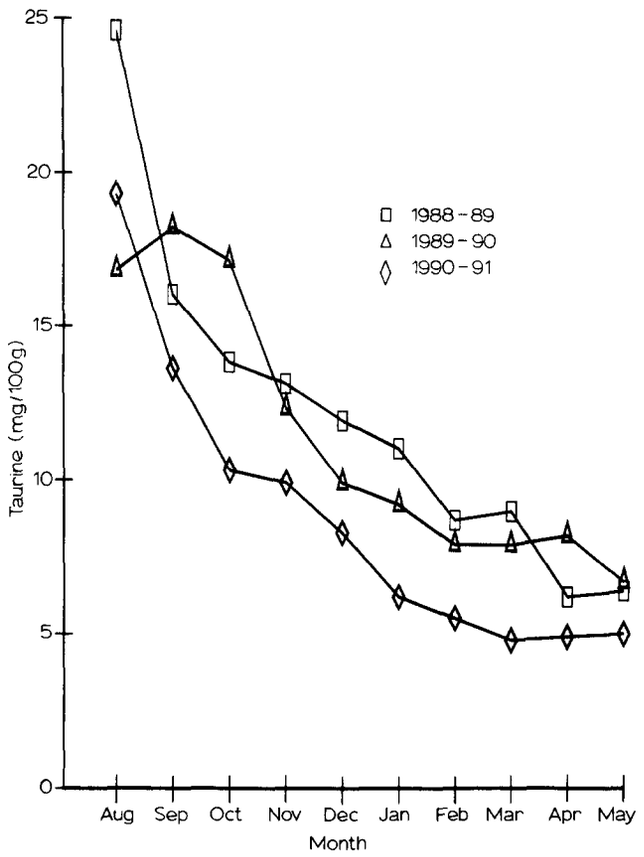

Fig. 3. Temporal comparisons of taurine in skim milk powder from a single factory during a three-year production period.

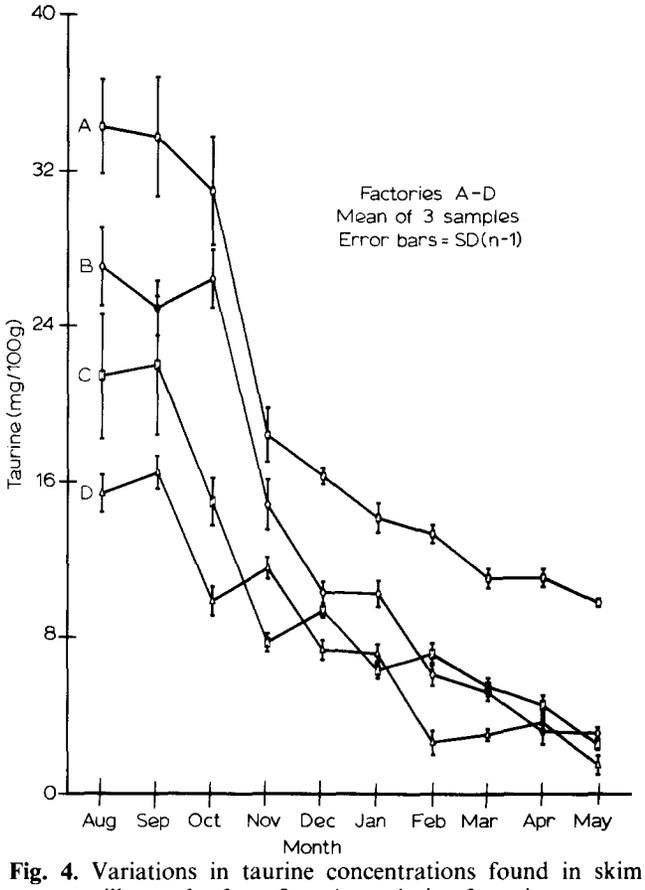

Fig. 4. Variations in taurine concentrations found in skim milk powder from four Australasian factories.

mature milk 0·8–2·8 mg per 100 g), pooled herd milk (0·3–1·8 mg per 100 g) and spray-dried wholemilk powder (1·7–10·4 mg per 100 g) throughout a full production season, as illustrated in Fig. 2. These results indicate that parturition is accompanied by a several-fold increase in taurine concentration, followed by a steady decrease as milk collection continues. The same pattern was exhibited in both colostrum and mature milk of a single cow or in pooled herd milk and derived milk powder. Pooled colostrum milk was not available for inspection as it is withheld from use in dairy product manufacture.

Losses of taurine during processing of milk to milk powder were estimated by comparison of levels in silo milk with product on a total solids basis. In all cases losses were less than 6%, suggesting that degradation is minimal. This conclusion has been further supported in supplemented infant formulae, since comparison of taurine added prior to manufacture with concentrations observed in the finished products revealed a recovery averaging 96%.

The response to seasonal variables was examined in a single manufacturing plant over sequential production seasons (Fig. 3). The taurine concentrations decreased throughout each year in a reasonably constant manner throughout the three-year study. A survey of skim-milk powder manufactured at four geographical locations within New Zealand and Australia is presented in Fig. 4 where similar seasonal patterns are apparent, although taurine concentrations differed considerably between sites.

Taurine distribution in a wide range of powdered Australasian dairy products has been surveyed and reported in Table 3. The data include the taurine status of products with a variety of compositions such as whey powders (12–16% protein), whey protein concentrates (35–80% protein), casein and caseinates (87–94% protein) and buttermilk powder (2·4–13·1% protein).

Table 3. Mean taurine concentrations in various Australasian milk products[a]

Product	*N*	Mean (mg per 100 g)	Mean (mg per 100 g MSNF)	Mean (mg per 100 g protein)
Wholemilk powder	36	4·28 (0·8–8·7)	6·18 (1·2–12·5)	15·48 (2·9–33·3)
Skim milk powder	40	6·75 (2·7–15·5)	7·08 (2·9–16·2)	17·94 (7·5–40·9)
Buttermilk powder	24	11·13 (2·2–23·0)	13·67 (3·4–24·6)	29·68 (6·2–57·6)
Whey powder	24	18·75 (10·3–32·9)	19·75 (10·9–34·6)	135·12 (78·0–238·4)
Whey protein concentrate	44	4·28 (1·0–9·2)	4·69 (1·1–9·7)	6·51 (1·3–15·0)
Casein/caseinate	44	1·62 (1·0–2·50)	1·72 (1·1–2·7)	1·82 (1·2–2·8)

[a] Ranges in parentheses. *N* = number of observations, milk powders = 40.

Perishable products such as milk, butter and cultured products were not included in this survey for practical reasons. Samples were selected in a random fashion to ascertain which products were taurine-rich. The number of samples per region was limited to four to avoid any dominance of the data sets and were collected during the summer months (January–April) when seasonal effects were at a minimum. There are substantial differences between product types, especially those subjected to extensive compositional alterations during production.

DISCUSSION

The intricacies of amino acid profiling in biological specimens are summarised in the compendial reports of Deyl *et al.* (1986), and Ersser and Davey (1991). A number of techniques have been described for the estimation of taurine, either uniquely or in combination with other free amino acids, including colorimetry (Lau *et al.*, 1990), fluorimetry (Goodman & Shihabi, 1987), gas chromatography (Kataoka *et al.*, 1984) and notably, ion-exchange chromatography (Erbersdobler *et al.*, 1983; Nishikawa *et al.*, 1984; Donovan & Lönnerdal, 1989; Laidlaw *et al.*, 1990; Nicolas *et al.*, 1990). Developments involving reversed-phase HPLC have attempted to avoid some of the inconveniences of these other approaches, predominantly utilising *o*-phthalaldehyde (OPA) pre-column derivatisation procedures (Larsen *et al.*, 1980; Fleury & Ashley, 1983; Ali Qureshi *et al.*, 1984; Elkin, 1984; Eslami & Stuart, 1984; Halfpenny & Brown, 1985; Hirschberger *et al.*, 1985; Eslami *et al.*, 1987; Rajendra, 1987; Anderson *et al.*, 1988; Porter *et al.*, 1988; Pamblanco *et al.*, 1989; Murai *et al.*, 1990). In view of the instability of the OPA-taurine derivative, alternative reagents have also been investigated, including the nitrobenzo-2-oxa-1,3-diazoles (Watanabe & Imai, 1984; Palmerini *et al.*, 1987), 9-fluorenylmethyl chloroformate (Haynes *et al.*, 1991), dabsyl chloride (Chang *et al.*, 1981), fluorescamine (Saidi & Warthesen, 1990; Sakai & Nagasawa, 1992), phenylisothiocyanate (Gunawan *et al.*, 1990; Sherwood *et al.*, 1990; Fierabracci *et al.*, 1991) and dansyl chloride (Tapuhi *et al.*, 1981; Biondi *et al.*, 1986; Subba Rao, 1987). Some post-column methodologies have also been reported, using OPA (Hirai *et al.*, 1987) or thiamin (Yokoyama & Kinoshita, 1991) although HPLC hardware requirements are greater.

The highly fluorescent dansyl derivative exhibits stability and chromatographic advantages compared to other chromophores. Confirmation of the suitability of dansylation has been demonstrated for clinical samples (Quesada *et al.*, 1986; Saller & Czupryna, 1989; Amiss *et al.*, 1990) and supplemented infant formulae (Hischenhuber, 1988), and was the preferred method in this study. Protein hydrolysis was proven unnecessary for the estimation of taurine and only complicated the analysis due to its low concentration compared with many protein-bound amino acids. The described strategy involved protein precipitation and dansylation of the free amino acids using well-proven techniques. Deproteination by other common practices, such as those described for biological fluids by Uhe *et al.* (1991), were found less suitable for dairy products than Carrez solutions. Separations of the DNS-taurine derivatives were achieved within ten minutes without interference using simple isocratic HPLC without the need for gradient techniques. While it was necessary to clean the column of late-eluting materials, in practice it was possible to achieve four successive injections before this became mandatory, thereby substantially improving sample throughput. The derivatised extracts could be stored refrigerated, without degradation, for two days prior to analysis, giving further advantages to this technique as suitable for routine use.

Taurine concentrations in colostrum (2·9–13·4 mg per 100 g) and mature milk (0·1–1·8 mg per 100 g) collected from an individual animal were in general agreement with other reports (Rassin *et al.*, 1978; Erbersdobler *et al.*, 1983; Erbersdobler & Trautwein, 1984; Nishikawa *et al.*, 1984; Donovan & Lönnerdal, 1989; Pasantes-Morales *et al.*, 1989; Saidi & Warthesen, 1990). These data, combined with those for pooled herd milk collected across the 1990–91 production season, confirmed a gradual decline in taurine concentration following the initiation of lactation. This trend has been shown in other reports to be species-specific, and indeed less pronounced for humans, where breast milk appears to contain significantly higher and more consistent levels (3·8–5·1 mg per 100 g) throughout lactation (Rassin *et al.*, 1978; Atkinson *et al.*, 1980; Erbersdobler *et al.*, 1983; Harzer *et al.*, 1984; Nishikawa *et al.*, 1984; Pamblanco *et al.*, 1989; Shubat *et al.*, 1989), reflecting in part, taurine's complex biochemical role in human development, a subject currently under intense study in many laboratories. The taurine content of other mammalian milks has received limited study (Hayes & Sturman, 1981) although this situation may improve where the milk is used in human nourishment (Taha & Kielwein, 1990).

Recoveries of endogenous taurine during the manufacture of whole milk powder, have been found to be quantitative. Not unexpectedly, taurine levels in dried product paralleled the same seasonal trend as the source milk with an overall decline of almost one order of magnitude. Although in general agreement with the work of Saidi & Warthesen (1990), this observation is contrary to the conclusions of Pasantes-Morales *et al.* (1989), where processed fluid milk was reported to contain non-detectable levels.

A random survey of milk-powder samples collected during the summer months facilitated certain comparisons to be made between product types while overcoming any influence of seasonal bias. Thus, dry wholemilk shows a lower overall taurine level compared with the equivalent skim-milk product. This may, in part, be rationalised through geographic variation, but a more dominant factor is lipid content, since taurine is not associated with the fat phase. Expression

on a milk-solids–non-fat (MSNF) basis largely corrects this distortion and produces more closely correlated levels (Table 3). Normalisation on a protein basis achieves additional correlative benefits in view of the higher 'intact' protein content in skim- as compared with whole-milk powders despite the absence of taurine as a protein structural component. In addition, samples of commercial whey-derived lactose were found to contain no measurable taurine, further suggesting that expression on a protein basis may more accurately reflect its metabolic origins and assist in the interpretation of nutritional surveys.

Manufacturing procedures seem important in governing the taurine content of various dairy products because it is easily solubilised and can take various pathways during milk processing. Whey-based materials revealed relatively high taurine loadings, particularly on a protein basis, in agreement with reports of Erbersdobler and Trautwein (1984) who demonstrated correlations between the whey protein and taurine contents of infant formulae. Whey protein concentrates showed a lower and more variable taurine content than spray-dried whey. These speciality products should ideally be studied on an individual rather than collective basis because of the diversity of manufacturing procedures used and the wide variety of end-product compositions. Buttermilk powders, often regarded as by-products, also have variable compositions so a wide scatter in taurine data is not surprising.

It remains unconfirmed in this study whether the caseins are poor 'carriers' of taurine relative to whey proteins. Although both rennet and lactic caseins have low and equivalent levels, this may be a reflection of the severity of the processing, where precipitation and extensive washing cycles probably remove associated taurine. Significantly, purified α-lactalbumin, also subject to severe production protocols, contained a negligible taurine contribution, although recent evidence suggests that supplementary processes (electrodialysis, ion-exchange and ultrafiltration) influence the recovery of non-protein nitrogen fractions (Donovan & Lönnerdal, 1989). Demineralisation has also been reported to reduce the taurine composition of whey powders (Hischenhuber, 1988).

Annual trends in taurine content of skim-milk powder, were similar over three consecutive seasons at a single manufacturing site (Fig. 3) with minor variations probably reflecting normal production, sampling and analytical precision. The absence of dietary supplementation and the dominance over individual animal variations by the pooling of large volumes of milk are expected to contribute to this observed repetitive pattern.

A survey of four geographically disparate Australasian anhydrous skim-milk production sites revealed individual differences, superimposed upon a consistent decline in taurine throughout the season (Fig. 4). These differences seem correlated to the severity of thermal processing, since plant A is based on a low-heat process, plants B and C used medium-heat regimes and D a high-heat regime. These observations appear supported by the recent studies of Saidi and Warthesen (1990), who correctly draw attention to the potential reactivity of the primary amine functionality of taurine with milk lactose during exposure to heat under controlled laboratory conditions.

REFERENCES

Ali Qureshi, G., Fohlin, L. & Bergström, J. (1984). Application of high-performance liquid chromatography to the determination of free amino acids in physiological fluids. *J. Chromatogr.*, **297**, 91–100.

Amiss, T. J., Tyczkowska, K. L. & Aucoin, D. F. (1990). Analysis of taurine in feline plasma and whole blood by liquid chromatography with fluorimetric detection and confirmation by thermospray mass spectrometry. *J. Chromatogr. Biomed. Applic.*, **526**, 375–82.

Anderson, G. M., Durkin, T. A., Chakraborty, M. & Cohen, D. J. (1988). Liquid chromatographic determination of taurine in whole blood, plasma and platelets. *J. Chromatogr.*, **431**, 400–5.

Atkinson, S. A., Anderson, G. H. & Bryan, M. H. (1980). Human milk: Comparison of the nitrogen composition in milk from mothers of premature and full-term infants. *Am. J. Clin. Nutr.*, **33**, 811–15.

Barbeau, A. & Huxtable, R. J. (1978) *Taurine and Neurological Disorders.* Raven Press, New York, USA.

Biondi, P. A., Negri, A. & Iopollo, A. (1986). High-performance liquid chromatographic determination of taurine in formulations as the dansyl derivative. *J. Chromatogr.*, **369**, 431–4.

Chang, J., Martin, P., Bernasconi, R. & Braun, D. G. (1981). High sensitivity amino acid analysis. Measurement of amino acid neurotransmitters in mouse brain. *Fed. Eur. Biochem. Soc.*, **132**, 117–20.

Chapman, G. E. & Greenwood, C. E. (1988). Taurine in nutrition and brain development. *Nutr. Res.*, **8**, 955–68.

Deyl, Z., Hyanek, J. & Horakova, M. (1986). Review: Profiling of amino acids in body fluids and tissues by means of liquid chromatography. *J. Chromatogr. Biomed. Applic.*, **379**, 177–250.

Donovan, S. M. & Lönnerdal, B. (1989). Non-protein nitrogen and trace protein in infant formulas. *Acta Paedriatr. Scand.*, **78**, 487–504.

Elkin, R. G. (1984). Measurement of free amino acids in avian blood serum by reverse-phase high-performance liquid chromatography as compared to ion-exchange chromatography. *J. Agric. Food Chem.*, **32**, 53–7.

Erbersdobler, H. F. & Trautwein, E. (1984). Determination of taurine in human milk, cows' milk and some milk products. *Milchwissensch.*, **32**, 722–4.

Erbersdobler, H. F., Greulich, H.-G. & Trautwein, E. (1983). Determination of taurine in foods and feeds using an amino acid analyser. *J. Chromatogr.*, **254**, 332–4.

Ersser, R. S. & Davey, J. F. (1991). Liquid chromatographic analysis of amino acids in physiological fluids: Recent advances. *Med. Lab. Sci.*, **48**, 59–71.

Eslami, M. & Stuart, J. D. (1984). Separation of orthophthalaldehyde/ethanethiol derivatives of taurine and closely eluting amino acids by high performance liquid chromatography. *J. Liquid Chromatogr.*, **7**, 1117–31.

Eslami, M., Stuart, J. D. & Dean, R. W. (1987). Analysis of taurine in blood plasma of epileptic patients using an improved isocratic HPLC method for amino acids. *J. Liquid Chromatogr.*, **10**, 977–95.

Fierabracci, V., Masiello, P., Novelli, M. & Bergamini, E. (1991). Application of amino acid analysis by high-performance liquid chromatography with phenyl isothiocyanate derivatisation to the rapid determination of free amino

acids in biological samples. *J. Chromatogr. Biomed. Applic.*, **570**, 285–91.

Fleury, M. O. & Ashley, D. V. (1983). High-performance liquid chromatographic analysis of amino acids in physiological fluids: On-line precolumn derivatization with *o*-phthaldialdehyde. *Anal. Biochem.*, **133**, 330–5.

Gaull, G. E. (1982*a*). Taurine in the nutrition of the human infant. *Acta Paediatr. Scand.*, **296**, 38–40.

Gaull, G. E. (1982*b*). Taurine nutrition in man. *Adv. Exp. Med. Biol.*, **139**, 89–95.

Geggel, H. S., Ament, M. E., Heckenlively, J., Martin, D. A. & Kopple, J. D. (1985). Nutritional requirements for taurine in patients receiving long-term parenteral nutrition. *New Engl. J. Med.*, **312**, 142–6.

Goodman, H. O. & Shihabi, Z. K. (1987). Automated analysis for taurine in biological fluids and tissues. *Clin. Chem.*, **33**, 835–7.

Gunawan, S., Walton, N. Y. & Treiman, D. M. (1990). High-performance liquid chromatographic determination of selected amino acids in rat brain by precolumn derivatisation with phenylisothiocyanate. *J. Chromatogr.*, **503**, 177–87.

Halfpenny, A. P. & Brown, Ph. R. (1985). Evaluation of conditions for the HPLC determination of plasma amino acids using precolumn derivatisation. *J. High Resolution Chromatogr.*, **5**, 243–7.

Harzer, G., Franzke, V. & Bindels, J. G. (1984). Human milk non-protein nitrogen components: Changing patterns of free amino acids and urea in the course of early lactation. *Am. J. Clin. Nutr.*, **40**, 303–9.

Hayes, K. C. (1988). Taurine nutrition. *Nutr. Res. Revs.*, **1**, 99–113.

Hayes, K. C. & Sturman, J. A. (1981). Taurine in metabolism. *Ann. Rev. Nutr.*, **1**, 401–25.

Haynes, P. A., Sheumack, D., Greig, L. G., Kibby, J. & Redmond, J. W. (1991). Applications of automated amino acid analysis using 9-fluorenylmethyl chloroformate. *J. Chromatogr.*, **588**, 107–14.

Hirai, T., Ohyama, H. & Kido, R. (1987). A direct determination of taurine in perchloric acid–deproteinized biological samples. *Anal. Biochem.*, **163**, 339–42.

Hirschberger, L. L., De La Rosa, J. & Stipanuk, M. H. (1985). Determination of cysteinesulphinate, hypotaurine and taurine in physiological samples by reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, **343**, 303–13.

Hischenhuber, C. (1988). High performance liquid chromatographic and thin-layer chromatographic determination of taurine in infant formulas. *Deutsche Lebensmittel-Rundschau*, **84**, 117–20.

Kataoka, H., Yamamoto, S. & Makita, M. (1984). Quantitative gas–liquid chromatography of taurine. *J. Chromatogr.*, **306**, 61–8.

Laidlaw, S. A., Grosvenor, M. & Kopple, J. D. (1990). The taurine content of common foodstuffs. *J. Parentral Enteral. Nutr.*, **14**, 183–8.

Larsen, B. R., Grosso, D. S. & Chang, S. Y. (1980). A rapid method for taurine quantitation using high performance liquid chromatography. *J. Chromatogr. Sci.*, **18**, 233–6.

Lau, O.-W., Luk, S.-F. & Chiu, T. P. Y. (1990). Spectrophotometric determination of taurine in food samples with phenol and sodium hypochlorite as reagents and an ion-exchange clean-up. *Analyst*, **115**, 653–5.

Marquez, F. J., Quesada, A. R., Sánchez-Jiménez, F. & Núñez De Castro, I. (1986). Determination of 27 dansyl amino acid derivatives in biological fluids by reversed-phase high-performance liquid chromatography. *J. Chromatogr. Biomed. Applic.*, **380**, 275–83.

Murai, S., Sato, H., Masuda, Y. & Itoh, T. (1990). Very rapid and simple assay of taurine in the brain within 2 minutes by HPLC with electrochemical detection. *J. Pharmacological Methods*, **23**, 195–202.

Nicolas, E. C., Pfender, K. A., Aoun, M. A. & Hemmer, J. E. (1990). Determination of taurine in infant formulas using ultrafiltration and cation-exchange chromatography. *J. Assoc. Off. Anal. Chem.*, **73**, 627–31.

Nishikawa, I., Yoshida H., Ahiko, K. & Ueda, A. (1984). Free amino acid concentrations of human and cow's milk and effect of taurine supplementation to infant milk formulas on serum taurine levels of infants. *J. Jap. Soc. Nutr. Food Sci.*, **37**, 301–9.

Paauw, J. & Davis, A. T. (1990). Taurine concentrations in serum of critically injured patients and age- and sex-matched healthy control subjects. *Am. J. Clin. Nutr.*, **52**, 657–60.

Palmerini, C. A., Fini, C., Cantelmi, M. G. & Floridi, A. (1987). Assessment of taurine in plasma and urine by anion-exchange high-performance liquid chromatography with pre-column derivatisation. *J. Chromatogr.*, **423**, 292–6.

Pamblanco, M., Portoles, M., Paredes, C., Ten, A. & Comín, J. (1989). Free amino acids in pre-term and term milk from mothers delivering appropriate or small for gestational age infants. *Am. J. Clin. Nutr.*, **50**, 778–81.

Pasantes-Morales, H. & Flores, R. (1991). Taurine content of Mexican beans. *J. Food Comp. Anal.*, **4**, 322–8.

Pasantes-Morales, H., Quesada, O., Alcocer, L. & Sánchez Olea, R. (1989). Taurine content in foods. *Nutr. Rep. Int.*, **40**, 793–801.

Porter, D. W., Banks, M. A., Castranova, V. & Martin, W. G. (1988). Reversed-phase high performance liquid chromatographic technique for taurine quantitation. *J. Chromatogr.*, **454**, 311–16.

Rajendra, W. (1987). High performance liquid chromatographic determination of amino acids in biological samples by precolumn derivatisation with *o*-phthaldialdehyde. *J. Liquid Chromatogr.*, **10**, 941–55.

Rana, S. K. & Sanders, T. A. B. (1986). Taurine concentrations in the diet, plasma, urine and breast milk of vegans compared with omnivores. *Brit. J. Nutr.*, **56**, 17–27.

Rassin, D. K., Sturman, J. A. & Gaull, G. E. (1978). Taurine and other free amino acids in milk of man and other mammals. *Early Hum. Dev.*, **2**, 1–13.

Rassin, D. K., Gaull, G. E., Järvenpaa, A.-L. & Räihä, N. (1983). Feeding the low-birth-weight infant: II. Effects of taurine and cholesterol supplementation on amino acids and cholesterol. *Prediatrics*, **71**, 179–86.

Saidi, B. & Warthesen, J. J. (1990). Analysis and heat stability of taurine in milk. *J. Dairy Sci.*, **73**, 1700–6.

Sakai, T. & Nagasawa, T. (1992). Simple, rapid and sensitive determination of plasma taurine by high-performance liquid chromatography using pre-column derivative formation with fluorescamine. *J. Chromatogr.*, **576**, 155–7.

Saller, C. F. & Czupryna, M. J. (1989). γ-Amino butyric acid, glutamate, glycine and taurine analysis using reversed-phase HPLC and ultraviolet detection of dansyl chloride derivatives. *J. Chromatogr. Biomed. Applic.*, **487**, 167–72.

Sherwood, R. A., Titheradge, A. C. & Richards, D. A. (1990). Measurement of plasma and urine amino acids by high-performance liquid chromatography with electrochemical detection using phenylisothiocyanate derivatization. *J. Chromatogr. Biomed. Applic.*, **528**, 293–303.

Shubat, P. J., Parker, D. & Huxtable, R. J. (1989). Effect of suckling and diurnal influences on the concentrations of taurine and other free amino acids in milk. *Eur. J. Clin. Nutr.*, **43**, 675–80.

Sturman, J. A., Rassin, D. K. & Gaull, G. E. (1977). Taurine in development. *Life Sci.*, **21**, 1–22.

Subba Rao, G. N. (1987). Liquid chromatographic determination of taurine in vitamin premix formulations. *J. Assoc. Off. Anal. Chem.*, **70**, 799–801.

Taha, N. M. & Kielwein, G. (1990). Pattern of peptide-bound and free amino acids in camel, buffalo and ass milk. *Milchwissensch.*, **45**, 22–5.

Tapuhi, Y., Schmidt, D. E., Lindner, W. & Karger, B. L.

(1981). Dansylation of amino acids for HPLC analysis. *Anal. Biochem.*, **115**, 123–9.

Trautwein, E. A. & Hayes, K. C. (1990) Taurine concentrations in plasma and whole blood in humans: Estimation of error from intra- and interindividual variation and sampling technique. *Am. J. Clin. Nutr.*, **52**, 758–64.

Uhe, A. M., Collier, G. R., McClennan, E. A., Tucker, D. J. & O'Dea, K. (1991). Quantitation of tryptophan and other plasma amino acids by automated pre-column *o*-phthalaldehyde derivatisation high-performance liquid chromatography: Improved sample preparation. *J. Chromatogr. Biomed. Applic.*, **564**, 81–91.

Watanabe, Y. & Imai, K. (1984). Sensitive detection of amino acids in human serum and dried blood disc of 3 mm diameter for diagnosis of inborn errors of metabolism. *J. Chromatogr. Biomed. Applic.*, **309**, 279–86.

Yokoyama, T. & Kinoshita, T. (1991). High-performance liquid chromatographic determination of taurine in biological fluids by post-column fluorescence reaction with thiamine. *J. Chromatogr.*, **568**, 212–18.