LOSS OF L-ASCORBIC ACID IN COMMERCIAL DRINKING MILK CAUSED BY MILK PROCESSING AND STORAGE TIMES

Nannapat Nalame, Wasana Chaisri and Witaya Suriyasathaporn

Ruminant Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand

Abstract. The goals of this study were to determine L-ascorbic acid concentrations in various milk products, and to evaluate the effect of storage time on L-ascorbic acid in milk. Commercial plain milk samples were obtained from either a raw-food market or a supermarket, in Mae Hia, Mueang District, Chiang Mai Province, Thailand, during July, 2008. The types of milk were separated based on fat percentages (non fat-0%, low fat-1.5%, full fat-3%), and their method of processing (pasteurization, UHT). All samples were collected, transported, and measured for their L-ascorbic acid concentrations on the same day. The expiration date, type of milk, and source of milk were recorded. Pasteurized milk had higher L-ascorbic acid levels than UHT milk ($p<0.05$), but no differences of L-ascorbic acid levels were seen among the milk fat percentage groups. The L-ascorbic acid level was significantly positively related to time before the expiration date of the milk, indicating that increased storage time of milk is related to decreased L-ascorbic acid concentration in the milk. Longer milk storage times resulted in lower- L-ascorbic acid levels and pasteurized milk has higher L-ascorbic acid levels than UHT milk.

INTRODUCTION

L-ascorbic acid is essential for the formation of intracellular substances of skeletal tissues and maintenance of the normal function of these tissues. It also stimulates phagocytic activity, the reticuloendothelial system and formation of antibodies (Robinson, 1966; Roche, 1976). Ascorbic acid is an antioxidant that reduces oxidative stress on the oxidation-reduction system in the cellular oxidation process. It is involved in recycling other antioxidants, such as alpha-tocopheral (Roche, 1976).

Correspondence: Witaya Suriyasathaporn, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, 50100, Thailand.
Tel: 66 (0) 5394 8023, Fax: 66 (0) 5327 4710
E-mail: suriyasathaporn@hotmail.com

Milk is essential for promoting bone and dentine growth. Milk is rich in several nutrients that are essential for health, especially in babies. Babies can obtain ascorbic acid by drinking milk (Kraus, 1998; Padayatty *et al*, 2003; Parker, 2003). Breast milk has a higher level of vitamin C than commercial milk, but is easily destroyed by heat (O'Neil *et al*, 2006). When babies are 4-5 months old, commercial milk is often used instead of breast milk (Kraus, 1998). In Thailand, most commercial milk is either pasteurized or UHT milk and comes in different fat concentrations. Therefore, the goals of this study were to compare L-ascorbic acid concentrations in various forms of drinking milk. The effect of storage time on L-ascorbic acid levels in milk was also investigated.

MATERIALS AND METHODS

The ascorbic acid standard used in the study had a purity of 99.7% and was obtained from Merck (Darmstadt, Germany). The *meta*-phosphoric acid used in the study had a purity of 33.5-36.5% and was purchased from Fluka (Buchs, Switzerland). The acetic acid was purchased from Merck (Darmstadt, Germany) and methanol was purchased from Labscan (Dublin, Ireland). The reagents were of analytical grade except for the methanol which was HPLC grade. Water was double distilled and purified with a Millipore Milli-Q (Millipore, USA).

Commercial plain milk samples were obtained from either a raw-food market or a supermarket, in Mae Hia, Mueang District, Chiang Mai Province, Thailand in July, 2008. The milk was separated into groups bases on fat percentages (nonfat-0%, low fat-1.5%, full fat-3%), and by method of processing (pasteurization, UHT). All samples were collected, transported, and measured for L-ascorbic acid levels in the same day. The expiration date, type of milk and source of milk were recorded.

L-ascorbic acid determination was followed the method described by Romeu-Nadal *et al* (2006). The milk samples were thawed to room temperature, protected from light, then mixed. To analyze ascorbic acid levels, 300 µl milk and 300 µl 0.56% (w/v) *meta*-phosphoric acid solution were added to a centrifuge tube, shaken for 30 seconds and centrifuged 3,000*g* for 10 minutes at 10ºC.

Chromatographic measurements were carried out using a Shimadzu Model LC-10 AD VP-10 pump system and a SPD-10 AV VP (Shimadzu, Japan), an UV-vis detector. Separation was performed using an Inersil® ODS-3 C18 (250x4.6 mm ID, 5 µm particle size) (GL Sciences, Japan) analytical column connected to an Inersil® C18 (50 mm x 4.6 mm, 5 µm) guard column (GL Sciences, Japan). Fifty microliters of filtrate sample was directly injected into the HPLC system. Isocratic chromatographic separation was carried out using the mobile phase which consisted of Milli-Q water and acetic acid (0.1% v/v) which was then mixed with methanol in a relative proportion of 95:5 (v/v). The eluent flow-rate was set at 0.7 ml/minute and the column temperature was 25ºC. The elute was monitored for UV absorbance at 254 nm. Quantification was carried out using an external standard.

Standard stock solutions were prepared by dissolving ascorbic acid in 0.56% (w/v) *meta*-phosphoric acid solution and Milli-Q water and stored at 4ºC. The stock solution was diluted with *meta*-phosphoric acid to the concentration ranges of 0.39-100 µg/ml.

Means and standard error of means were used to describe the L-ascorbic acid data. Time before expiration date was defined as 14 minus the difference between the expiration date and the sampling date. A Student's *t*-test was used to compare the concentrations of L-ascorbic acid in the pasteurized milk and UHT milk. Differences in L-ascorbic acid levels at the studied fat levels (full-fat-3%, low-fat-1.5%, non-fat-0%) were evaluated with the one-way ANOVA. A correlation between L-ascorbic acid levels and storage time for pasteurized milk samples was evaluated with simple linear regression. The significance level was set at $p<0.05$.

RESULTS

The chromatogram for ascorbic acid is seen in Fig1A. It shows the relative retention time with this HPLC system is 5.367 minutes. The linearity of the calibration curve using the standard solution is seen in Fig 1B. Determination of the standard stock solution concentration at different dilutions showed the relative between peak area and L-ascorbic acid concentration was

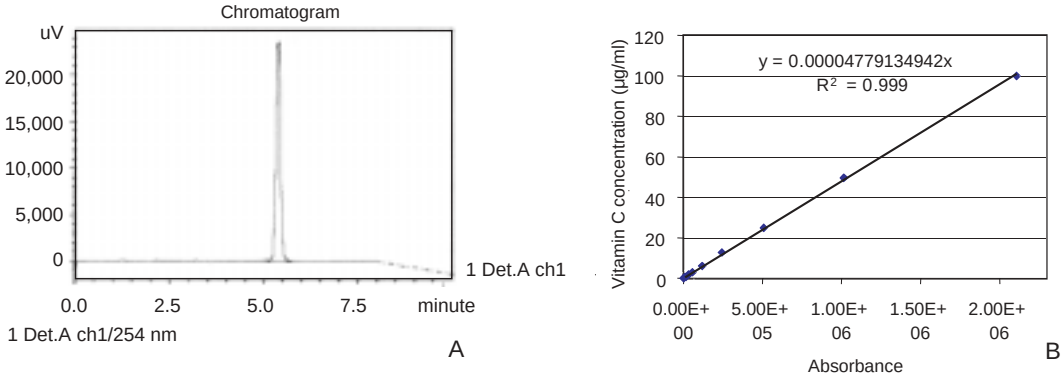

Fig 1–Determination of L-ascorbic acid using HPLC: A. chromatogram of ascorbic acid standard, and B. the linearity of the calibration curve with the standard solution.

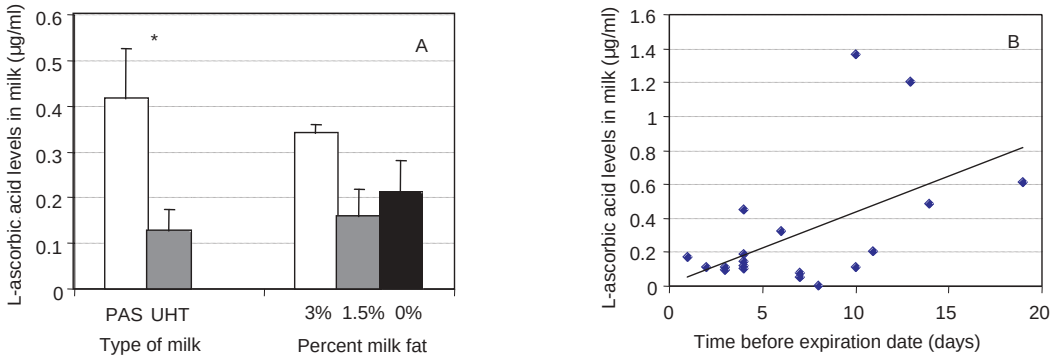

Fig 2–L-ascorbic acid levels. A. by type of milk processing and percentages of milk fat, B. and by time before expiration date indicates the means were significantly different.

y = 0.00004779134942x [y = peak area, x =L-ascorbic acid concentration (µg/ml), R^2 = 0.99938564037492].

Thirty-one milk samples were used in this study: 23 pasteurized and 8 UHT milk samples. One sample (4.3%) of pasteurized milk and 4 samples (50%) of UHT milk had undetectable levels of L-ascorbic acid. Fig 2 shows the results of the method of milk processing, percentages of milk fat (2A) and time to expiration date (2B) on L-ascorbic acid concentrations. The L-ascorbic acid levels ranged from 0.5-2 µg/ml. Pasteurized milk had higher L-ascorbic acid levels than UHT milk (p<0.05), but no differences in L-ascorbic acid levels were seen among the various milk fat percentage groups. The L-ascorbic acid level was positively related to the time before expiration of the milk (Fig 2B). The Pearson's correlation coefficient for the correlation between the two variables is 0.52.

DISCUSSION

The relative retention time with the HPLC system and the linearity of the calibration curve were both good, as seen in Fig 1B. The linearity determination coefficient of 0.999 in this study is similar to previous reports (Romeu-Nadal *et al*, 2006). Romeu-Nadal also validated the method and found

the HPLC method to be reliable, reproducible, and sensitive for detecting L-ascorbic acid in milk. This indicates the accepted levels of reliability of the HPLC system for determining the L-ascorbic acid level in the study.

In our study L-ascorbic acid levels ranged from 0.5-2 μg/ml, similar to the L-ascorbic acid levels measured by high-performance liquid chromatography (Romeu-Nadal *et al*, 2006). The levels of L-ascorbic acid in pasteurized milk were higher than in UHT milk, which may be related to the shorter storage time and lower temperature during production of pasteurized milk compared to UHT milk. The UHT heating process uses a higher temperature than pasteurization. Heat treatment results in an increase in ascorbic acid conversion to dehydroascorbic acid and diketogulonic acid (Romeu-Nadal *et al*, 2008).

A correlation was found between L-ascorbic acid level and time to expiration of the milk. Increased storage time of milk was related to decreased L-ascorbic acid levels in milk. This supports the theory that L-ascorbic acid levels were lower in UHT milk because UHT milk had longer storage times than pasteurized milk. Oxygen is the main component responsible for the loss of L-ascorbic acid during storage since it causes oxidation to dehydroascorbic acid. The loss of L-ascorbic acid during storage was probably due to the oxygen in the package (Oamen and Seartzel, 1989). Its function as an antioxidant may be negatively related to peroxidation levels in milk when stored in the refrigerator. This is supported by the finding that MDA, as an oxidative marker in human breast milk, is increased within 24 hours of being placed in the refrigerator (Miranda *et al*, 2004). L-ascorbic acid in milk decreases with increasing storage times and pasteurized milk has higher L-ascorbic acid levels than UHT milk.

ACKNOWLEDGEMENTS

The authors thank the Faculty of Veterinary Medicine and Intervet Thailand, Co Ltd, for their funding of the project.

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