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Effect of pasteurisation on ascorbic acid, dehydroascorbic acid, tocopherols and fatty acids in pooled mature human milk

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Abstract

Ascorbic and dehydroascorbic acids (vitamin C), tocopherols (vitamin E) and unsaturated fatty acids are heat-sensitive and therefore, their concentrations in human milk could be affected by pasteurisation. Here we determined the concentrations of ascorbic acid plus dehydroascorbic acid, ascorbic acid alone, and α- and γ-tocopherols, and the percentages of fatty acids in samples of human milk after pasteurisation by a slow (62.5 °C, 30 min) or fast heating (100 °C, 5 min) procedure. Both methods led to a significant decrease in the concentrations of ascorbic acid plus dehydroascorbic acid (12% and 29%), ascorbic acid (26% and 41%), α-tocopherol (17% and 34%) and γ-tocopherol (13% and 32%), respectively. However, milk fatty acids, including the polyunsaturated long-chain fatty acids, were unaffected by the two methods. On the basis of these observations, we recommend that human milk be treated using a slow pasteurisation. In addition, we propose ascorbic acid as a marker of the degree of heat treatment.

Keywords: Ascorbic acid; Fatty acid; Pasteurisation; Tocopherol

1. Introduction

Breast milk is an ideal nutrient for term and pre-term infants up to 6 months of age (The Committee on Nutrition of the American Academy of Pediatrics, 1978). It improves host defences, digestion and absorption of nutrients, gastrointestinal function, and neurodevelopment (Schanler, Hurst, & Lau, 1999).

In the absence of an adequate supply of breast milk, mothers are offered term breast milk from human milk banks, which collect, process, and store milk from healthy lactating women. Banked human milk is an alternative for the care and treatment of premature and low-birth-weight neonates, and sick newborns and infants with severe infectious disease, immunodeficiency, serious intestinal illness, intractable diarrhoea, and heterologue protein intolerance (Góes, Torres, Donangelo, & Trugo, 2002).

To avoid the transmission of infectious microorganisms, donor milk must be pasteurised before it is given to infants. Several pasteurisation methods have been developed for this purpose (Henderson, Fay, & Hamosh, 1998; Israel-Ballard et al., 2005; Lepri, Del Bubba, Maggini, Donzelli, & Galvan, 1997; Resto et al., 2001). Pasteurisation, most often by the Holder Technique (62.5 °C, 30 min), following the procedure of the Human Milk Banking Association of North America (HMBANA; http://www.hmbana.org, 2005), results in the loss of variable amounts of milk IgA, IgM, IgG, lactoferrin, several vitamins, and other components. Fast-heat pasteurisation (100 °C, 5 min) is an alternative method currently used in the United States to treat breast milk (Resto et al., 2001).

Ascorbic acid and tocopherols are crucial for antioxidant activity and immunomodulation (Jensen, 1995). Many of the disorders common to pre-term infants are

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thought to be due to an imbalance between pro- and antioxidants, in favour of the former (Hanna et al., 2004).

Long-chain polyunsaturated fatty acids (LC-PUFAs), specifically arachidonic acid (C20:4 n-6, AA) and docosahexaenoic acid (C22:6 n-3, DHA), are critical during the perinatal period, in which the brain and retina are developing, and affect visual acuity and learning capacity (Innis, 2004). Total fat content and fatty acids in human milk are unaltered by pasteurisation at 62.5 °C for 30 min (Fidler, Sauerwald, Demmelmair, & Koletzko, 2001; Henderson et al., 1998; Lepri et al., 1997).

Ascorbic acid and tocopherols are sensitive to light, oxygen and temperature (Miquel, Alegrıa, Barberá, & Farré, 2004); therefore their concentrations could be affected by pasteurisation (Brátová & Vávra, 1981; Jandal, 1996; Trifonova et al., 1981). However, few studies, performed many years ago, have addressed the stability of ascorbic acid and tocopherols in human milk (Brátová & Vávra, 1981; Erb, Brice, Lhopitault, & Assy Seka, 1981; Randoin & Perroteau, 1950; Trifonova et al., 1981; Zoeren-Grobben, Schrijver, Van den Berg, & Berger, 1987) in response to pasteurisation. Here we assessed the effect of pasteurisation using a slow-heat (62.5 °C, 30 min) and a fast-heat (100 °C, 5 min) method on the composition of ascorbic acid plus dehydroascorbic acid, ascorbic acid alone, α- and γ-tocopherols, and fatty acids in human milk.

2. Materials and methods

2.1. Sample Collection

Identical volumes (60 ml) of mature human milk samples were collected from both breasts by means of a Chicco manual breast pump (Chicco®, Italy), following the manufacturer's instructions, from 10 healthy mothers aged 20–35 years, at the extraction unit of the Department of Nutrition (Facultad de Farmacia, Universidad de Barcelona). Informed consent was obtained from the participating mothers. Human milk was collected in sterile opaque bottles during the first expression of the morning. It was then immediately pooled and divided into six groups, with 10 aliquots in each group, to be processed as follows: unpasteurised (two groups of 10 aliquots), slow-heat method (two groups of 10 aliquots), and fast-heat method (two groups of 10 aliquots). Unpasteurised aliquots were frozen immediately at −80 °C (maximum 1 month) and thawed to around 22 °C in a water bath protected from light at the time of processing.

2.2. Pasteurisation methods

We evaluated two pasteurisation methods, slow-heat and fast-heat, which are currently used to treat human milk in the United States. Pasteurisation treatments were done in duplicate (10 aliquots in each treatment). For the slow-heat procedure, pasteurisation was performed using the Holder technique, following the HMBANA protocol (2005), by submerging the glass tubes in a shaking water bath maintained at a temperature of 62.5 °C for 30 min. For the fast-heat method (Resto et al., 2001), glass tubes were submerged with agitation for 5 min at 100 °C in the same water bath. Once pasteurised, samples were submerged immediately at −20 °C for 10 min in an ice slurry and then frozen at −80 °C (maximum 1month) until analysed. To examine milk compounds, the aliquots were thawed to around 22 °C in a water bath protected from light, and then mixed.

2.3. Determination of ascorbic and dehydroascorbic acids

The concentrations of ascorbic plus dehydroascorbic acids and of ascorbic acid alone were measured following the direct method described by Romeu-Nadal, Morera-Pons, Castellote, and López-Sabater (2006a). For this purpose, dithiothreitol was used to reduce dehydroascorbic acid to ascorbic acid. The latter was resolved by reversed-phase high-performance liquid chromatography using a mobile phase of Milli-Q water with acetic acid (0.1% v/v) and methanol in a relative proportion of 95:5 v/v. The analytical column used was a Tracer Spherisorb ODS2 C_{18} (250 × 4.6 mm ID, 5 μm particle size) protected with a guard column (Tracer, C_{18}, 5 μm), both from Tracer Analitica (Tecknokroma, Barcelona, Spain). An UV–Vis detector, SPD-10 AV VP (Shimadzu, Kyoto, Japan) and an HP-3365 Series II Chemstation were used. Detection was performed at 254 nm.

2.4. Tocopherol determination

α-Tocopherol and γ-tocopherol were separated and quantified with reversed-phase high-performance liquid chromatography using a mobile phase of acetonitrile: methanol: dichloromethane (60:38:2 v/v) following the direct method described by Romeu-Nadal, Morera-Pons, Castellote, and López-Sabater (2006b). Human milk, with the addition of an internal standard (α-tocopherol acetate), was diluted in hexane. The dried sample was reconstituted in a dichloromethane:acetonitrile (3:1) solution. The analytical column was a Pinnacle II C_{18}(50 × 2.1 mm ID, 3 μm particle size) protected by a guard column (C_{18}, 1 cm) from Restek (Bellefonte, PA). An UV–Vis detector, SPD-10 AV VP Shimadzu (Kyoto, Japan) and an HP-3365 Series II Chemstation were used. Detection was performed at 292 nm.

2.5. Fatty acid determination

Fatty acid methyl esters (FAMEs) were prepared with sodium methylate and methanolic boron trifluoride (BF_3) and dissolved in hexane, following López-López, Castellote, and López-Sabater (2001). Fatty acids were separated and quantified with fast gas chromatography.

Fast gas chromatographic analyses were performed on a Shimadzu GC-2010 gas chromatograph (Kyoto, Japan)

equipped with a flame ionisation detector and a Shimadzu AOC-20i Autoinjector. FAMEs were separated on a capillary column (10 m × 0.10 mm ID) coated with a Varian VF-23ms stationary phase (high cyanopropyl phase, 0.10 μm film thickness) from Varian (Palo Alto, USA).

The split–splitless injector was used in split mode with a split ratio of 100:1. The injection volume of the sample was 1 μl. The injector and detector temperatures were kept at 250 °C and 270 °C, respectively. The initial oven temperature was 120 °C, increased at 35 °C/min until 175 °C (kept 0.5 min), increased at 20 °C/min until 250 °C. Helium was used as the carrier gas, with a linear velocity of 59.4 cm/s (at 120 °C) at a pressure of 482 kPa; detector gas flows were H_2 at 50 ml/min, air at 400 ml/min and nitrogen make-up gas at 50 ml/min. Sampling frequency was 50 Hz. Data acquisition and processing were performed with Shimadzu Chemstation software for gas chromatographic systems.

2.6. Statistical analysis

Data are presented as means ±SD. Tukey's test were performed for multiple comparisons between groups. SPSS 12.0 (SPSS, Chicago, IL) statistical software was used.

3. Results

To examine the effect of pasteurisation on the ascorbic and dehydroascorbic acids and fatty acid composition of human milk, these compounds were analysed before and after heat treatments. When reporting total vitamin C levels we measured both ascorbic acid and dehydroascorbic acid. The former is the main biologically active form but the latter, an oxidation product, also exhibits biological activity, since it can be easily converted into ascorbic acid in the human body (Packer et al., 1997).

Pasteurisation by the slow-heat and fast-heat methods resulted in a significant decrease ($P < 0.05$) in the sum of ascorbic acid and dehydroascorbic acid by about 12% and 29%, respectively (Table 1). Losses were higher for ascorbic acid alone, about 26% and 41%, respectively.

Both heat treatments significantly decreased the concentrations of α-tocopherol and γ-tocopherol (Table 1). Loss of tocopherols caused by the slow-heat method ranged from 13% to 17%, and for the fast-heat procedure from 32% to 34%.

Table 2
Effect of the slow-heat and fast-heat methods on fatty acids (%) in human milk[a]

Fatty acid	Unpasteurized	Slow-heat	Fast-heat
C8:0	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
C10:0	0.95 ± 0.06	0.94 ± 0.05	0.96 ± 0.05
C12:0	5.08 ± 0.16	4.94 ± 0.29	4.84 ± 0.26
C14:0	5.60 ± 0.30	5.44 ± 0.16	5.49 ± 0.19
C14:1	0.09 ± 0.00	0.09 ± 0.01	0.09 ± 0.01
C15:0	0.15 ± 0.01	0.15 ± 0.00	0.15 ± 0.00
C15:1	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C16:0	19.8 ± 0.32	19.4 ± 0.31	19.5 ± 0.18
C16:1 n-7 and n-9	1.50 ± 0.03	1.49 ± 0.04	1.50 ± 0.03
C17:0	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01
C17:1	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
C18:0	7.19 ± 0.25	7.05 ± 0.14	7.16 ± 0.15
C18:1 n-9	38.1 ± 0.78	38.6 ± 0.30	38.4 ± 0.56
C18:2 n-6	18.0 ± 0.21	18.3 ± 0.43	18.1 ± 0.11
C18:3 n-6	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
C18:3 n-3	0.64 ± 0.02	0.65 ± 0.02	0.64 ± 0.01
C20:0	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
C20:1 n-9	0.32 ± 0.02	0.31 ± 0.01	0.31 ± 0.02
C21:0	0.05 ± 0.00	0.03 ± 0.00	0.06 ± 0.00
C20:2 n-6	0.25 ± 0.02	0.24 ± 0.01	0.25 ± 0.01
C20:3 n-6	0.32 ± 0.02	0.33 ± 0.02	0.33 ± 0.02
C20:4 n-6	0.47 ± 0.02	0.47 ± 0.03	0.47 ± 0.01
C22:0	0.07 ± 0.01	0.07 ± 0.00	0.08 ± 0.01
C22:1	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
C22:2	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
C22:4 n-6	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
C22:5 n-6	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C24:1	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C22:5 n-3	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
C22:6 n-3	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01

[a] Mean of twenty measurements ± standard deviation.

The losses of ascorbic acid and tocopherols in the fast-heat treatment were greater than in the slow-heat treatment. Significant differences in both ascorbic acid and tocopherol were found between the two pasteurisation methods (Table 1).

No substantial differences in the fatty acid composition of unpasteurised and pasteurised milk were detected (Table 2).

4. Discussion

Few studies have addressed the effects of pasteurization on ascorbic acid and tocopherols in human milk. We found that, like ascorbic acid, tocopherol concentrations

Table 1
Effect of the slow-heat and fast-heat methods on the content (mg/l) of ascorbic and dehydroascorbic acids and α- and γ-tocopherols in human milk[a]

Treatment	Ascorbic and dehydroascorbic acids	Ascorbic acid	α-Tocopherol	γ-Tocopherol
Unpasteurized	35.5 ± 1.33	34.2 ± 0.89	4.41 ± 0.16	0.47 ± 0.02
Slow-heat method	31.0 ± 1.41[b]	25.5 ± 0.69[b]	3.67 ± 0.13[b]	0.41 ± 0.02[b]
Fast-heat method	25.0 ± 1.14[b,c]	20.1 ± 0.59[b,c]	2.91 ± 0.08[b,c]	0.32 ± 0.01[b,c]

[a] Mean of 20 measurements ± standard deviation.
[b] Significant difference from unpasteurised samples ($P < 0.05$).
[c] Significant difference between slow- and fast-heat method ($P < 0.05$).

decreased after slow-heat and fast-heat pasteurisation procedures.

The loss of ascorbic acid in human milk caused by heat treatment may result from an increase in the conversion rate of ascorbic to dehydroascorbic acid and then to diketogulonic acid (Naidu, 2003). The total mean ascorbic acid concentration detected in our fresh samples was similar with that reported by Buss, McGill, Darlow, and Winterbourn (2001). The losses of ascorbic acid induced by the slow-heat method (26%) are also consistent with the results of studies that used a similar treatment on human milk (Brátová & Vávra, 1981; Trifonova et al., 1981). However, the decreases detected in our study were lower than the 36% drop reported by Zoeren-Grobben et al. (1987) and the 39% decrease described by Randoin and Perroteau, who used a shorter pasteurisation cycle (65 °C, 20 min).

The α-tocopherol levels detected in our study were similar to those reported by Hoppu et al. (2005) in human milk (3.7–4.8 mg/l). α-Tocopherol losses observed in human milk pasteurised by the slow-heat method (17%) were lower than the decrease found by Erb et al. (1981) in human milk pasteurised at 73 °C for 10 min (34%).

Our observation that ascorbic acid and tocopherols are lost during pasteurisation indicates that donor milk has less antioxidant activity than milk received directly from a mother. The maximum decrease in ascorbic acid and tocopherols was detected in the fast-heat method. Our findings are consistent with those of Trifonova et al. (1981).

Our results show that ascorbic acid is more unstable than tocopherols in response to heat treatment. Therefore, ascorbic acid measurements may be good indicator of heat treatment degree. Ascorbic acid has been proposed as a biomarker of oxidative stress in biological samples (Lykkesfeldt, Loft, & Poulsen, 1995).

Our observation that LC-PUFAs are unaffected by pasteurisation is consistent with the findings of other studies (Fidler et al., 2001; Henderson et al., 1998; Lepri et al., 1997) and indicates that infants receiving donor milk are not deprived of these crucial milk components. The stability of these LC-PUFAs during pasteurisation may be due to the high antioxidant activity of human milk (Henderson et al., 1998). In particular, arachidonic acid and docosahexaenoic acid, detected in this study at 0.47% and 0.26% respectively, were similar to the levels reported by Gibson and Kneebone (0.40% and 0.32%), Lepri (0.42% and 0.21%) and Henderson (0.52% and 0.21%) in pooled human milk.

In conclusion, to limit the loss of ascorbic acid and tocopherols in human milk during pasteurisation, we recommend treating the milk at 62.5 °C for 30 min instead of at 100 °C for 5 min. In addition, we propose ascorbic acid as a marker of the degree of heat treatment of human milk.

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