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SCIENTIFIC PAPER

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INFLUENCE OF KOMBUCHA INOCULUM ON THE FATTY ACID COMPOSITION OF FERMENTED MILK PRODUCTS

Dairy products obtained by the fermentation of cow's milk with kombucha cultivated on stinging nettle and peppermint tea at two different fermentation temperatures were studied in order to investigate influence of inoculum on fatty acid composition. Analysis of fatty acid methyl esters was performed by gas chromatography. The saturated, mono and polyunsaturated fatty acid contents were within the ranges of 62.86-70.42, 27.85-32.62 and 1.56-4.25% of total fatty acids, respecttively. Among all investigated samples, milk fermented at temperature of 43°C with kombucha cultivated on stinging nettle tea showed the most desirable fatty acid composition with the lowest saturated fatty acid and the highest mono and polyunsaturated fatty acid contents.

Key words: fatty acids • kombucha • fermented milk products

INTRODUCTION

The nutritional importance of milk constituents places milk above other nutritive substances, because it contains all of the main nutrient groups (Lorand, 1913).

Milk fat is the most variable component of the milk constituents (Kadegowda, 2008). It is also an important dietary source of nutrients and energy, but during the past several decades milk fat has been considered as a risk factor for coronary heart diseases (CHD) and reduction in fat intake has been recommended. However, it has been cleared that types of fat have a more important role in determining risk of CHD than total amount of fat in the diet (Hu *et al.*, 2001). Various fatty acids have different effects on plasma lipids: short and medium chain fatty acids do not affect plasma lipoproteins, while consumption of saturated fatty acids (SFA), specifically saturated fats with 12-16 carbon atoms tend to increase plasma total and low density lipoprotein (LDL) cholesterol levels (He *et al.*, 2007). The fatty acid composition of milk fat typically comprises 70% saturated fatty acids, 25% monounsaturated fatty acids, and 5% polyunsaturated fatty acids. The carbon number of the fatty acids, their degree of unsaturation, and their positional distribution within the triacylglycerol molecules influence the nutriational and physical properties and consumer acceptance of foods containning milk fat (Bobe *et al.*, 2007). Milk is ideal for human nutrition because of high content of short chain fatty acids which can be more easily attacked by the digestive enzymes. Although milk fat contains a relatively small amount of unsaturated fatty acids, it is an important source of essential fatty acids, especially arachidonic acid (Salamon *et al.*, 2009). Additionally, milk fat is a significant source of conjugated linoleic acids (CLA), which have recently been recognised as a nutrient that exerts important physiological effects (Gulati *et al.*, 2000).

Kombucha or the tea fungus is a symbiotic culture of acetic acid bacteria and fungi capable of producing a refreshing beverage with many beneficial effects on human health, by means of fermentation of sugared tea (Dufresne and Farnworth, 2000). Black and green tea are typical substrates for kombucha cultivation, however, it can be cultivated on different atypical nutrients such as coca-cola, wine, vinegar, extract of *Echinacea*, *Mentha* or molasses from sugar beat processing (Malbaša *et al.*, 2009).

Fermentation milk products contain all important food ingredients in such relation that a human body can optimally use them and because of that they belong to a group of very important food in human nutrition. They, as well as milk, contain all the basic ingredients needed for growth of a human body, for development, reproduction, maintenance and satisfying energy needs. During fermentation there is a change of some constituent of milk and with creating of new constituents, fermented products get new features compared to milk (Vitas *et al.,* 2010).

Composition of dairy products manufactured by adding pure cultures is determined to the greatest extent by the composition of the raw milk, since the cultures produce rather aroma materials and they affect fatty acid composition to a smaller extent. Kravić *et al.* (2011) noted changes in composition and contents of some

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fatty acid during fermentation of cow's milk with kombucha starters cultivated on differrent inoculums, but still small number of researches exist about inoculum effect on the fatty acid composition of kombucha fermented milk products. Therefore, the aim of this study was to examine fatty acid composition of dairy product obtained by addition of kombucha cultivated on peppermint and stinging nettle tea to cow's milk at two different fermentation temperatures: 40ºC and 43ºC.

MATERIALS AND METHODS

Inoculum

Two different kombucha inoculums were prepared. The first inoculum was prepared with peppermint extract as follows: in 1 dm^3 of boiling tap water 70 g sucrose and 2.25 g peppermint tea (tea in bulk purchased at a local health food store) was added. After boiling for 5 minutes, the tea was cooled to room temperature, strained, and then 100 cm^3 inoculum from a previous fermentation was added. The glass jar was covered with fabric bandwidth for air. Kombucha incubation was performed at room temperature for 7 days. The second inoculum was prepared with stinging nettle extract (tea in bulk purchased at a local health food store) under the same conditions as the inoculum with peppermint extract.

Production of fermented milk products

Pasteurized and homogenized milk with 1.6% milk fat, from the producer "AD IMLEK" Belgrade, Department "Novosadska mlekara", Novi Sad, was used for the laboratory manufacture of fermented milk products by adding 10% (v/v) kombucha inoculum. Kombucha was added in milk at two different temperatures: 40ºC and 43ºC, until a pH value of 4.5 was reached. Obtained gels were cooled to the temperature of 8ºC and homogenized by mixing.

Lipid extraction

The extraction of fat was carried out as described by Havemose *et al.* (2004) with minor modifications. Fat was extracted from kombucha milk products (4 cm^3) by adding methanol (4 cm^3) and chloroform (4 cm^3). The mixture was shaken vigorously for 1 min and then centrifuged for 10 min. The lower phase containing the lipid fraction was isolated and evaporated to dryness under nitrogen.

Preparation of fatty acid methyl esters

The methylation of fatty acid extracted from milk-based kombucha products was carried out as described by Kravić *et al.* (2010) with minor modifications. Previously extracted fats were dissolved in 2.4 cm^3 of hexane. An aliquot (0.6 cm^3) of 2 mol dm^{-3} methanolic KOH solution was added. The tube was capped and vigorously shaken for 20 s and allowed to boil one min in water bath at 70°C. After 20s of shaking 1.2 cm^3 of 1 mol dm^{-3} HCl was added and gently stirred. After phase separation the upper phase containing the fatty acid methyl esters was decanted and 2 µl was used for further analysis.

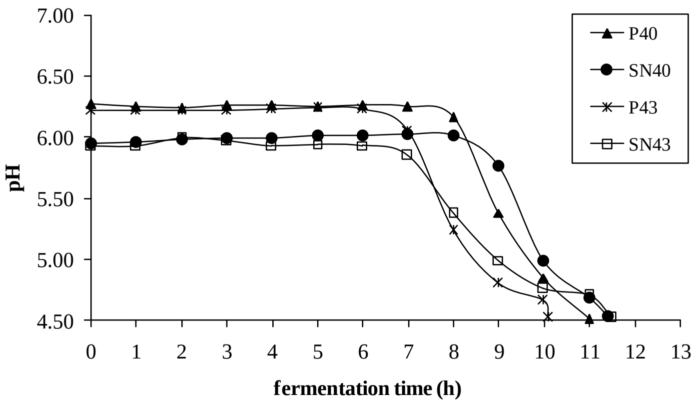

Figure 1. FERMENTATION PATTERN OF KOMBUCHA FERMENTED MILK PRODUCTS

Slika 1. FERMENTACIONI DIJAGRAM PROIZVODNJE KOMBUHA FERMENTISANIH PROIZVODA

Gas chromatography analysis

The analysis of fatty acid methyl esters was performed on a Becker 409 gas chromatograph equipped with a flame ionization detector (FID). Chromatographic resolution was achieved using a stainless steel column (3 m × 3 mm i.d.) packed with 10% SP-2330 on 100/120 mesh Chromosorb W AW (Supelco). The inlet temperature was 240ºC, and the carrier gas was nitrogen (99.9%) with constant flow rate of 15 cm^3 min^{-1}. Injected sample volumes were 2 µl. The analyses were performed at isothermal run with oven temperature of 180ºC. Identification of individual fatty acid methyl esters was based on relative retention times of commercial standard FAME Mix RM-6 (Supelco) and on dependence of Kovats index of relative retention times. Quantitative determination of separated fatty acid methyl esters was done using method 100%.

RESULTS AND DISCUSSION

Changes of pH value during fermentation of milk

Kombucha fermentation was monitored by measuring pH. Course of fermentation of milk with 1.6% milk fat in the production process of kombucha fermented milk products is shown in Figure 1. Samples were labelled as following, milk fermented with kombucha cultivated on peppermint tea: at a temperature of 40ºC - P40 and at a temperature of 43ºC - P43; milk fermented with kombucha inoculum cultivated on stinging nettle tea: at temperature of 40ºC – SN40 and at temperature of 43ºC – SN43. Fermentation was stopped after reaching the pH value of 4.5. Sample P43 achieved the desired pH value for 10.10 hours, sample P40 for 11.00 hours and samples SN40 and SN43 for 11.50 hours.

Fatty acid composition of kombucha fermented milk products

The fatty acid composition of the milk and resulted four kombucha milk products are given in Table 1, as relative ratio of total fatty acid content. The presented results represent the

Table 1. FATTY ACID COMPOSITION OF MILK AND KOMBUCHA FERMENTED MILK PRODUCTS

Tabela 1. SASTAV MASNIH KISELINA MLEKA I KOMBUHA FERMENTISANIH MLEČNIH PROIZVODA

Sample	**M1.6**	**SN40**	**SN43**	**P40**	**P43**
Fatty acid content (% of total fatty acid)					
C4:0	1.23±0.07	1.31±0.08	1.27±0.05	1.24±0.04	1.17±0.07
C6:0	0.82±0.04	0.84±0.05	0.77±0.06	0.69±0.04	0.73±0.06
C8:0	0.37±0.03	0.23±0.02	0.18±0.01	0.27±0.02	0.32±0.02
C10:0	2.82±0.07	2.34±0.06	2.24±0.07	6.28±0.08	2.54±0.05
C11:0	0.24±0.02	0.10±0.01	0.17±0.01	0.23±0.02	0.24±0.02
C12:0	3.50±0.10	3.40±0.11	3.51±0.12	3.25±0.11	3.64±0.09
C13:0	0.19±0.02	0.09±0.01	0.58±0.03	0.14±0.01	0.08±0.01
C14:0	12.04±0.41	11.93±0.31	11.41±0.43	12.66±0.37	14.54±0.43
C14:1	2.81±0.11	2.73±0.09	2.23±0.08	2.26±0.11	2.13±0.12
C15:0	0.25±0.02	0.26±0.02	0.24±0.01	0.16±0.02	2.07±0.12
C16:0	32.36±0.43	32.87±0.62	31.23±0.82	32.21±0.71	34.50±0.52
C16:1	2.26±0.12	2.50±0.16	2.08±0.11	2.14±0.11	2.23±0.12
C17:0	0.27±0.02	0.33±0.03	0.34±0.03	0.22±0.01	0.66±0.05
C17:1	0.34±0.03	0.34±0.03	0.80±0.06	0.29±0.02	0.37±0.03
C18:0	11.59±0.18	11.45±0.33	10.94±0.27	10.21±0.17	9.95±0.26
C18:1	25.95±0.42	26.34±0.51	27.52±0.71	24.57±0.48	23.12±0.41
C18:2	2.79±0.07	2.93±0.11	4.25±0.14	3.19±0.09	1.56±0.07
SFA	65.68	65.13	62.86	67.55	70.42
MUFA	31.35	31.91	32.62	29.26	27.85
PUFA	2.79	2.93	4.25	3.19	1.56
UFA	34.14	34.83	36.87	32.45	29.40
AI	2.46	2.41	2.18	2.65	3.28

SFA – saturated fatty acids / zasićnene masne kiseline
MUFA – monounsaturated fatty acids / mononezasićene masne kiseline
PUFA – polyunsaturated fatty acids / polinezasićene masne kiseline
UFA – unsaturated fatty acid / nezasićene masne kiseline
AI – atherogenic index / aterogeni indeks

mean ± standard deviation of three replications for each sample.

As can be seen from Table 1 predominant fatty acids in milk and kombucha fermented milk products were palmitic (C16:0), followed by oleic acid (C18:1), myristic acid (C14:0) and stearic acid (C18:0), which together accounted for around 83% of total fatty acids. Samples P40 and P43 showed a higher relative content of SFA and a lower relative content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) compared to control milk. The relative content of SFA in samples SN40 and SN43 was lower than in control milk, while the relative content of MUFA in the same samples slightly increased. The relative content of PUFA was near two-fold higher in sample SN43 compared to control milk and slightly higher in sample SN40. Among investigated kombucha fermented milk products, those fermented at temperature of 43ºC with kombucha cultivated on stinging nettle tea showed the most desirable fatty acid composition with the lowest SFA content and the highest MUFA and PUFA contents compared to control milk sample and the other kombucha fermented milk products.

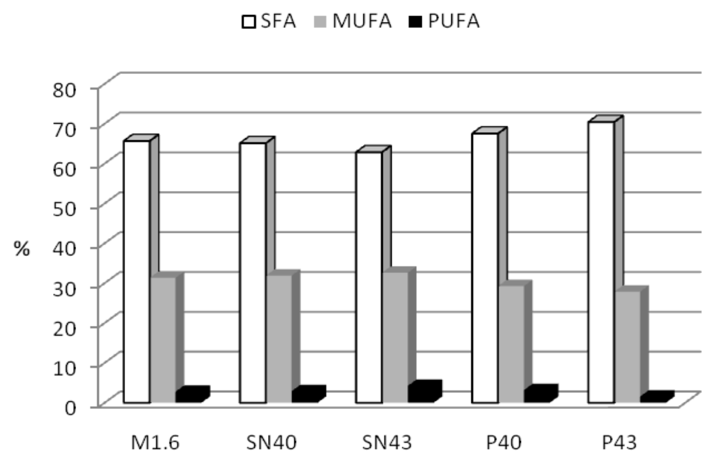

Figure 2. TOTAL CONTENT OF SATURATED, MONO AND POLYUNSATURATED FATTY ACIDS OF MILK AND KOMBUCHA FERMENTED MILK PRODUCTS

Slika 2. UKUPAN SADRŽAJ ZASIĆENIH, MONO I POLINEZASIĆENIH MASNIH KISELINA U MLEKU I KOMBUHA FERMENTISANIM MLEČNIM PROIZVODIMA

Saturated fatty acids with a chain length of C12:0-C16:0 are atherogenic, stearic acid is neutral, and oleic and polyunsaturated fatty acids have a lipid lowering effect (Kravić *et al.*, 2011). The sum of lauric (C12:0), myristic and palmitic acids was the highest in milk fermented with kombucha cultivated on peppermint tea at temperature of 43ºC (52.68%), which was higher than in control milk, while the lowest atherogenic fatty acids content was observed in sample SN43 (46.14%). In addition, sample SN43 showed the highest contents of oleic and linoleic (C18:2c) acids. Amount of stearic acid was ranged from 9.95% to 11.59%. Medium chain capric acid (C10:0) was about two-fold higher in sample P40 compared to milk and other kombucha fermented milk products.

The atherogenic index is the sum of concentrations of C12:0, C16:0, and 4 x C14:0 divided by the concentration of total unsaturated fatty acids (Kravić *et al.*, 2011) and it was proposed as a dietary risk indicator of lipids for cardiovascular diseases. According to this equation, all unsaturated fatty acids, regardless of their double-bond number, position, or configuretion, are considered to be equally effective in decreasing the risk for atherogenicity, primarily for lack of reliable information to assign more suitable coefficients to the individual acids. The results of this investigation indicate that milk fermented at temperature of 43ºC with kombucha cultivated on stinging nettle tea has the lowest atherogenic index compared to all examined samples.

In Figure 2 total contents of SFA, MUFA and PUFA of control milk and kombucha fermented milk products is showed. It is evident that saturated fatty acid content slightly increases while polyunsaturated fatty acid content decreases with rising of fermentation temperature in samples obtainned by fermentation of milk with kombucha inoculum prepared with peppermint extract. Otherwise, in samples obtained by fermentation of milk with kombucha inoculum prepared with stinging nettle extract saturated fatty acid content decreases while polyunsaturated fatty acid content increases with rising of fermentation temperature.

CONCLUSION

This study demonstrates that milk fermented at a temperature of 43ºC with kombucha cultivated on stinging nettle tea probably has a more health-promoting fatty acid composition compared to milk and milk fermented with kombucha cultivated on peppermint tea. This product showed lower content of saturated fatty acids and higher content of unsaturated fatty acids compared to the same samples. It also had lower atherogenic index.

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IZVOD

UTICAJ INOKULUMA KOMBUHE NA SASTAV MASNIH KISELINA U FERMENTISANIM MLEČNIM PROIZVODIMA

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Mlečni napici proizvedeni fermentacijom kravljeg mleka pomoću kombuhe kultivisane na čaju koprive i mente na dve različite temperature fermentacije su analizirani u cilju ispitivanja uticaja inokuluma na sastav masnih kiselina. Analiza metil estara masnih kiselina izvedena je primenom gasne hromatografije. Sadržaj zasićenih, mono i polinezasićenih masnih kiselina kretao se u opsegu 62,86-70,42; 27,85-32,62 i 1,56-4,25%, redom. Među svim ispitivanim uzorcima, mleko fermentisano na 43ºC inokulumom kombuhe kultivisane na čaju koprive, pokazalo je najpovoljniji masno-kiselinski sastav sa najnižim sadržajem zasićenih masnih kiselina i najvišim sadržajem mono i polinezasićenih masnih kiselina.

Ključne reči: masne kiseline • kombuha • fermentisani mlečni proizvodi