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FORMATION OF SOME ORGANIC ACIDS DURING FERMENTATION OF MILK

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The formation of benzoic, sorbic and nucleic acids during fermentation of milk with different commercial lactic acid bacteria starters was the object of this study. The changes of these organic acids content during storage of fermented milk were investigated as well. It was determined that benzoic acid content in fermented milk was 14–23 mg/kg, sorbic acid content was 0.06–0.085 mg/kg and nucleic acids content was 70–80 mg/100 mL. In comparison with raw milk the benzoic, sorbic and nucleic acids content in fermented milk was higher 5.1, 4.0 and 2.5 times, respectively. The most intensive formation of organic acids was detected during 3–6 h of milk fermentation, *i.e.* during the log phase. The organic acids content in fermented milk was affected by the type of commercial lactic acid bacteria starters while the highest concentration of the organic acids was detected in the milk fermented by starter La-5 containing *Lactobacillus acidophilus* bacteria. However, no influence of a starter type was noticed on the changes of organic acids during storage of fermented milk. In all cases the benzoic and sorbic acids content decreased during storage and no changes of the nucleic acids content were detected.

INTRODUCTION

Recently, a great interest has been paid to biologically active compounds in food products. These compounds are present in food in very small amounts if compared with the main food components, therefore they are referred to as micronutrients. Milk and milk products contain mineral elements, vitamins, enzymes, free amino acids and organic acids which can be classified as micronutrients [Kato *et al.*, 1989; Renner *et al.*, 1991]. A number of organic acids are known to occur in milk, including lactic, citric, orotic, sialic, benzoic, sorbic and others. The major organic acid in raw milk is citric acid [Belitz & Grosch, 1999]. During storage it disappears rapidly as a result of the action of bacteria. Lactic and acetic acids are products of lactose degradation. Orotic acid is an intermediary product in biosynthesis of pyrimidine nucleotides. Other acids, such as benzoic and sorbic acids, are present in milk in less amounts [Kato *et al*., 1992; Lee *et al*., 1995]. However they are important due to their preservative properties. Together with other biologically active compounds of milk, such as imunoglobulins, lysozyme and lactoferrin, they prevent the growth of various microorganisms in it [Monk *et al*., 1995; Lui *et al*., 1995], increasing the quality of a product during storage.

During fermentation of milk, the concentration of some organic acids (lactic, propionic, acetic) increases, while the concentration of the other organic acids (hippuric, orotic, citric) decreases [Dellaglio, 1988; Driessen & Puhan, 1988]. Depending on the microorganisms involved, fermentation of milk proceeds *via* glycolysis pathway with the almost exclusive formation of lactic acid, *via* pentose phosphate pathway with formation of lactic and acetic acids [Belitz & Grosch, 1999]. It is known that the formation of benzoic, sorbic and nucleic acids also takes place in the fermentation process of milk in significantly lower amounts [Fernandez-Garcia & McGregor, 1994; Damir *et al*., 1992]. Nevertheless their role as natural preservatives is very important for the increasing quality of the fermented milk products, especially for extending their shelf life. Therefore these organic acids are the objectives of our study.

It has been reported that benzoic acid content in milk ranges from 2 to 5 mg/kg [Obentraut, 1982]. Moreover, according to Sieber *et al*. [1990, 1995], in fermented milk products and cheeses its content is significantly higher – up to 50 mg/kg and is influenced by the microorganisms used in the production of these products [Sajko *et al*., 1984; Kisza *et al*., 1984]. Benzoic acid is characterised by strong antibacterial properties [Daeschel, 1997]. It is known that in the presence of benzoic acid the growth of yeast and moulds decreases to a significant extent [Renterghem & Waes, 1987]. There are reports that benzoic acid lowers the concentration of nitrozo compounds and mycotoxins in dairy products [Massey *et al*., 1982; Niyomca & Suttajit, 1988].

Sorbic acid properties are quite similar to those of benzoic acid. It can also be attributed to natural preservatives [Giese, 1994; Fernloef, 1994]. The content of sorbic acid in milk is markedly lower compared with benzoic acid. Regretfully, investigations on the sorbic acid content in fermented milk products are very limited.

A separate group of organic acids are nucleic acids. They

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also inhibit the growth of microorganisms and viruses, therefore, they prevent spoilage of food products during their shelf life. According to some investigations [Adigamov, 1986], a higher total content of nucleic acids in foods yields higher availability of these products. Russian authors find out that the total amount of nucleic acids in dairy products is approximately 30–40 mg/kg [Serebrianikova & Aristova, 1983] and depends on the content of proteins. It is also determined that the content of nucleic acids in raw milk depends on the cows' health. The higher content of nucleic acids is found in mastitic cows' milk [Zielinski *et al*., 1991]. In fermented dairy products the content of nucleic acids is considerably higher [Urbiene, 2001].

The aim of this study was both to determine the formation of benzoic, sorbic acids and nucleic acids during fermentation of milk with different commercial lactic acid bacteria starters and to investigate the changes of these acids during the storage of fermented milk.

MATERIALS AND METHODS

Raw milk from an experimental farm of the Lithuanian University of Agriculture was used for the manufacture of fermented milk. Milk was pasteurized at a temperature of 80°C for 15–20 s and inoculated by commercial lactic acid bacteria starters obtained from Christian Hansen Lab (Denmark). Three different commercial dairy starters were used: YC-180, composed of *Lactobacillus delbrueckii subsp. lactis, Streptococcus thermophylus* and *Lactobacillus delbrueckii subsp. bulgaricus*; ABT-2, composed of *Lactobacillus acidophilus, Bifidobacteria* and *Streptococcus thermophylus;* and La-5, composed of *Lactobacillus acidophilus* bacteria. The temperature of inoculation was 32±1°C. Fermented milk was stored for 6 days at 6±1°C.

Benzoic and sorbic acids were separated by distillation and determined spectrophotometrically. The benzoic acid content was measured at 295 nm wavelength, the sorbic acid content – at 258 nm [AOAC, 2000].

Nucleic acids were separated by heating a sample with chloric acid ($HClO_3$) and their total content was measured spectrophotometrically at wavelengths of 270 and 290 nm according to the method described by Serebrianikova [Serebrianikova & Aristova, 1988].

Titratable acidity was determined by titration of milk with 0.1 mol/L NaOH and expressed in Thorner degrees (°T). The pH was measured by means of a pH-meter.

At least three replicates of each measurement were made.

RESULTS AND DISCUSSION

During fermentation of milk and storage of fermented milk the changes of acidity were measured. The results showed that the most intensive changes of acidity occurred in the milk fermented by a lactic acid bacteria starter La-5 and the slowest changes were noticed for the milk fermented by a starter YC-180 (Table 1). Acidity changes could be evaluated as an indirect characteristic of the growth of lactic acid bacteria. For that reason, the conclusion could be made that under given conditions the most active were *Lactobacillus acidophilus* bacteria which were the main component of La-5. In all samples the changes of acidity were in accordance with the classical laws. The first phases of the development of lactic acid bacteria (lag and log) could be observed according to the acidity changes. During the first 3 h of fermentation an increase in acidity was negligible (lag phase). During the

TABLE 1. Acidity changes during milk fermentation with different starters.

Fermentation time (h)	Starter					
	La–5		ABT–2		YC–180	
	Acidity (°T)	pH	Acidity (°T)	pH	Acidity (°T)	pH
0	17–18	6.49–6.52	17–18	6.49–6.52	17–18	6.49–6.52
1.0	17–18	6.50–6.51	17–18	6.50–6.51	17–18	6.50–6.51
2.0	24–25	6.24–6.27	21–22	6.31–6.34	19–21	6.41–6.42
3.0	40–42	5.01–5.02	39–40	6.21–6.22	29–30	6.29–6.30
4.0	60–62	5.16–5.18	51–53	5.29–5.30	36–37	6.19–6.24
5.0	72–73	4.90–4.93	66–67	4.98–5.01	49–50	5.34–5.36
6.0	82–84	4.71–4.77	75–76	4.82–4.84	61–62	5.01–5.04
7.0	91–92	4.69–4.70	85–86	4.73–4.75	74–75	4.80–4.82

TABLE 2. Acidity changes during storage of fermented milk produced with different starters.

Storage time (days)	Starter					
	La–5		ABT–2		YC–180	
	Acidity (°T)	pH	Acidity (°T)	pH	Acidity (°T)	pH
1	110–115	4.60–4.62	96–98	4.70–4.71	90–92	4.70–4.71
2	118–120	4.58–4.60	99–101	4.63–4.65	91–93	4.68–4.69
3	118–120	4.57–4.60	99–102	4.63–4.65	91–93	4.68–4.69
4	125–127	4.52–4.58	105–108	4.60–4.61	95–96	4.64–4.66
5	126–130	4.52–4.58	105–108	4.60–4.62	95–96	4.63–4.66
6	127–130	4.52–4.58	110–112	4.60–4.63	96–98	4.63–4.66

following 3 h the acidity changed markedly (log phase), and later the increase in acidity slowed down because the bacteria development entered into the stationary phase.

The changes of acidity during the storage of fermented milk samples at the temperature of $6\pm1^{o}C$ are presented in Table 2. The slight changes of acidity were detected during the storage. The biggest increase in acidity was noticed during the first 12 h of the storage.

Benzoic acid changes

It is known that during the fermentation of milk lactic acid bacteria convert hippuric acid (naturally present in milk) into benzoic acid [Sieber *et al*., 1995; Horak *et al*., 1996]. The changes of benzoic acid content during fermentation of milk with various lactic acid bacteria starters are presented in Figure 1. The analysis of the results showed that the content of benzoic acid increased in all samples. Comparing the variation of benzoic acid content with the acidity changes during fermentation of milk it could be noticed that the development of benzoic acid was related to an increase in acidity of the samples. The most intensive period of benzoic acid development was coincident with the period when the biggest increase in acidity was observed. As far as the increase in acidity indicated the growth of lactic bacteria in the sample, we suppose that there was a relationship between the increase of benzoic acid content and the development of lactic acid bacteria during fermentation of milk. At the end of the fermentation process, when the development of lactic acid bacteria and changes of acidity of the samples decreased, the intensity of benzoic acid variation also decreased. Such behaviour was characteristic for all three samples inoculated by starters La-5, ABT-2 and YC-180. In fact, the highest benzoic acid content was determined in the sample fermented by La-5. It was 5.1 times higher than the initial benzoic acid content at the beginning of the fermentation process and increased from 5 mg/kg up to 24 mg/kg. The sample inoculated with YC-180 contained 18 mg/kg of benzoic acid at the end of the fermentation, and when compared to the initial content it was 4.0 times higher. The fermentation of a milk sample with ABT-2 caused the lowest increase in benzoic acid – up to 16 mg/kg.

The changes of benzoic acid content during storage are shown in Figure 2. The content of benzoic acid during storage decreased in all samples. During the first three days the decrease in benzoic acid was insignificant, while during the following three days its content decreased considerably: in the sample fermented by La-5 – to 14 mg/kg; in the sample fermented with YC-180 – to 11 mg/kg and in the sample fermented with ABT-2 – to 9 mg/kg. It should be noticed that the benzoic acid content in the fermented milk after storage for 6 days had almost reached the initial content of benzoic acid in milk before fermentation.

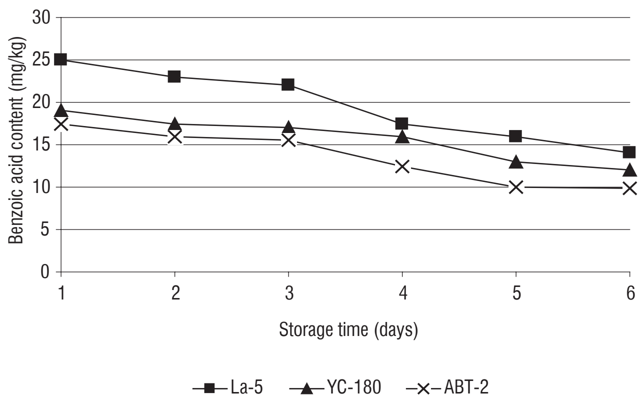

FIGURE 2. Changes of benzoic acid content during storage of fermented milk produced with different starters.

Sorbic acid development

The sorbic acid content variations during fermentation of milk with different lactic acid bacteria starters were investigated in the second part of this work. The results showed (Figure 3) that sorbic acid content in fermented milk was very low compared with that of benzoic acid. It increased during fermentation of the milk. The effect of a starter used for fermentation on sorbic acid content in fermented milk was detected. Sorbic acid development was related to an increase in acidity during fermentation. The most intensive development of sorbic acid was determined from 3 to 6 h of the fermentation process. The highest sorbic acid content – 0.09 mg/kg, was detected in the milk fermented by *Lactobacillus acidophilus* (La-5). The use of a lactic acid bacteria starter ATB-2 caused the development of 0.07 mg/kg of sorbic acid. The lowest sorbic acid content – 0.06 mg/kg, was reported in the milk fermented by a starter YC-180.

The influence of storage on sorbic acid content in fermented milk is shown in Figure 4. The sorbic acid con-

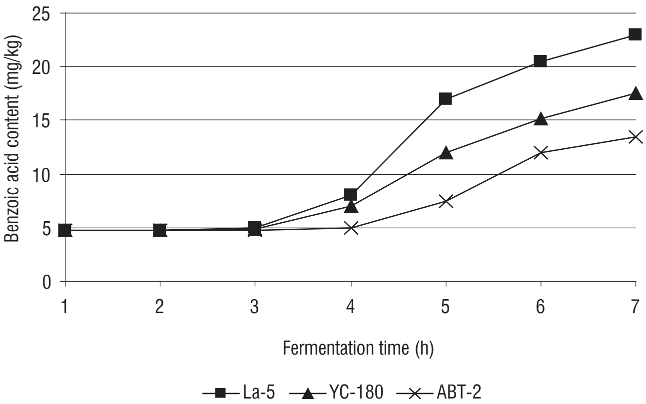

FIGURE 1. Benzoic acid development during fermentation of milk with different starters.

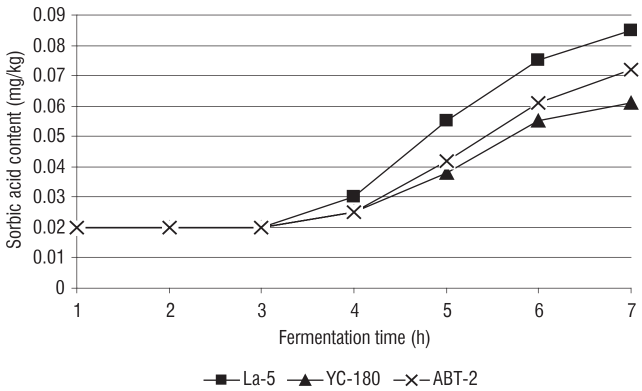

FIGURE 3. Sorbic acid development during fermentation of milk with different starters.

tent had slightly decreased during the storage. The rate of decrease was influenced neither by the type of bacteria used for fermentation nor by the period of storage.

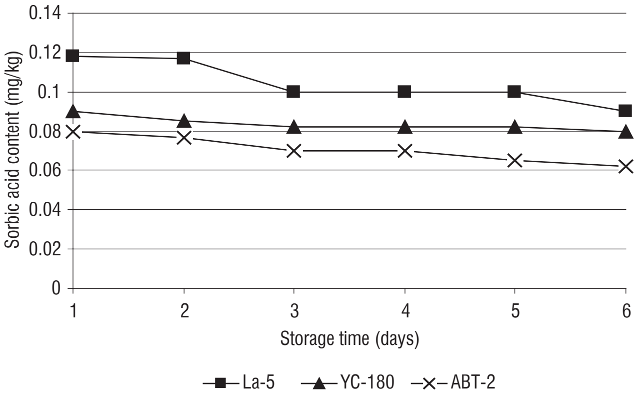

FIGURE 4. Changes of sorbic acid content during storage of fermented milk produced with different starters.

Nucleic acids changes

The results of the changes of the total nucleic acids content during fermentation and storage of fermented milk are presented in Figures 5 and 6. The slight formation of nucleic acids was observed during the first 3 h of fermentation. Starting from the 4th h of fermentation the increase in nucleic acids was more expressed and reached 10–12 mg/100 mL every hour. It could be noticed that the type of a starter used for the fermentation of milk had only a slight influence on the total nucleic acids content. The nucleic acids content in milk fermented by La-5 was 80 mg/100 mL, whereas in that fermented with ABT-2 and YC-180 was nearly the same, *i.e.* 70 mg/100 mL.

Nucleic acids develop in relationship with the activity of lactic acid bacteria. It is known that lactic acid bacteria induce inconsiderable proteolysis of proteins and the intensity of the proteolysis process is related with the intensity of bacteria development. The result of these processes is the increased free amino acids content in fermented milk [Damir *et al*., 1992]. We suppose that the variation of the nucleic acids content is also related to the intensity of lactic acid bacteria development. The changes (Figure 6) of the total nucleic acids content have not been detected during the storage of fermented milk.

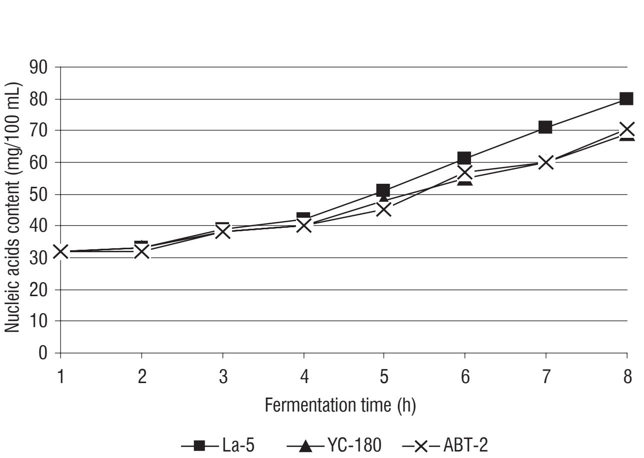

FIGURE 5. Nucleic acids development during fermentation of milk with different starters.

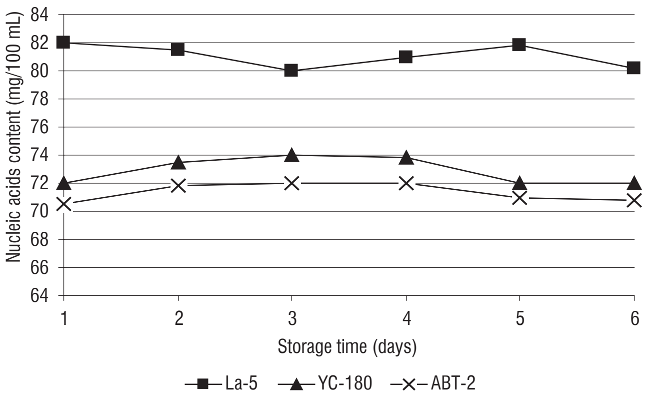

FIGURE 6. Changes of nucleic acid content during storage of fermented milk produced with different starters.

CONCLUSIONS

1. The content of benzoic, sorbic and nucleic acids increased during fermentation of milk in comparison with their content in raw milk.
2. The formation of benzoic, sorbic and nucleic acids in fermented milk depended on the phase of the development of lactic acid bacteria and was the most intensive in the log phase.
3. The benzoic, sorbic and nucleic acids content in fermented milk was affected by the type of commercial lactic acid bacteria starters and the highest concentration of organic acids was detected in the milk fermented by a starter La-5 containing *Lactobacillus acidophilus* bacteria.
4. The contents of benzoic acid and sorbic acid decreased during the storage of fermented milk, while no changes of the nucleic acids content were detected. During the storage, the changes of the organic acids content were not influenced by the microorganisms involved in milk fermentation.

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