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# Changes of Lactic and Benzoic Acid Concentrations in Milk During Post-Collection Period

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**Abstract:** The objective was to evaluate the concentrations of lactic and benzoic acids in milk during 6 h after collection. Ten composite milk samples from 10 healthy lactating dairy cows were collected. At 0-6 h after collection, milk samples were analyzed for the concentrations of lactic acid by titration method and for the concentrations of benzoic acid by high-performance liquid chromatography. Results revealed that average concentrations of lactic acid at 0 h were 129.6±7.0 mg $dL^{-1}$ and the concentrations gradually increased during the 6 h post-collection period. At 6 h after collection, the concentrations were 158.9±4.3 mg $dL^{-1}$. Similar results were also observed for benzoic acids. At 0 and 6 h after collection, average concentrations were 0.09±0.06 and 0.13±0.03 ppm, respectively. These results confirmed that lactic and benzoic acids were normally found in fresh milk and their concentrations were increased when the measurement time increased.

**Key words:** Benzoic acid, lactic acid, milk, cows, samples, Thailand

## INTRODUCTION

In general, cow milk contains small amounts of organic acids such as acetic, butyric, citric, orotic and lactic acids (Izco *et al*., 2002). These organic acids is important for monitoring growth and activity of bacterial and these acids may affect the flavor and aroma of dairy products (Marsili *et al*., 1981). Hippuric acid also a naturally found organic acid is present in cow milk (Patton, 1953; Sieber *et al*., 1995) and the concentration may be up to 50 mg $kg^{-1}$ (Sieber *et al*., 1995). In skim milk, hippuric acid concentration ranged from 3.1-6.4 mg $dL^{-1}$ (Patton, 1953).

Lactic acids in milk could be produced by lactic acid producing bacteria (Aziz *et al*., 2009; Ali, 2011). Aziz *et al*. (2009) reported that the prevalence of lactic isolates was highest in cow milk (75%) followed by buffalo milk (68%) and sheep milk (55%).

During fermentation process, some organic acids such as lactic acid increased while some such as hippuirc acid decreases (Dellaglio, 1998; Driessen and Puhan, 1998) It is known that milk lactic acid bacteria convert naturally found hippuric acids into benzoic acid (Sieber *et al*., 1995). It has been reported that milk benzoic acid concentration ranges from 2-5 mg $kg^{-1}$ (Obentraut, 1982). Taking these all together it may be postulated that increased activity of lactic acid bacterial in the milk would induce the change of hippuric acid to benzoic acid. Urbiene and Leskauskaite (2006) reported that benzoic acid content is higher for fermented milk than for raw milk.

To understand the changes of lactic acid and benzoic acid in milk sample during post-collecting period, the aim of this study was to determine the concentrations of milk lactic and benzoic acids hourly starting from 0-6 h after sampling.

## MATERIALS AND METHODS

**Milk samples:** Milk samples were collected from 10 healthy, lactating dairy cows raised in the Demonstrating Dairy Farm of the Faculty of Veterinary Medicine, Kasetsart University, Nakhonpathom, Thailand. At sampling, 150 mL of composited milk from each cow were collected into dry and clean plastic bottle and were transported to the laboratory immediately within 15 min after collection. At laboratory, the milk samples were kept in the bottle at room temperature (25-28°C) and each milk sample was analyzed for lactic and benzoic acid concentrations at 0-6 h after collection.

**Lactic acid determination:** Lactic acid concentrations were determined using AOAC (1990) method. In brief,

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20 mL of milk sample was added by 40 mL of boiled and cooled distilled water. The diluted milk samples were then added by 2 mL of phenolphthalein (1% in ethanol). The mixture was titrated with 0.1 M NaOH until the pink color persisted for 30 sec thereafter, one more drop of NaOH was added and the final volume of NaOH added was noted. Calculation of the lactic acids was performed using the formula as described by Fabro *et al*. (2006).

**Benzoic acid determination:** Before determination of benzoic acid, 25 mL of milk samples were transferred into a 50 mL flask. Thereafter, 5 mL of 1 M zinc acetate and 5 mL of 0.25 M potassium hexacyanoferrate were added into the flask. The mixture was diluted to 50 mL by distilled water. After shaking for 1 min, the mixture was filtered through the filter paper. Benzoic acid concentrations were determined in the filtered solution using High-Performance Liquid Chromatography (HPLC) as described by Qi *et al*. (2009).

**Statistical analysis:** Data were tested for their normal distribution using Sapiro Wilk (Patrie and Watson, 1999). Mean concentration of both lactic and benzoic acids at 0 h were compared with mean concentrations at 1-6 h after collection using paired t-test. The significant difference was preset at $p \leq 0.05$.

## RESULTS AND DISCUSSION

The concentration of lactic acid in milk measured during 6 h from collection is shown in Fig. 1. Average concentrations of lactic acid in milk from 10 dairy cows are shown in Fig. 1. At 0 h of collection, average lactic acid concentration in milk was 129.6±7.0 mg $dL^{-1}$ (SD). The concentrations slowly increased during 2 h after collection thereafter, the concentrations gradually increased to 158.9±4.3 mg $dL^{-1}$ at 6 h after collection. Although, researchers did not culture for lactic acid producing bacteria in the milk samples, Aziz *et al*. (2009) reported that lactic acid bacteria identified in cow milk samples include *Streptococcus thermophilus*, *Lactococcus lactis* sp. *lactis*, *Lactobacillus delbrueckii* sp. *bulgaricus* and *Lactococcus lactis* sp. *cremoris*. When these bacteria are used as lactic acid bacteria starter for fermented milk, the pH of the milk declined following fermentation time (Urbiene and Leskauskaite, 2006). Furthermore, benzoic acid concentrations are increased during 7 h after fermentation with lactic acid bacteria starters (Urbiene and Leskauskaite, 2006). The increased concentrations of lactic acid in the milk observed in this study would probably due to the fermentation of lactic acid bacteria, naturally presented in the milk. The concentration of benzoic acid in milk measured during the 6 h from collection is presented in Fig. 2. At 0 h of collection, average benzoic acid concentration in milk was 0.09±0.06 ppm which is relatively lower than a previous study by Obentraut (1982). The researcher reported that the concentration of benzoic acid in raw milk approximately ranged from 2-5 ppm. Thereafter, benzoic acid concentrations in milk gradually increased from 0.09±0.06 ppm at 0 h to 0.13±0.03 ppm at 6 h after collection. Qi *et al*. (2009) reported that benzoic acid widely occurs in milk and milk products in China at the low levels. These researchers found that pasteurized milk contained 0.2 mg $kg^{-1}$ of benzoic acid which was a little higher than the levels observed in this study (0.13 ppm). From the study of Urbiene and Leskauskaite (2006), the initial benzoic acid concentration in milk before inoculated with lactic acid bacteria was 5.0 mg $kg^{-1}$ and the concentrations increased

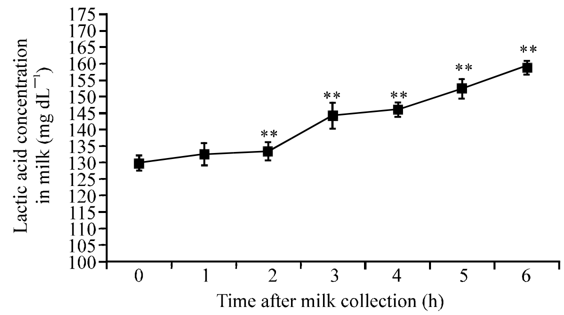


Fig. 1: Lactic acid concentration (mg $dL^{-1}$) in composite milk (n = 10) measured at 0-6 h after collection. Data represented means and SEM as error bars. Asterisks indicated that the concentration at each hour significantly differed from the concentration at 0 h ($p \leq 0.05$)

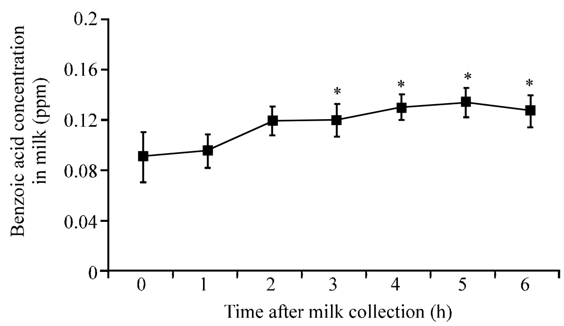


Fig. 2: Benzoic acid concentration (ppm) in composite milk (n = 10) measured at 0-6 h after collection. Data represented means and SEM as error bars. Asterisks indicated that the concentration at each hour tended to differ from the concentration at 0 h ($p \leq 0.1$)

upto 24 mg $kg^{-1}$ after inoculated for 7 h. It was suggested that increased lactic acid concentrations, produced by lactic acid bacteria would lead to increased benzoic acid concentrations as also suggested by Sieber *et al.* (1995). It was possible that increased acidity of the milk by lactic acid would favor the commonly found hippuric acid in the milk changing to benzoic acid. It is recommended that fresh milk should be ideally stored at a temperature of 4°C in order that activity of bacteria is reduced (Fellows and Hampton, 1992). However, in Thailand, cooling the milk at 4°C immediately after collection is not well-practiced and is hardly possible to be practiced by small-holder farmers. The times required for transporting the milk from the farms to the collecting centers or to the dairy cooperatives are varied. Taking together with the high temperature climate of Thailand, it is most likely that the lactic acid bacteria normally found in the milk would increase their activity which might ease convert hippuric acid to benzoic acid as observed in this study.

## CONCLUSION

Fresh milk from small-holder farms in Thailand contained lactic acid and benzoic acid and the concentrations of these organic acids in the milk increased when the measurement time increased.

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