EFFECT OF STORAGE TIME ON THE FATTY ACID PROFILE OF KOMBUCHA FERMENTED MILK PRODUCT

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Abstract
Kombucha fermented milk products are the new functional dairy products that are obtained by applying non-conventional starter culture-kombucha. The health aspect of fermented milk beverages depends on the fatty acid composition of milk fat, as one of the most important components of these products. In this investigation kombucha fermented milk product was produced from milk with 2.8% milk fat using 10% (v/v) kombucha inoculums cultivated on black tea. Effect of storage time on fatty acid profile of kombucha fermented milk product was investigated. The fatty acid composition of kombucha fermented milk product was determined after production and during 7, 14 and 21 days of storage. Fatty acids analysis was performed by capillary gas chromatography - mass spectrometry after previous extraction of lipids and derivatization. The main fatty acids encountered in kombucha fermented milk product were: palmitic, oleic, stearic and myristic with mean contents of 32.63; 26.65; 11.11 and 10.80%, respectively. During 14 days of storage content of saturated fatty acids (SFA) slightly increased, while contents of monounsaturated (MUFA) and polyunsaturated fatty acids decreased (PUFA). However, after 21 days of storage the content of SFA, MUFA and PUFA in kombucha fermented milk product was very similar with those obtained after production of fermented beverage. Results showed that the storage time has not significant influence on the fatty acid profile of kombucha fermented milk product.

Introduction
Fermented milk products possess various nutritional and therapeutic properties. They are characterized by easy digestibility, appropriate dietary properties (changed colloidal structure of fat and protein compared to milk, as a consequence of lactic acid production), good sensory properties, extended shelf life and a wide range of products that are enriched with the addition of fruits, grains, vitamins, mineral materials [1]. Recent studies presented the technological and nutritional potential of kombucha as an innovative starter culture in dairy industry [2-4]. Kombucha is a symbiotic association of several yeast species (*Schizosaccharomyces*, *Saccharomycodes*, *Saccharomyces*, *Zygosaccharomyces*, *Candida*, *Pichia*, *Kloeckera*, *Brettanomyces* and *Torulopsis*) and acetic acid bacteria (*Acetobacter* and *Gluconobacter*) whose metabolic activity on tea sweetened with sucrose produces a pleasant refreshing beverage [1-3, 5]. Kombucha fermented product contains ethanol, carbon dioxide, a high concentration of acid (gluconic, acetic and lactic) and a number of other health-promoting metabolites. Therefore, it is considered to be beneficial beverage in cases of: digestive ailments, diabetes, hypercholesterolaemia, high blood pressure, combating stress and cancer as well as body vitalisation, among others [2]. As the result of milk fermentation by kombucha inoculums, products similar to yoghurt or kefir are produced. Milk fat, as one of the most important components of fermented milk beverages, have great influence on the health aspect

of these products. Milk lipids represent a good dietary source of the liposoluble vitamins such as α-tocopherol, retinol, and β-carotene, as well as the essential fatty acids. The fatty acid composition of milk is not only effective on physical properties, oxidative stability and organoleptic quality of dairy products, but also has positive effects on human health. Various fatty acids have different effects on plasma lipids. While short and medium chain fatty acids do not affect plasma lipoproteins, consumption of saturated fatty acids specifically, saturated fats with 12–16 carbon atoms tend to increase plasma total and low density lipoprotein (LDL) cholesterol levels. Fatty acid composition of milk fat, especially owing to short-chain fatty acids present in relatively big amount, is ideal for the human organism because triacylglycerol’s containing short-chain fatty acids can be more easily attacked by the digestive enzymes. Milk fat contains relatively small amount of unsaturated fatty acids, despite this it can contain considerable amount of essential fatty acids needed to satisfy the requirements of the human organism. Besides, milk fat can contain conjugated linoleic acids in considerable quantity, which have according to the latest researches many useful physiological effects. Thus, recent clinical studies have focused on the bioactive fatty acids such as butyric acid, oleic acid and conjugated linoleic acid which may show key roles in the prevention of certain diseases [6]. The fatty acid content of milk is affected by numerous factors such as the geographical location, the breed type and the genetic and physiological factors of the animals. In addition, milk processing conditions like the heat treatment, the added starter culture and the storage conditions also affect the fatty acid composition of different dairy products. Therefore, the aim of this work was to study the effect of storage time on fatty acid composition of kombucha fermented milk.

Experimental

Pasteurized and homogenized milk with 2.8% milk fat (Dairy Subotica, Subotica, Serbia), was used for the laboratory manufacture of kombucha fermented milk. Kombucha inoculum was obtained by cultivation of Kombucha on black tea (1.5 g/l) with sucrose concentration of 70 g/l. The tea was cooled at the room temperature, after which inoculum from a previous fermentation was added in concentration of 10%. Incubation was performed at 25°C for 7 days [4]. Kombucha inoculum in concentration of 10% was applied for milk fermentation. The fermentation was performed at 42°C and it lasted until the pH value of 4.5 was reached. Milk gel was then cooled to the temperature of 4°C, homogenized by mixer, packed in polypropylene glasses and stored in refrigerator at 4°C [2]. The extraction of fat from kombucha fermented milk product was carried out as described by Havemose et al. [7], whiles the methylation of fatty acid and was performed according to the methodology of Kravić et al. with minor modifications [8]. Fatty acid profile of the kombucha fermented milk product was determined by gas chromatography-mass spectrometry in accordance to our previously published experimental results [9].

Results and discussion

The fatty acid composition of the kombucha fermented milk product after production (0 day) and during 7, 14 and 21 days of storage are given in Table 1, as relative ratio of total fatty acid content. The presented results represent the mean of three replications for each sample. The distribution of saturated, monounsaturated and polyunsaturated fatty acids in analysed samples is presented in Figure 1.

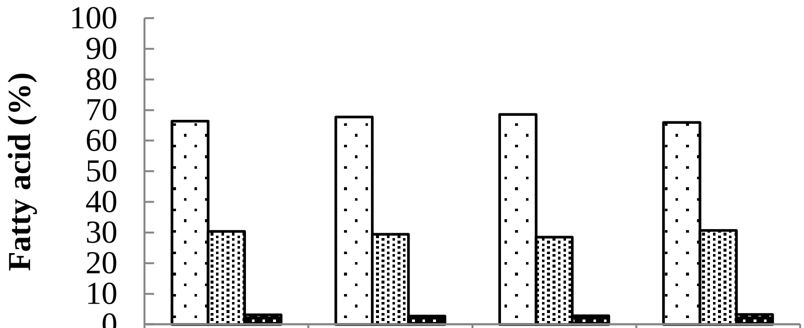
As can be seen from Table 1 predominant fatty acids in all analysed samples of kombucha fermented milk product were palmitic (C16:0), oleic (C18:1c), stearic (C18:0) and myristic acid (C14:0), with mean contents of 32.63; 26.65; 11.11 and 10.80%, respectively. These fatty acids comprised about 81% of the total fatty acids. These results are in accordance with the results presented by other research [1, 10]. The content of branched-chain fatty acids, characteristic minor fatty acids of ruminants, was ranged from 1.29% to 1.53%, and this percentage was made up of five different acids: iso- and anteiso-C15, iso- and anteiso-C17 and iso-C16. Recent studies have shown that branched-chain fatty acids have anti-cancer activity. The cytotoxicity of branched-chain fatty acids was comparable to that of conjugated linoleic acid, a generally lesser component of milk fat which has received much greater attention as a potential anti-cancer agent. These fatty acids inhibited fatty acid synthesis in tumour cells, which is recognised as a useful route for developing cancer treatments since cancer cells are more dependent on fatty acid biosynthesis than healthy cells [11]. The highest content of branched-chain fatty acids was detected in kombucha fermented milk product after 21 day of storage.

Table 1. Fatty acid profile of kombucha fermented milk product during storage

Fatty acid	0 day	7 day	14 day	21 day
4:0	1.22	1.19	1.07	1.15
6:0	1.11	1.08	1.03	1.12
8:0	0.78	0.76	0.75	0.80
10:0	2.22	2.19	2.20	2.18
12:0	2.89	2.93	2.96	2.82
14:0	10.88	11.24	11.44	10.89
14:1	0.74	0.73	0.79	0.86
15:0i	0.18	0.19	nd	0.20
15:0a	0.39	0.36	0.40	0.41
15:0	1.14	1.09	1.15	1.16
16:0i	0.21	0.20	0.20	0.22
16:0	33.01	34.13	34.54	32.50
16:1	1.45	1.40	1.46	1.56
17:0i	0.22	0.20	0.33	0.30
17:0a	0.37	0.34	0.37	0.40
17:0	0.60	0.56	0.62	0.66
17:1	nd	nd	nd	0.21
18:0	11.15	11.34	11.56	10.96
18:1t	2.13	1.64	1.02	2.29
18:1c	26.13	25.69	26.26	25.65
18:2c	2.86	2.73	2.88	2.98
18:3n3	0.32	nd	nd	0.35
20:0	nd	nd	nd	0.18
20:1	nd	nd	nd	0.15

nd – not detected; t-trans; c-cis; i-iso; a-anteiso

Results presented in Fig 1. show that during 14 days of storage content of saturated fatty acids slightly increased, while contents of monounsaturated and polyunsaturated fatty acids decreased. After 21 days of storage the content of SFA; MUFA and PUFA in kombucha fermented milk product was very similar with those obtained at starting point, i.e. immediately after production of fermented beverage. Results showed that the storage time has not significant influence on the fatty acid profile of kombucha fermented milk product.



	0 day	7 day	14 day	21 day
SFA	66.37	67.80	68.61	65.94
MUFA	30.45	29.47	28.51	30.73
PUFA	3.18	2.73	2.88	3.33

Figure 1. Distribution of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in kombucha fermented milk product during storage

Conclusion

This study examined the fatty acid profile of novel functional dairy products, kombucha fermented milk product, during storage. Based on the results it can be concluded that predominant fatty acids in all samples were saturated fatty acids. The most common was palmitic acid, followed by stearic and myristic acid. Of unsaturated fatty acids, the dominant were monounsaturated fatty acids, primarily oleic. The obtained results showed that fatty acid composition of kombucha fermented milk product was not changed during 21 days of storage.

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