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Full Length Research Paper

Biochemical changes occurring during fermentation of camel milk by selected bacterial starter cultures

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The biochemical changes in amino acids, water soluble vitamins, soluble sugars and organic acids occurring during fermentation (at 43°C for 6 h) of camel milk inoculated with *Streptococcus thermophilus* 37, *Lactobacillus delbrueckii sub* sp*. bulgaricus* CH2, *Lactococcus lactis, Lactobacillus acidophilus* and mixed yogurt culture (*S. thermophilus* and *L. bulgaricus* 1:1) were studied. Amino acids analysis revealed slight insignificant increase in alanine, leucine, histidine, lysine and argninie, while valine, methionine and tyrosine were slightly decreased. The fermentation process significantly decreased vitamin C contents, while it had no significant effect on riboflavin and thiamine content. Single as well as mixed culture fermentation resulted in decrease in lactose concentration with parallel increase in glucose and galactose. Organic acids analysis revealed the presence of lactic acid, formic acid and acetic acid, with yogurt culture showing the highest concentration of lactic acid, formic acid and acetic acid, while *L. lactis* showed lowest concentration.

Key words: Camel milk, fermentation, amino acids, organic acids, vitamins, sugars.

INTRODUCTION

Sudan has the second largest number of camels in Africa, which is estimated to be 2.7 million. They belong to the one-humped dromedary kind, and reached the country from Arabia. They are found in the northern states and mostly owned by the nomads who inhabit the semi-desert zone. Despite the large camel population in Sudan, camel milk is not fully utilized because of its salty taste and high acidic nature. Traditionally, camel milk in Sudan is consumed either as fresh raw milk or fermented sour milk by nomadic tribes. However, recently, there is an increasing trend for the consumption of fermented milk products as ethno medicine such as for diabetes treatment (Agrawal et al., 2005) and curing Leshmaniasis and protozoa disease (Dirar, 1993).

The traditional method for preparing fermented camel milk consists of heating milk to the boiling point, then cooling it to body temperature, and a small amount of previously fermented milk is added as a starter. The milk is well stirred and kept overnight at ambient temperature, and by the next morning it would be curdled (Aggarwala, 1961; Kambe, 1986). Although the composition of camel milk, growth behavior of some lactic acid bacteria in camel milk and the production of some dairy products from the milk have been studied by many investigators, yet there is no information regarding changes such as amino acids, soluble vitamins, soluble sugars and organic acids that take place during fermentation of camel milk. The present research was carried out to study the biochemical changes that occur in the camel milk during fermentation by selected starter cultures.

MATERIALS AND METHODS

Sources and maintenance of cultures

The lyophilized pure culture strains of *Streptococcus thermophilus* 37, *Lactobacillus delbrueckii* sp. *bulgaricus* CH2, *Lactococcus lac-*

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tis, Lactobacillus acidophilus and mixed yogurt culture (*S. thermophilus* and *L. bulgaricus* 1:1) used in this study were obtained from Chr. Hansen's Laboratorium (Hørsholm, Denmark A/S). The working cultures were prepared by adding 100 mg of lyophilized cultures to 100 ml of previously reconstituted and sterilized (121°C/15 min) skim milk with total solids of 11% and incubated overnight at 25°C. One percent inocula from overnight coagulated cultures were propagated three times before being used in growth studies. Cultures were grown and maintained in sterile reconstituted non-fat dry milk (NDM) containing 11% solids (wt/wt) with weekly transfers. Purity of cultures was routinely checked by performing gram stains.

Preparation of fermented milk

Fresh whole camel milk from *Camelus dromedarius* was obtained from a private herd. The milk was immediately cooled and kept at 5 ± 1°C during transportation to the laboratory. The whole camel milk was pasteurized in 500-ml quantities at 80°C for 15 min in a water bath and immediately cooled to 5 ± 1°C in an iced bath. The milk samples (500 ml) were equilibrated for one hour at the fermentation temperature (43°C) in a water path before inoculation with the starter cultures. The cultures were sub-cultured using 1% inocula (10^6 - 10^7 cfu/ml) in sterile 11% reconstituted NDM and incubated at 37°C for 18 - 24 h and propagated at least three times before experimentation involving camel milk as the medium of growth. Each milk was inoculated with 5% (10^6 - 10^7 cfu/ml) of *S. thermophilus 37, L. delbrueckii ssp. bulgaricus CH2, L. lactis, L. acidophilus* and mixed yogurt culture (*S. thermophilus and L. bulgaricus* 1:1). The contents were thoroughly mixed after inoculation and incubated at 43°C in a shaker water-bath for 6 h. After incubation period, the fermented camel milk were analyzed for amino acids, vitamins (C, B1 and B2) , sugars (glucose, galactose and lactose) and organic acids.

Determination of amino acids

Amino acid composition was determined with Moore and Stein (1963) method. Fermented samples were duplicated by transferring one gram of sample into a 15 ml ampoule, adding 10 ml 6 N HCl, sealing the vial under vacuum and digesting at 110°C for 24 h. Amino acids analysis was performed on reverse phase-high-pressure liquid chromatography (RP-HPLC) (Shimadzu 34 LC – 10 AD, Shimadzu corporation, Kyoto, Japan).

Determination of vitamins

Vitamin C was determined by 2,6-dichloroindo phenol titrimetric method, while thiamine (Vitamin B_1) and riboflavin (vitamin B_2) were determined by fluorometric method as described in AOAC (1984).

Determination of sugars

Soluble sugar analysis was performed according to the method of Pirisino (1983). 5 ml of fermented milk, 5 ml of water and 20 ml of HPLC grade acetonitrile were added to a 50 ml round-bottom glass centrifuge tube, shaken for 1 min and centrifuged for 10 min at 5000 rpm (Universal Centrifuge Model PLC-012, Germany Industrial Corp). To obtain a clear supernatant, the supernatant was filtered by passing it through a 0.45-µm-membrane filter. The clear supernatant was analyzed for lactose, glucose and galatose using HPLC Shimadzu LC. NH_2 from Shimadzu, Kyoto-Japan. Sugar standards were purchased from Sigma (Sigma Chemical Co., St. Louis, Mo). Results were reported as percentage (w/w).

Determination of organic acids

The organic acids and ethanol were determined according to Marssili et al. (1980). 10 ml of fermented milk were centrifuged at 10000 rpm for 20 min. The supernatant was filtered through membrane filter 0.45 µm diameter 25 mm (Schleichen ∝ Schiill-Germany) and analyzed for lactic acid, formic acid, acetic acid and ethanol by HPLC Shimadzu LC. NH_2 was from Shimadzu, Kuoto-Japan, using an organic acid column PL Hi-plex H (from Polymer Laboratories Amherst, M.A. 01002, U.S.A) fast acid column. Results were reported as percentage (w/w).

Statistical analysis

Each sample was analyzed in triplicate and the values were then averaged. The statistical analysis was performed with the Statistical Analysis System (SAS) program (SAS, 1990). Analysis of variance (ANOVA) and Duncan's multiple range tests were used to analyze the data. (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Amino acids of fermented camel milk

The amino acid composition of camel milk fermented by the five starter cultures and the unfermented milk along with the recommended FAO requirements for pre-school children (2 - 5 years) are presented in Table 1. The results show that there are no significant differences (P > 0.05) among the five cultures in the values of the individual amino acids except for phenylalanine and proline which were significantly decreased in milk fermented by *L. lactis* and *S. thermophilus*, respectively. There were slight significant increase in glycine, alaninie, leucine, lysine and argninie, while there were slight decrease in valine, methionine and tyrosine in comparison to unfermented camel milk.

The values of amino acids in this study are much higher than those given by Rao et al. (1978) who studied the amino acid of Labneh (a concentrated yogurt product consumed routinely in the middle East) made from goat and cow milk. This variation in the amino acid composition may be due to differences in preparation procedure, source of milk (goat or cow) and the type of final product. Many studies have shown that concentrations of most of the amino acids slightly increase due to fermentation. Muradyan et al. (1986) reported that fermentation of milk by thermophilic lactic streptococci or acidophilic rods enriched the final products with at least 4 amino acids (cysteine, valine, proline and arginine). The contents of essential amino acids such as valine, threonine, methionine, isoluecine, leucine, histidine, lysine and phenylalanine + tyrosine in the fermented camel milk were found to be higher than those of the FAO/WHO/UNU (1985). These findings confirm the excellent nutritional quality of fermented camel milk protein.

Vitamins (C, B1 and B2) of fermented camel milk

Vitamin C, riboflavin and thiamine contents of the fer-

Table 1. Amino acids profile (g/100g) of camel milk fermented for 6 hours at 43ºC by selected starter and unfermented Camel milk

Amino acids (g/100g)	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	Yogurt culture (1:1) *S. thermophilus* and *L. bulgaricus*	Unfermented camel milk	FAO / WHO reference value*
ASP	6.96 (±1.44)a	7.84 (±0.52)a	7.85 (±0.11)a	6.99 (±0.16)a	7.85 (±0.23)a	6.89±0.16	
THR	4.62 (± 0.95)a	5.25 (±0.45)a	5.12 (±0.07)a	4.62 (±0.17)a	5.20 (±0.13)a	4.21±0.24	3.4
SER	4.14 (±0.83)a	4.68 (±0.31)a	4.75 (±0.13)a	4.22 (±0.19)a	4.66 (±0.03)a	4.28±0.21	
GLU	17.02 (±3.09)a	19.24 (±1.21)a	19.44 (±0.46)a	17.22 (±0.52)a	19.39 (±0.47)a	18.86±0.21	
PRO	10.95 (±0.12)a	10.88 (±0.33)a	11.28 (±0.11)a	10.22 (±0.15)b	11.17 (±0.21)a	11.26±0.04	
GLY	1.68 (±0.37)a	1.88 (±0.11)a	1.86 (±0.02)a	1.68 (±0.03)a	1.91 (±0.11)a	1.32±0.10	
ALA	2.83 (±0.71)a	3.24 (±0.17)a	3.14 (±0.04)a	2.82 (±0.05)a	3.21 (±0.20)a	2.27±0.08	
VAL	5.54 (±1.16)a	6.18 (±0.31)a	6.02 (±0.09)a	5.80 (±0.12)a	6.54 (±0.33)a	6.93±0.28	3.5
METH	2.54 (±0.58a	2.87 (±0.23)a	2.82 (±0.02)a	2.62 (±0.06)a	2.88 (±0.05)a	3.03±0.04	(M+C) 2.5**
ILEU	4.78 (±0.97)a	5.33 (±0.29)a	5.08 (±0.05)a	4.88 (±0.16)a	5.69 (±0.00)a	5.14±0.05	2.8
LEU	8.86 (±1.87)a	10.09 (±0.64)a	9.90 (±0.11)a	9.01 (±0.24)a	10.20 (±0.50)a	8.42±0.00	6.6
TYR	3.32 (±0.79)a	3.75 (±0.23)a	3.65 (±0.08)a	3.41 (±0.23)a	3.34 (±0.13)a	4.39±0.11	
PHY	4.57 (±1.03)a	3.96 (±0.63)a	3.38 (±0.02)b	4.35 (±0.11)a	4.89 (±0.18)a	4.65±0.21	6.3
HIS	2.79 (±0.64)a	3.40 (±0.51)a	3.42 (±0.05)a	3.29 (±0.04)a	3.78 (±0.37)a	2.3 1±0.30	1.9
LYS	7.55 (±1.69)a	8.22 (±0.00)a	7.61 (±0.11)a	7.62 (±0.26)a	8.28 (±0.53)a	6.68±0.23	5.8
ARG	3.66 (±0.79)a	4.06 (±0.25)a	4.90 (±1.31)a	4.46 (±0.27)a	4.71 (±0.24)a	3.47±0.18	

● Values are means ± SD of three replicates.
* Amino acid requirements patterns as suggested by FAO / WHO /UNU (1985) for pre- school children (2-5 years).
** Methionine + Cysteine.
● Means not sharing a common following letter in a raw are significantly different at $p<0.05$.

mented camel milk by five starter cultures are shown in Figure 1. Vitamin C contents were 3.66, 5.55, 7.42, 7.35, and 7.34 and thiamine content was 0.295, 0.291, 0.286, 0.285 and 0.280, while riboflavin content was 0.352, 0.361, 0.368, 0.384, 0.343 mg/kg, respectively for camel milk fermented by *L. acidophilus, L. bulgaricus, L. lactis*, *S. thermophilus* and mixed cultures of *L. bulgaricus* and *S. thermophilus* (1:1), respectively. After fermentation at 43°C for 6 h by five starter cultures, vitamin C decreased significantly, whereas riboflavin and thiamine contents showed slight decreases when compared to the unfermented camel milk (Figure 1). The decrease of vitamin C was higher in the milk fermented by *L. bulgaricus* followed by mixed culture (1:1) of *L. bulgaricus* and *S. thermophilus*, *S. thermophilus*, *L. lactis*, and *L. acidophilus*. This finding is consistent with those reported by Oberman (1985) who found that lactic acid bacteria fermentation resulted in a marked decrease in vitamin B6, B12 and vitamin C level, while only small changes in vitamin A, B1, B2 and niacin took place. Baranova et al.

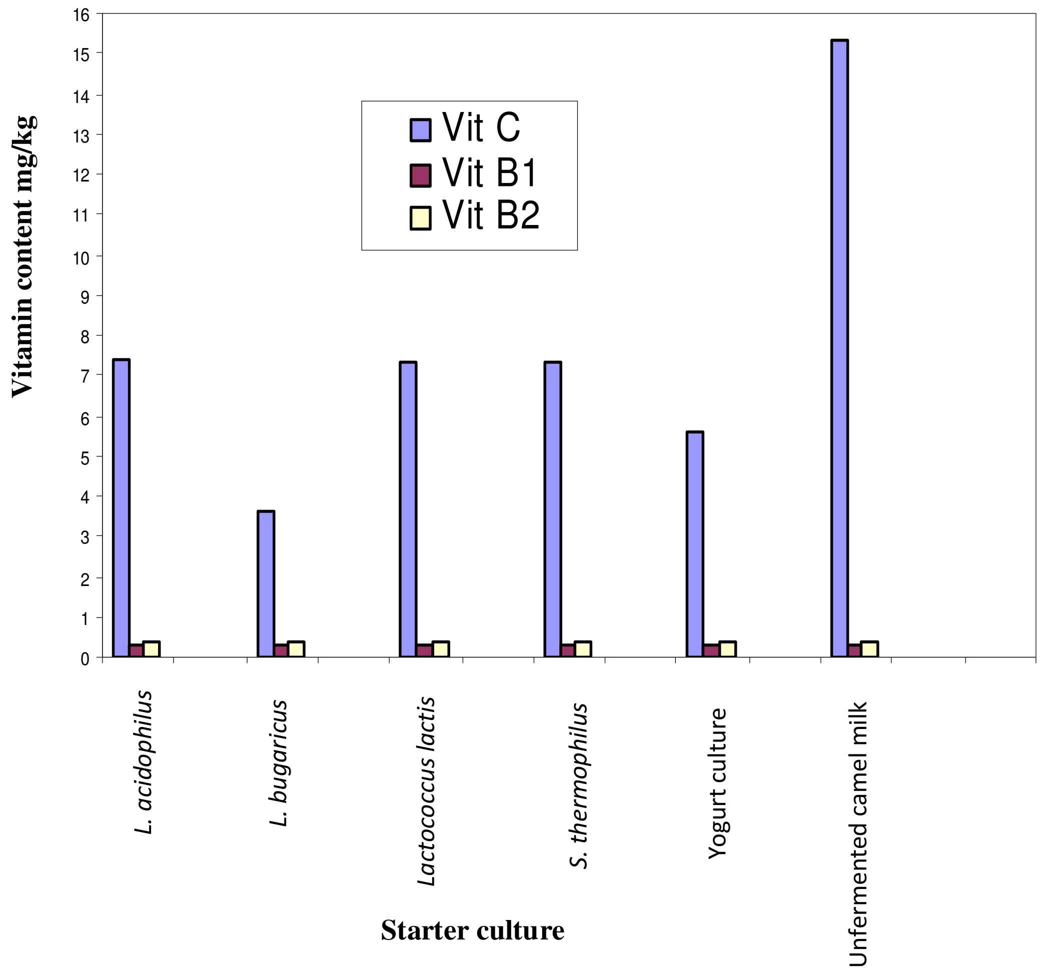

Figure 1. Vitamin C, B1 and B2 content of unfermented and fermented camel milk at 43°C for 6 h by selected starter cultures.

(1998) also reported that fermentation of goat milk by selected lactic acid bacteria significantly decreased vitamin C, but resulted in slight decrease in B1 and B2 and did not influence tocopherol contents.

An increase in folic acid content and a slight decrease in vitamin B12 was found in fermented milk compared to raw milk (Alm, 1984c). In the same trend, Saidi and Warthesen (1993) observed reduction in riboflavin content in yogurt fermented for 5 h. Bonczar and Regula (2003) found that the vitamin C and ascorbic acid contents decreased in ewe's milk after pasteurization and in yogurts during storage period, but increased with increasing amount of starter culture. In contrast, Khamagaeva et al. (1986) reported an increase in the content of thiamin and riboflavin by 27 and 18%, respectively, when the milk was inoculated with the combination of starter micro- flora, bifidobacteria, *L. bulgaricus* and kefir starter at a ratio of 1:0.5:0.5 in fermented milk products. Several researchers observed that during fermentation, the lactic acid bacteria require vitamins for growth, and certain micro-organisms produce vitamins at a higher rate than others. The changes in the vitamin content are dependent on the type of microorganisms, time and temperature of incubation.

Available sugars in fermented camel milk

The lactose, glucose and galactose contents of fermented and unfermented camel milk are presented in Figure 2. Since no much literature is available on the sugars content of fermented camel milk, comparisons are made with results of researchers who studied fermented milks from other animals. In general, lactose content was reduced significantly, whereas glucose and galactose contents were markedly increased due to fermentation by different starter cultures. After 6 h fermentation of camel milk, the lactose contents were 3.75, 3.45, 3.04, 2.85 and 2.86% and glucose contents was 0.268, 0.155, 0.297, 0.276 and 0.422%, while that of galactose contents was 0.82, 0.59, 0.083, 0.119 and 0.824%, for camel milk fermented by *L. acidophilus, L. bulgaricus, L. lactis*, *S.*

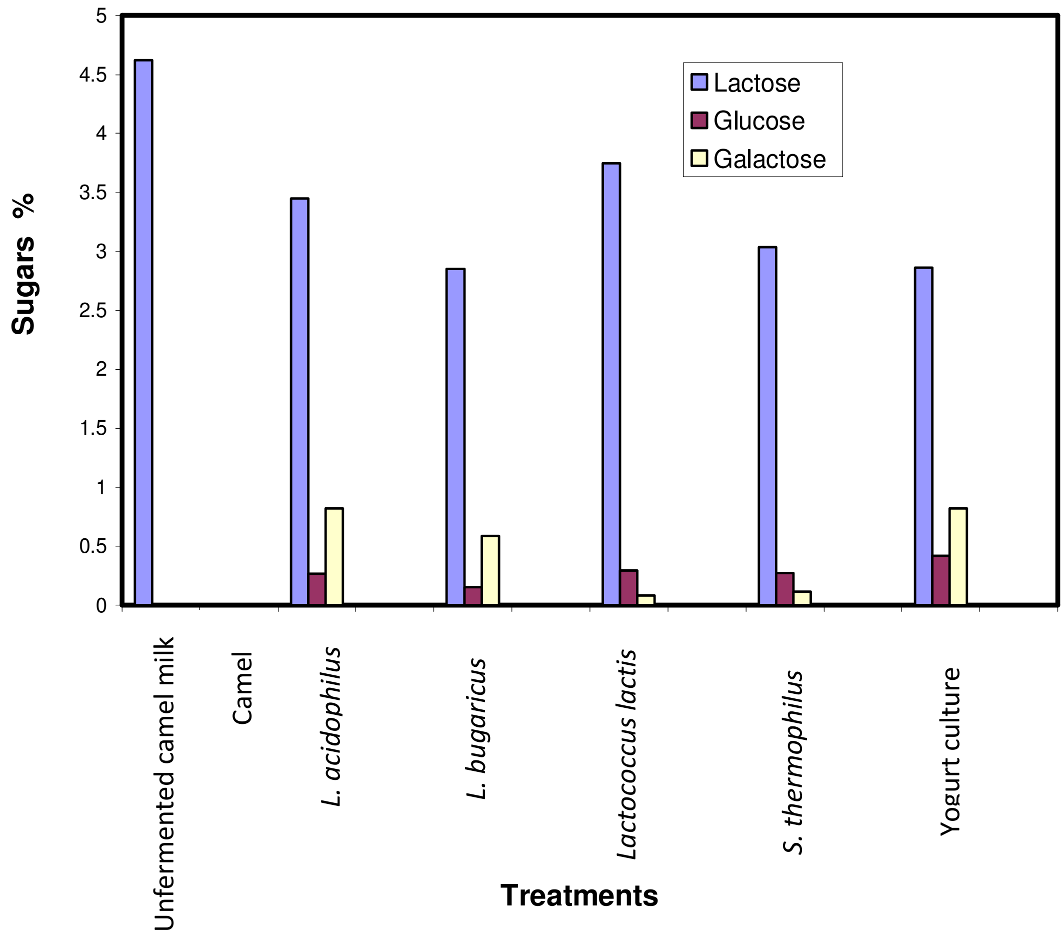

Figure 2. Lactose, glucose and galactose contents of unfermented and fermented camel milk at 43°C for 6 h by selected starter cultures.

thermophilus and mixed strains of *L. bulgaricus* and *S. thermophilus* (1:1), respectively.

The residual lactose concentration in fermented milk fermented by *L. bulgaricus* (2.85%) was equal to that fermented by mixed strains of *L. bulgaricus* and *S. thermophilus* (1:1) (2.86%), but both were lower than those fermented by *S. thermophilus* (3.04%), *L. acidophilus* (3.45%) and *L. lactis* (3.75%). The variation in the hydrolysis of lactose may be due to the strains of lactic acid bacteria used and the growth temperature. The present results indicated no significant difference among the five cultures with respect to the amount of glucose. The amount of the galactose were similar for *L. acidophilus* (0.82%), and mixed yogurt cultures (0.82%), but slightly lower for *L. bulgaricus* (0.59%) and much lower in the milk fermented by *S. thermophilus* (0.083%) than those fermented by the other strains. These results are in accordance with that reported by Toba et al. (1983) who found a decrease in the lactose content from 6.53 to 4.22% and increase in glucose and galactose in yogurt prepared by *L. bulgaricus* and *S. thermophilus.* Similarly, Brein (1999) studied the sugar profile of cultured dairy products in the United Kingdom (UK), and found that most lactic acid fermentations resulted in a decrease in lactose and increase in galatose. In agreement with the present finding, Saitmuratova and Sulaimanova (2000) found that the carbohydrates content of *Shubat* (fermented camel milk) was 3 - 5 times lower than those of unfermented camel milk.

Organic acids and ethanol contents in fermented camel milk

The lactic acid, formic acid, acetic acid and ethanol concentrations in the fermented camel milk products are shown in Table 2. Three organic acids (lactic acid, formic acid and acetic acid) were detected, while ethanol was not detected in the final fermented camel milk products. The concentration of lactic acid were 0.6, 0.73, 0.23, 0.47 and 0.85% and those of formic acid were 0.024, 0.026, 0.014, 0.026 and 0.031%, while those of acetic acid were 0.021, 0.025, 0.009, 0.020 and 0.025% for camel milk fermented by *L. acidophilus, L. bulgaricus, L. lactis*, *S.*

Table 2. Organic acids and ethanol concentration (%) of camel milk fermented for 6 hours at 43℃ by selected starter cultures.

Chemical components	*L. acidophilus*	*L. bulgaricus*	*L. lactis*	*S. thermophilus*	Yogurt culture*
Lactic acid	$0.6 \pm 0.028_c$	$0.73 \pm 0.028_b$	$0.23 \pm 0.014_e$	$0.47 \pm 0.014_d$	$0.85 \pm 0.014_a$
Formic acid	$0.024 \pm 0.028_a$	$0.026 \pm 0.001_a$	$0.014 \pm 0.002_b$	$0.026 \pm 0.001_a$	$0.031 \pm 0.001_a$
Acetic acid	$0.021 \pm 0.001_a$	$0.025 \pm 0.001_a$	$0.009 \pm 0.001_b$	$0.020 \pm 0.001_a$	$0.025 \pm 0.001_a$
Ethanol	ND	ND	ND	ND	ND

Values are means ± SD of three replicates; ND = Not detected; * combination of *S. thermophilus and L. bulgaricus* 1:1; means not sharing a common value following letter in a row are significantly different at $p < 0.05$.

thermophilus and mixed strains of *L. bulgaricus* and *S. thermophilus* (1:1), respectively. The lactic acid content was higher in milk fermented by yogurt culture followed by that of *L bulgaricus L. acidophilus S. thermophilus* and *L. lactis*. The concentration of formic acid and acetic acid in milk fermented by *L. lactis* were lower than those fermented by the other cultures, while no significant differences ($p > 0.05$) were observed between the other starter cultures. Formation of volatile acids during fermentation of Swedish fermented milk products showed that acetic acid and ethanol were low in yogurt than in bifidus milk (Alm, 1981). Kato et al. (1992) studied organic acids during fermentation of skim milk with lactic acid bacteria, and detected seven organic acids. In a similar study, Damir et al. (1992) found more than six organic acids during kishk fermentation, with lactic acid been the highest while formic acid the lowest.

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