Analysis and Heat Stability of Taurine in Milk[1]

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ABSTRACT

A method based on formation of the fluorescamine derivative of taurine and HPLC was developed for analysis of taurine in milk. Taurine in milk ranged from 2.4 to 12.0 mg/L. The degradation of taurine in taurine-fortified milk and in a buffered taurine and lactose solution (pH 6.7) was determined by heating at 80, 100, and 120°C. First-order reaction kinetics were observed for taurine losses in milk and buffered solution. Activation energies were 20.5 and 21.0 kcal/mol for milk and buffered solution, respectively. The taurine loss in milk seems to proceed through browning with the same degradation rate as lysine.
(Key words: taurine, heat stability, analysis)

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is an end product of sulfur amino acid metabolism found in low levels in cow's milk. Considerable evidence exists for the importance of taurine during growth and development of humans and other mammals (9, 34). The possible importance of taurine to neurotransmission, retinal function, cardiac function, muscle function, and epilepsy has been reviewed (12, 13, 15, 28). In the newborn of many species, including humans, taurine synthesis is limited and seems to be a dietary essential for the very young infant (9, 14). The need for taurine supplementation in baby foods has been suggested by Martensson and Finnstrom (21) and Subba Rao (35), who reported that taurine is added to many vitamin premix formulations used in infant nutritional preparations to provide the same measure of nutrition that human milk provides.

Normal cow's milk, from which most formulas are prepared, contains only small amounts of taurine (1, 29), and little is known about the heat stability of taurine in milk. Because it is a primary amine, taurine might react with the reducing sugar lactose during the heating or storage of milk. Erbersdobler et al. (7) reported a range of 3 to 14 mg/L and 18 to 129 mg/L for taurine contents in cow's milk and human milk, respectively, whereas Matsuyama et al. (23) found 1.6 and 46.9 mg/L as average values in cow's milk and human milk, respectively.

Various methods have been reported for the estimation of taurine (6, 8, 19, 20, 24, 25, 26, 31, 33, 35). Some of these methods lack sensitivity or are time consuming and, therefore, may not be suitable for routine determination of taurine (3). Application of HPLC for the quantification of taurine has been limited and most reports have been restricted to using ion-exchange chromatography as reported by Larsen et al. (19).

The objectives of this research were to develop an HPLC method for the quantification of taurine in milk, to investigate its heat stability in milk, and to determine the reactivity of taurine with lactose in buffered solution.

MATERIALS AND METHODS

Milk Samples

The milk samples tested in this study were from the US and Morocco. Taurine in skim

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milk was determined from four retail skim milk samples obtained in St. Paul, Minnesota, and two skim milk samples from the University of Minnesota herd. Taurine in Moroccan milk was also determined using eight retail whole milk samples obtained in Rabat. Samples were analyzed in duplicate.

Sample Treatments

To determine the effect of temperature on taurine stability in milk, a taurine-fortified milk was prepared by adding 20 or 30 mg of taurine (Sigma Chemical Co., St. Louis, MO) to 1 L of skim milk purchased at a retail store. The milk was fortified to give a level of taurine high enough to measure its degradation accurately. The sample was warmed and mixed to dissolve taurine. Fortified samples (5-ml portions) were placed in Pyrex tubes (16 × 125 mm) with Teflon-lined screw caps. The tubes were held in a covered water bath at either 80 or 100°C. Duplicate samples were removed every 10 h over a 50-h period for 80°C and every 4 h over a 16-h period for 100°C. For 120°C, milk samples were placed in glass test tubes that were sealed with a torch and heated in an oil bath. Duplicate samples were removed every 30 min over a 2-h period.

To test the effect of temperature on the extent of browning of taurine, a solution of taurine at a concentration of 10 mg/L and lactose at a concentration of 40 g/L was prepared at pH 6.7 using a phosphate buffer system (.2 *M*) as reported by Gomori (11). Samples of 5 ml of buffered taurine-lactose solution were heated at the same temperatures and in the same manner as the milk samples. Duplicate samples were removed every 2 h over a 12-h period and every hour over a 6-h period for 80 and 100°C, respectively. At 120°C, duplicate samples were removed after 10, 20, 30, and 45 min.

Preparation of Milk Samples for Analysis

Milk samples of 5 ml were transferred into test tubes. One milliliter of TCA (12% wt/vol) was added, and the samples were mixed using a mixer and centrifuged at 800 × *g* for 20 min. The supernatants were decanted into 2-dr vials and used in the fluorescamine reaction. Recovery studies were done in which known amounts (5 and 10 mg/L) of taurine were added to skim milk. Taurine recoveries averaged 96% with a standard deviation of 4.8% when three samples for each concentration were used.

Reaction with Fluorescamine

The derivatization procedure was based on the method used by Datta and Naryanaswami (3) and Udenfriend et al. (36). Milk sample supernatant (200 µl), buffered taurine-lactose (200 µl), or taurine standard (200 µl) were placed in a 2-dr vial with 2.0 ml of .2 *M* borate buffer (pH 9.0). Fluorescamine (200 µl; Sigma Chemical Co.) solution in acetone (3 mg/ml) was added, and the vial contents were mixed. The reaction with fluorescamine is rapid and was completed after gentle mixing. The sample was filtered through a .45-µm membrane filter, and 20 µl were used for chromatographic analysis.

High Performance Liquid Chromatography

The chromatograph equipment was a Model 501 pump (Waters Associates, Milford, MA), a Model 7120 injector with a 20- µl sample loop (Rheodyne, Berkeley, CA), a Fluorichrom fluorescence detector (Varian, Walnut Creek, CA), and an SP 4270 recorder integrator (Spectra Physics, Piscataway, NJ). The excitation filter was a 400-nm interference filter, and the emission was through a 3-70-cutoff filter and 4-76-band filter. The fluorescent taurine derivative was separated using an end-capped octyl column (250 × 4.5 mm i.d.) with 5-µm particle size (IBM Instruments, Inc., Wallingford, CT) and a mobile phase of 70% .05 *M* phosphate buffer (pH 2.1) and 30% acetonitrile at a flow of 1.0 ml/min. A second mobile phase with the same composition, but to which was added tetrabutylammonium phosphate (.005 *M*) was used for further identification of taurine. This provided an additional mobile phase based on ion-pairing chromatography for the separation of the fluorescamine derivative of taurine. Taurine was quantified by comparing the peak heights of the samples to the peak heights of standard taurine solutions. The standards used were 1.6, 3.2, 6.4, 12.8, and 16.0 mg/L. The response of the fluorescence detector was linear over the range of concentrations used.

Color Measurement

The optical density of taurine-lactose buffered solutions was measured at 450 nm (27, 32) on a Cecil CE 303 spectrophotometer.

Data Treatment

Linear regression with transformation of the dependent variable (taurine retention) was used to determine reaction order and rate constants for heat degradation of taurine in milk and buffered solution. Linear and semilogarithmic relationships were compared to determine if the reaction order was zero or first order.

RESULTS AND DISCUSSION

Taurine in Milk

A reverse-phase HPLC method utilizing fluorescamine for derivatization was developed to measure taurine in milk. A typical chromatogram of the taurine derivative in milk using a mobile phase of 30% acetonitrile and 70% phosphate buffer (pH 2.1) is given in Figure 1. The identity of taurine was confirmed by co-chromatography with standards, the addition of standard solutions to the milk samples, and by using a second mobile phase to which the ion-pairing tetrabutylammonium phosphate was added. A typical chromatogram showing taurine derivative at 21 min when using the ion-pairing mobile phase is given in Figure 2. The identity of the other peaks is not known except in the case of ammonia, which gave a retention time of about 16 min with both mobile phases.

Taurine concentrations in the US milk samples ranged from 2.4 to 12.0 mg/L with an average concentration of 6.1 mg/L and a standard deviation of 4.4 mg/L. Taurine in Moroccan milk ranged from 3.0 to 6.5 mg/L with an average concentration of 4.6 mg/L and a standard deviation of 1.2 mg/L. The coefficient of variation due to variations in the analytical procedure was between 1.2 and 5.7% with an average of 3%.

The taurine concentrations in USA and Moroccan milk were not statistically different at the 5% level. The samples showed variation, but the levels found are in agreement with those reported by several authors. Erbersdobler and Trautwein (5) and Erbersdobler et al. (7)

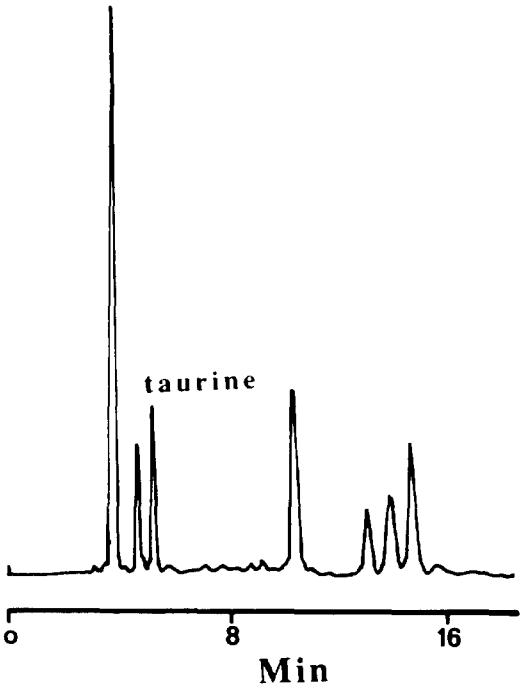

Figure 1. Chromatogram of taurine in milk extract. The taurine derivative elutes at 5.5 min.

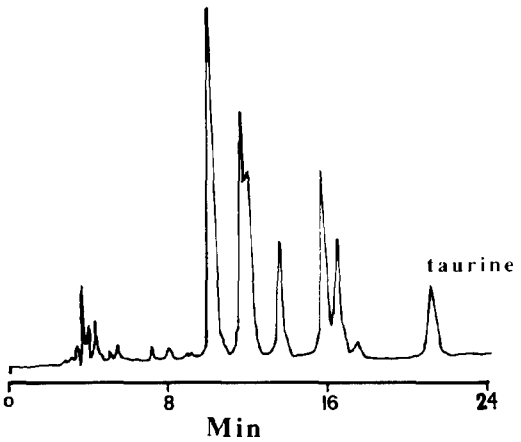

Figure 2. Chromatogram of taurine in milk extract with an ion-pairing mobile phase. The taurine derivative elutes at 21 min.

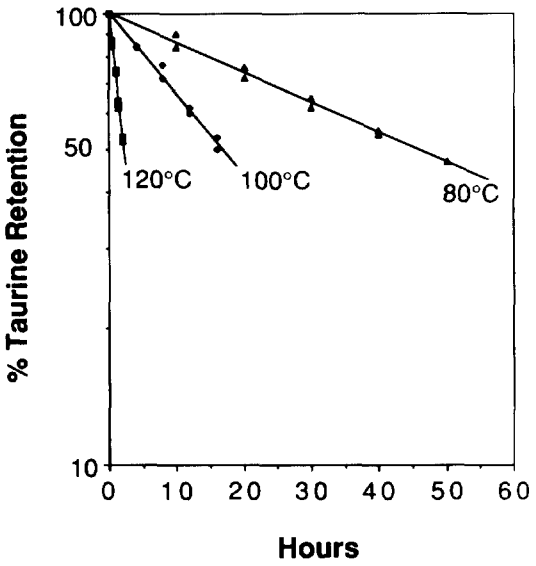

Figure 3. Taurine retention in milk heated at 80, 100, and 120°C.

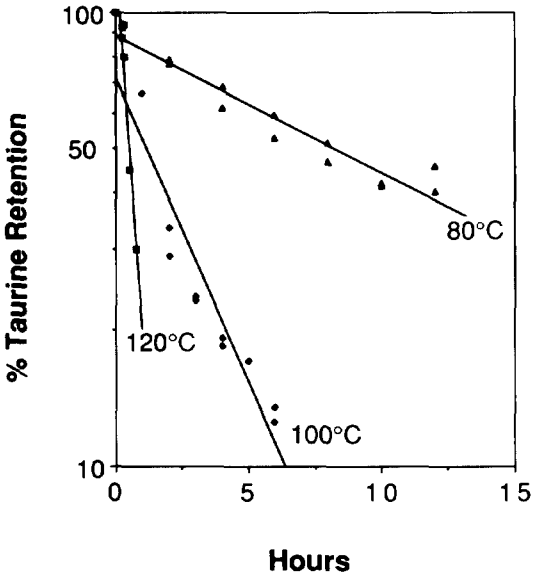

Figure 4. Taurine retention in buffered lactose solution (pH 6.7) heated at 80, 100, and 120°C.

reported taurine content ranges of 3 to 14 mg/L and 4 to 11 mg/L, respectively. Ghadimi and Pecora (10) found 28.5 and 7.1 mg/L in colostrum and mature milk, respectively. Roe and Weston (30) reported 151 mg/L as an average taurine value, but this level seems high relative to other literature values. Physiological variation of the taurine content in the various milk samples may explain some variability, since Ghadimi and Pecora (10) and Erbersdobler and Trautwein (5) reported, respectively, that the cow's colostrum contained 88 and 84 mg/L of taurine.

Heat Stability of Taurine

Taurine-fortified milk and a buffered solution containing taurine and lactose were used to determine heat effects of taurine. The milk was fortified to give a concentration of taurine high enough to measure its degradation beyond at least 50% loss in order to accurately establish the proper reaction order and rate constants. The added taurine could be expected to behave in the same way as the natural taurine, since this compound exists naturally as a free amino acid in milk and animal tissues.

Data for taurine retention in milk system and a buffered solution at 80, 100, and 120°C are presented in Figures 3 and 4. Lines were determined using linear regression based on first order kinetics. The taurine degradation was better described (higher r^2) by semilogarithmic relationship rather than linear relationship. The r^2 for milk at 80, 100, and 120 °C were respectively .95, .96, and .98 for semilogarithmic relationships and .94, .96, and .96 for linear relationships. The r^2 for buffered solution at 80, 100, and 120°C were respectively .92, .91, and .98 for semilogarithmic relationships and .87, .87, and .92 for linear relationships. Regression lines in Figure 3 were calculated using 10, 8, and 8 data points for 80, 100, and 120°C, respectively, whereas regression lines in Figure 4 were calculated using 12, 12, and 8 data points for 80, 100, and 120°C, respectively. Each sampling time has two data points.

Calculated first-order rate constants and r^2 for taurine retention in milk and in solution are reported in Table 1. The calculated rate constants show that taurine degradation is about five times faster in buffered solution than in milk. This may be due to the protective effect

TABLE 1. First-order rate constants (k) and r^2 values for taurine retention in milk and in buffered solution at 80, 100, and 120°C.

	T°C	k (h)	SE[1]	r^2
Milk	80	.015	.001	.95
	100	.042	.002	.96
	120	.320	.009	.98
Buffered solution	80	.069	.006	.92
	100	.308	.032	.91
	120	1.658	.088	.98

[1]Standard error for calculated k.

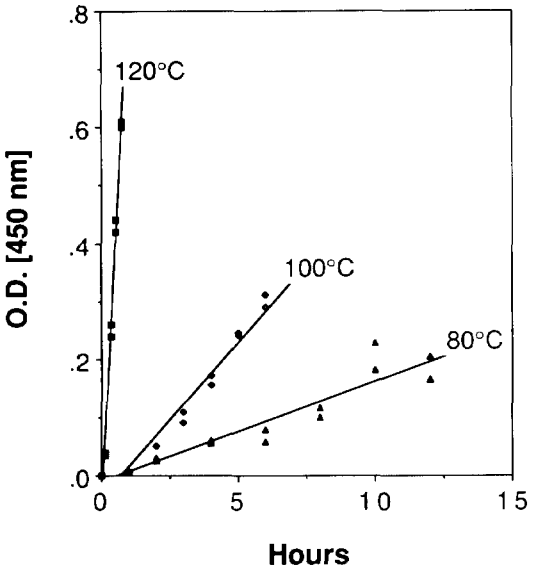

Figure 5. Optical density of taurine/lactose buffered solution heated at 80, 100, and 120°C.

of milk solids and the competing reactions of other amino compounds with the lactose in milk. These results suggest that taurine degradation in milk is limited under practical milk heat processing conditions. Sterilization at 120°C for 20 min caused on a 10% loss of taurine. This loss is similar to the loss of lysine in milk as reported by Burton (2). He reported a range for lysine loss of 3.3 to 11% under sterilization conditions similar to the ones used here.

The activation energies, E_a, obtained from the slopes of Arrhenius plots were quite similar for the two different heated systems. The value was 20.5 kcal/mol (r^2 = 0.98) for fortified milk and 21.0 kcal/mol (r^2 = 0.95) for buffered taurine and lactose solution. The calculated Q_{10} values at 100 to 110°C were 2.06 and 2.09, respectively, for milk and buffered solution.

Activation energies or Q_{10} have not been previously reported for taurine degradation, but those found in this experiment can be compared with the data found in studies on heat degradation of the lysine contained in milk proteins. Both have primary amino groups available for reaction. Mottar (22) studied the kinetics of lysine loss in milk over the temperature range 130 to 150°C in a pilot-scale plate heat exchanger. He found a Q_{10} of 2.14, which is close to the values obtained in this experiment. Burton (2) using the data of Horak and Kessler reported a Q_{10} of 2.1 for lysine loss. The E_a derived in present work is in the reported range for lysine degradation in whey powder (17). Because the values for E_a and Q_{10} are close to the values obtained in studies outlined before, they suggest that taurine and lysine must acquire about the same energy before they have the possibility of reacting. This also implies that the heat degradation of both amino acids proceeds with a similar mechanism. However, Kessler (16) reported an energy of activation of 26 kcal/mol for lysine in milk heated between 100 and 160°C. This value suggests that lysine acquires more energy than taurine before it undergoes degradation.

Studies have not been done to explain the losses of taurine in food during heat treatments, but nonenzymatic browning seems to be the main reaction for taurine loss. This is based on taurine structure and the similarity between lysine and taurine. The study of taurine loss through browning could not be measured in milk because of the presence of other amino compounds that also can undergo the Maillard reaction. Therefore, to evaluate the extent of taurine contribution to browning, a solution with a pH and lactose concentration similar to milk was used.

Data for optical density measured at 450 nm of taurine and lactose buffered solutions heated at 80, 100, and 120°C are presented in Figure 5. Lines were determined using linear regression based on zero-order kinetics. The formation of brown pigments was better described (higher r^2) by a linear relationship rather than a semilogarithmic relationship. The r^2 values at 80,

TABLE 2. Zero-order rate constants (k) and r^2 values for optical density (OD) increase in buffered solution at 80, 100 and 120°C.

Temperature (°C)	k OD/h	SE[1]	r^2
80	.017	.002	.89
100	.053	.003	.96
120	.901	.058	.97

[1]Standard error for calculated k.

100, and 120°C were, respectively, .89, .96, and .97 for linear relationships and .87, .84, and .79 for semilogarithmic relationships. Regression lines were calculated using 12 data points at 80 and 100°C and 8 data points at 120°C. Calculated zero-order rate constants and r^2 for the optical density increase in solution are reported in Table 2. Zero-order reaction kinetics for color formation during browning of buffered solutions of glucose and lysine have been reported by Petriella et al. (27). Labuza and Saltmarch (17) found that brown pigment formation (as obtained from measurement of optical density) in stored whey powder with a_w in the range .33 to .65 also followed a zero-order reaction kinetics.

The E_a, obtained from the slopes of Arrhenius plots for brown color development, was 27 kcal/mol (r^2 = .99). The calculated Q_{10} was 2.6 at 100 to 110°C. The E_a compared well with the values reported in other studies. Horak and Kessler found an E_a of 26 kcal/mol for milk browning over a temperature range of 25 to 150°C as reported by Burton (2). Labuza and Saltmarch (18) found an E_a of 27 kcal/mol over a temperature range of 93 to 121°C for goat milk browning. De Kanterewicz and Chirife (4) obtained an E_a of 26 kcal/mol for the browning of concentrated whey. Petriella et al. (27) calculated activation energies for color development in buffered glucose and lysine solutions in the range 23.5 to 28.3 kcal/mol.

The Q_{10} found for browning in this study also compared well with the values reported by Burton (2). He found a Q_{10} of 2.95 and calculated from the data of Horak and Kessler a Q_{10} of 2.41 for milk browning over a temperature range of 100 to 150°C. Because the browning values found for E_a or Q_{10} were close to the values reported by studies outlined previously, taurine degradation and subsequent color formation likely occur through the Maillard reaction.

When the combined results for taurine loss and browning in milk and in solution were compared with literature studies on lysine loss and browning, taurine degradation seems to occur in a way similar to lysine degradation. This degradation apparently proceeds at a similar rate of loss as lysine under the same conditions.

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