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Original scientific paper

INFLUENCE OF FERMENTATION TEMPERATURE ON THE CONTENT OF FATTY ACIDS IN LOW ENERGY MILK-BASED KOMBUCHA PRODUCTS

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The aim of this study was to investigate the influence of fermentation temperature on the fatty acids content in low energy milk-based products obtained by kombucha inoculums with herbal teas.

In this investigation low energy milk-based kombucha products were produced from milk with 0.8% milk fat using 10% (v/v) kombucha inoculums cultivated on winter savory, peppermint, stinging nettle and wild thyme.

The process of fermentation was conducted at two temperatures: 40°C and 43°C. Fermentation was stopped after the pH value of 4.5 was reached. Duration of the fermentation process was shorter by applying higher fermentation temperature.

Fatty acids content was determined by gas chromatography-mass spectrometry. Predominant fatty acids in all obtained products were saturated fatty acids, first of all the monounsaturated ones. The higher temperature resulted in the formation of lower amount of saturated fatty acids in the obtained milk-based kombucha products.

KEY WORDS: temperature, fatty acids, milk-based kombucha products, GC-MS

INTRODUCTION

Kombucha or tea fungus is a symbiotic association of acetic acid bacteria (genera *Acetobacter* and *Gluconobacter*) and autochthonous species of yeasts (genera *Saccharomyces*, *Zygosaccharomyces*, *Saccharomycodes*, *Torulaspora* and others). Although kombucha is most commonly prepared by biotransformation of sweetened black tea, other substrates such as: coca-cola, herbal teas, wine, beer, fruit drinks, milk, glucose, fructose and lactose, can be used too (1).

Fermented milk products are of great significance in nutrition of healthy and ill people. 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. These products are a source of proteins, calcium, B group vitamins, and the amount of carbo-

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hydrates and lactose is reduced, so these products can be consumed by lactose-intolerant people. The daily intake of these products allows the normal functioning of intestinal tract, and they can be applied in the treatment of diarrhea (2).

The composition of milk (whole, partially skimmed or skimmed) has a significant impact on the quality of fermented milk products. Taking into account the complex structure and composition of the fat globules, milk fat represents an essential component in the diet and in the formation of nutritional and sensory properties of fermented milk products (3). It is known that the atherogenic index (AI) was proposed as a dietary risk indicator of lipids for cardiovascular diseases. Lower value of AI indicates positive impact to human health (4).

Studies have shown that milk fermentation with kombucha inoculum, whereby the lactose is the source of carbon, can produce a high quality functional milk beverage (1).

In this work, four different herbal teas (winter savory, peppermint, stinging nettle and wild thyme) were used to prepare the extracts on which cultivation of kombucha was performed. The positive effects of these herbs on human health are well documented in the literature (5-8).

The objective was to investigate the influence of fermentation temperature on the content of fatty acids in low energy milk-based products obtained by means of kombucha inoculums with herbal teas.

EXPERIMENTAL

Milk

Pasteurized, homogenized milk with 0.8% milk fat, from the manufacturer AD IMLEK Beograd, branch Novosadska mlekara, Novi Sad was used for the production of fermented milk products in the laboratory.

Initial kombucha inoculum

Initial kombucha inoculum was the fermentation liquid obtained by cultivation of kombucha on the extracts prepared from winter savory, peppermint, stinging nettle and wild thyme, sweetened with 7% sucrose, during 7 days. Extracts were prepared by using bulk teas purchased in a health food store.

Production of kombucha inoculums on herbal extracts

Fermentation liquid used as inoculum for the fermentation of milk was obtained by cultivation of kombucha on cooled tea, which was prepared as follows: to 1 L of boiling tap water was added 70 g sucrose and 2.25 g of appropriate tea (winter savory (label WS), peppermint (label P), stinging nettle (label SN) and wild thyme (label WT). The prepared tea was cooled to room temperature, strained and then was added 100 mL of initial kombucha inoculum from a previous fermentation, respectively 10% of fermentation liquid. The glass jar was covered with fabric bandwidth for air. Kombucha incubation

was performed at room temperature for 7 days. The obtained kombucha inoculums (marked as WSI, PI, SNI and WTI) were used for fermentation of milk at 40 and 43°C.

Production of fermented milk products

Fermented milk products were produced from pasteurized, homogenized milk with 0.8% milk fat, as follows: to the 500 mL of milk, 10% (v/v) of the appropriate kombucha inoculum was added. Fermentation liquids of kombucha obtained by fermentation on extracts prepared from winter savory, peppermint, stinging nettle and wild thyme were used as starter cultures. The fermentation was performed at 40 and 43°C and it lasted until the pH value of 4.5 was reached. Gel was then cooled to the temperature of 8°C, homogenized by mixer, and the samples were stored in refrigerator. The obtained products were marked as WS40, P40, SN40, WT40, WS43, P43, SN43 and WT43 in dependence of the applied temperature.

Methods of analysis

The pH values of kombucha inoculums, milk and fermented milk products were determined using standard methods (9).

Composition and content of fatty acids of the obtained fermented milk products was determined by gas chromatography-mass spectrometry (GC-MS) (4). Samples for GC-MS were prepared in accordance to our previously published experimental results (4). Blank samples were obtained as follows: to 20 mL of milk with 0.8% milk fat was added 2 mL of the appropriate kombucha inoculum immediately followed by sample preparation for GC-MS, so there was no fermentation. Inoculums were added to the milk in the amount of 10% (v/v), respectively as in the production of fermented milk products. The obtained blanks were marked as WS*, P*, SN* and WT*. The blanks were made to compare the content of fatty acids before and after the fermentation and evaluate the influence of fermentation temperature on the measured values.

RESULTS AND DISCUSSION

Milk used for production of fermented milk products was very slightly acidic, as its pH value was 6.68.

Kombucha inoculums were moderately acidic, respectively considerably more acidic comparing to milk. The difference in pH values of the used kombucha inoculums comparing to milk was 2.29-3.38 pH units. Acidity of the inoculum depended on the tea used. The most acidic was SNI, while the lowest acidity had WSI. The difference in acidity of these two inoculums was 1.09 pH units. Acidity of the inoculums decreased in the following order: SNI>PI>WTI>WSI.

The dynamics of milk fermentation with different kombucha inoculums at 40 and 43°C is presented in Figures 1 and 2.

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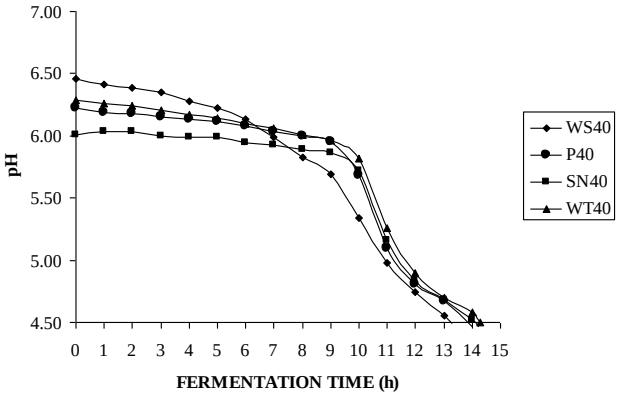

Figure 1. Fermentation process of milk with different kombucha inoculums at 40°C

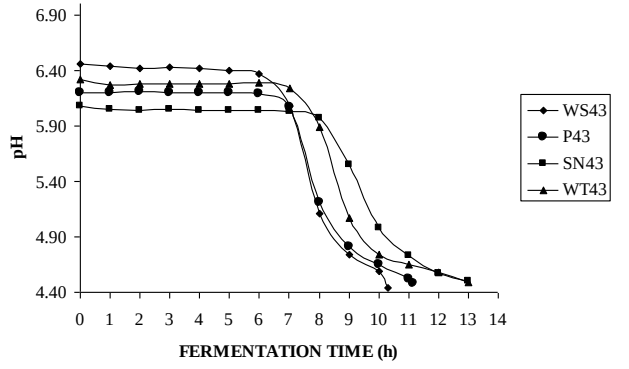

Figure 2. Fermentation process of milk with different kombucha inoculums at 43°C

The results presented in Figures 1 and 2 clearly show that the fermentation temperature of 43°C shortened the fermentation time to reach the desired value of pH (4.5). While the fermentation period at 40°C was shortest using inoculum WSI, 13.5 hours, the longest fermentation period at 43°C was 13 hours using SNI and WTI. The shortest fermentation, 10.5 hours, at 43°C was also observed using WSI. All fermentation curves in Figures 1 and 2 have a sigmoidal shape, which means that the dynamics of fermentation were similar on both temperatures with all applied inoculums.

The fatty acid composition of the blank samples and the four kombucha fermented milk products produced at temperature 40°C is given in Table 1. The distribution of saturated and unsaturated fatty acids in samples obtained at 40°C is presented in Figure 3.

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Table 1. Fatty acid composition of blank samples and kombucha fermented milk products produced at 40°C

Fatty acid	Sample							
	WS*	WS40	P*	P40	SN*	SN40	WT*	WT40
	Fatty acid content (% of total fatty acids)							
C4:0	1.22	1.92	1.55	1.22	1.46	2.40	2.54	1.87
C6:0	1.30	1.69	1.32	1.51	0.88	2.11	1.93	1.69
C8:0	0.99	1.31	0.95	0.83	0.84	1.32	1.34	1.34
C10:0	2.87	3.56	2.80	2.63	2.51	3.75	3.69	3.77
C12:0	3.71	4.42	3.74	3.59	3.56	5.03	4.41	4.62
C14:0	13.82	14.41	13.31	13.47	12.41	15.03	15.19	14.45
C15:0i	0.22	0.13	nd	nd	0.25	0.36	0.35	nd
C15:0a	0.52	0.62	0.51	0.59	0.54	0.71	0.73	0.69
C14:1	1.07	1.31	1.10	1.00	1.16	1.27	1.55	1.35
C15:0	1.55	1.99	1.63	1.76	1.76	1.95	2.34	1.99
C16:0i	0.34	0.33	nd	0.18	0.32	0.42	0.35	0.39
C16:0	29.61	29.21	28.93	31.04	26.29	29.15	29.22	29.47
C17:0i	0.50	0.47	0.51	0.48	0.52	0.55	0.50	0.49
C17:0a	0.51	0.48	0.52	0.52	0.54	0.56	0.37	0.60
C16:1	2.33	2.30	2.41	2.15	2.54	2.17	2.29	2.32
C17:0	1.22	1.12	1.17	1.17	1.29	1.04	0.97	1.16
C17:1	nd	nd	nd	nd	0.25	nd	nd	nd
C18:0	12.53	12.07	13.30	13.95	13.70	10.71	10.80	11.62
C18:1t	2.72	2.68	2.76	2.69	2.60	2.09	2.03	2.27
C18:1c	19.87	17.87	19.94	17.59	22.01	16.56	16.74	17.29
C18:2t	nd	nd	nd	nd	0.52	0.27	nd	nd
C18:2c	3.10	2.11	3.55	3.06	4.04	2.55	2.65	2.91
C20:0	nd	nd	nd	0.57	nd	nd	nd	nd
SFA	70.92	73.73	70.24	73.50	66.87	75.10	74.74	74.14
MUFA	25.98	24.16	26.22	23.44	28.56	22.08	22.61	23.23
PUFA	3.10	2.11	3.55	3.06	4.56	2.81	2.65	2.91
AI	3.05	3.47	2.89	3.34	2.40	3.79	3.74	3.52

nd-not detected
SFA-saturated fatty acids
MUFA-monounsaturated fatty acids
PUFA-polyunsaturated fatty acids
AI-atherogenic index
t-*trans*-isomer
c-*cis*-isomer
i-*iso*-isomer
a-*anteiso*-isomer

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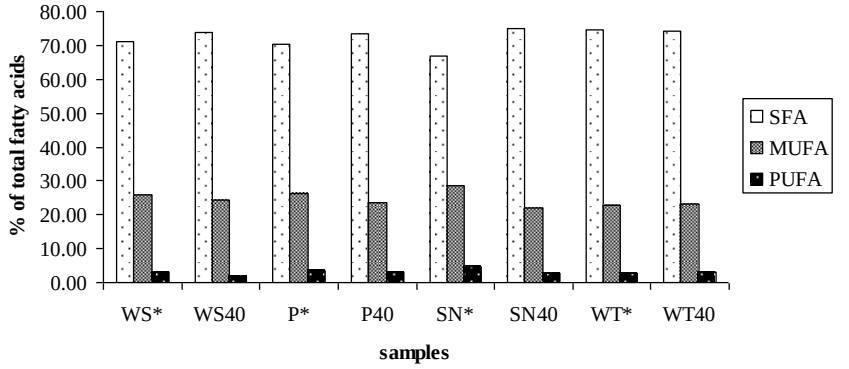

Figure 3. Distribution of saturated (SFA) and unsaturated (MUFA and PUFA) fatty acids in blank samples and kombucha fermented milk products obtained at 40°C

Based on the results given in Table 1 and Figure 3, it can be concluded that predominant fatty acids in all samples were saturated fatty acids. The most common was palmitic acid (C16:0), followed by myristic (C14:0) and stearic (C18:0). Of unsaturated fatty acids, the dominant were monounsaturated acids, primarily *cis*-oleic (C18:1c). By evaluating the fatty acid profiles, it can be established that only sample WT showed higher content of MUFA and PUFA compared to WT*.

As for the profile of saturated fatty acids in sample P40, it is obvious that in addition to the usual fatty acids from C4-C18, the fatty acid with C20 also occurred, but in amount below 1%.

All samples showed a higher AI, compared to the corresponding blank sample, except for sample WT40, but this difference is not significant.

The fatty acid composition of the blank samples and the four kombucha fermented milk products produced at temperatures 43°C are given in Table 2. The distribution of saturated and unsaturated fatty acids in samples obtained at 43°C is presented in Figure 4.

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Table 2. Fatty acid composition of blank samples and kombucha fermented milk products produced at 43°C

Fatty acid	Sample							
	WS*	WS43	P*	P43	SN*	SN43	WT*	WT43
	Fatty acid content (% of total fatty acids)							
C4:0	1.22	0.65	1.55	0.49	1.46	3.22	2.54	1.93
C6:0	1.30	1.35	1.32	1.02	0.88	2.44	1.93	1.62
C8:0	0.99	1.06	0.95	0.90	0.84	1.47	1.34	1.21
C10:0	2.87	2.81	2.80	2.89	2.51	3.90	3.69	3.18
C11:0	nd	0.17	nd	0.19	nd	nd	nd	nd
C12:0	3.71	3.95	3.74	3.89	3.56	4.43	4.41	4.07
C13:0	nd	0.24	nd	0.22	nd	nd	nd	nd
C14:0i	nd	nd	nd	0.14	nd	nd	nd	nd
C14:0	13.82	12.06	13.31	12.66	12.41	14.34	15.19	14.18
C15:0i	0.22	0.25	nd	0.31	0.25	0.36	0.35	0.37
C15:0a	0.52	0.58	0.51	0.65	0.54	0.71	0.73	0.78
C14:1	1.07	1.30	1.10	1.26	1.16	1.42	1.55	1.40
C15:0	1.55	1.89	1.63	2.05	1.76	2.15	2.34	2.14
C16:0i	0.34	0.36	nd	0.50	0.32	0.37	0.35	0.54
C16:0	29.61	25.92	28.93	26.39	26.29	28.76	29.22	28.95
C17:0i	0.50	0.57	0.51	0.66	0.52	0.47	0.50	0.59
C17:0a	0.51	0.80	0.52	0.87	0.54	0.53	0.37	0.62
C16:1	2.33	2.51	2.41	2.86	2.54	2.13	2.29	2.40
C17:0	1.22	1.46	1.17	1.56	1.29	1.09	0.97	1.30
C17:1	nd	0.54	nd	0.51	0.25	0.16	nd	0.43
C18:0	12.53	12.64	13.30	13.60	13.70	11.53	10.80	11.51
C18:1t	2.72	2.44	2.76	2.60	2.60	2.11	2.03	2.32
C18:1c	19.87	20.47	19.94	19.53	22.01	16.30	16.74	17.56
C18:2t	nd	0.74	nd	0.79	0.52	0.17	nd	0.39
C18:2c	3.10	4.14	3.55	2.67	4.04	1.93	2.65	2.49
C20:0	nd	0.15	nd	0.32	nd	nd	nd	nd
C20:1	nd	0.29	nd	0.45	nd	nd	nd	nd
C18:3	nd	0.57	nd	nd	nd	nd	nd	nd
C22:0	nd	0.07	nd	nd	nd	nd	nd	nd
SFA	70.92	67.00	70.24	69.31	66.87	75.78	74.74	73.00
MUFA	25.98	27.56	26.22	27.23	28.56	22.12	22.61	24.12
PUFA	3.10	5.45	3.55	3.47	4.56	2.10	2.65	2.89
AI	3.05	2.37	2.89	2.64	2.40	3.74	3.74	3.32

nd-not detected
SFA-saturated fatty acids
MUFA-monounsaturated fatty acids
PUFA-polyunsaturated fatty acids
AI-atherogenic index
t-*trans*-isomer
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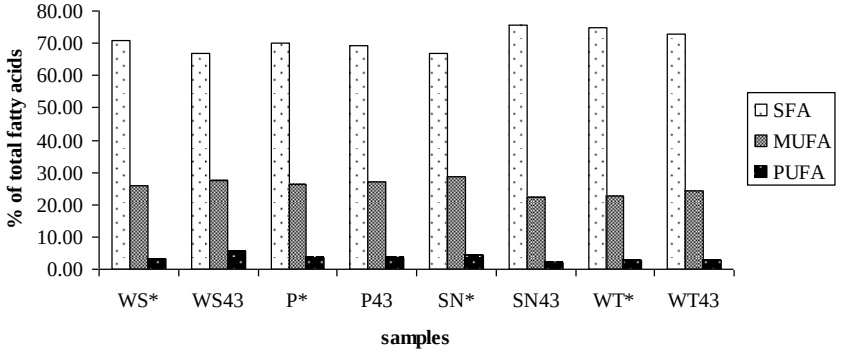

Figure 4. Distribution of saturated (SFA) and unsaturated (MUFA and PUFA) fatty acids in blank samples and kombucha fermented milk products obtained at 43°C

Based on the results given in Table 2 and Figure 4, it can be concluded that predominant fatty acids in all samples were saturated fatty acids. The most common was palmitic acid (C16:0), followed by myristic (C14:0) and stearic (C18:0). Of unsaturated fatty acids, the dominant were monounsaturated fatty acids, primarily *cis*-oleic (C18:1c). By evaluating the fatty acid profiles, it can be established that only sample SN showed a lower content of MUFA and PUFA compared to SN*.

As for the profile of saturated fatty acids in sample P43, it is obvious that in addition to the usual fatty acids from C4-C18, the fatty acid with C20 also occurred, but in amount below 1%. In sample WS43 saturated fatty acid with C20 also occurred, but in a very low amount (0.15%). Sample WS43 contained saturated fatty acid with C22, in an amount of 0.07%.

All samples showed a lower AI, compared to the corresponding blank sample, except the sample SN43, which is very important in view of human health. This means that the obtained products have the better quality in term of AI values in comparison to blank samples.

CONCLUSION

This study examined the possibility of obtaining high-quality fermented milk products at the fermentation temperatures of 40 and 43°C, using milk with 0.8% milk fat and kombucha inoculums cultivated on winter savory, peppermint, stinging nettle and wild thyme. Fermentation was stopped after pH value of 4.5 was reached. The pH values of milk and kombucha inoculums and content of fatty acids in the blank samples and the obtained fermented milk products were determined.

The most common saturated fatty acids were palmitic, myristic and stearic. Of unsaturated fatty acids, the dominant were monounsaturated fatty acids, primarily *cis*-oleic.

The common saturated fatty acids in samples P40 and WS43 were from C4-C18, and the C20 fatty acid below 1% also occurred. Sample WS43 showed the occurrence of the C22 saturated fatty acid.

Products obtained at higher temperature showed lower content of saturated fatty acids by 2.84%. It can be concluded that the temperature of 43°C fermentation process led to the higher formation of unsaturated fatty acids.

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УТИЦАЈ ТЕМПЕРАТУРЕ ФЕРМЕНТАЦИЈЕ НА САДРЖАЈ МАСНИХ КИСЕЛИНА У НИСКО-ЕНЕРГЕТСКИМ МЛЕЧНИМ ПРОИЗВОДИМА ОД КОМБУХЕ

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Циљ овог рада је било истраживање утицаја температуре ферментације на садржај масних киселина у ниско-енергетским млечним производима добијеним применом инокулума комбухе са биљним чајевима.

У овом раду су произведени ниско-енергетски млечни производи од комбухе коришћењем млека са 0,8% млечне масти уз додатак 10% инокулума комбухе култивисане на ртањском чају, нани, копривi и мајчиној душици.

Процес ферментације је изведен на две температуре: 40 и 43°C. Ферментација је заустављена након што је достигнута вредност pH од 4,5. Применом више температуре ферментације трајање процеса ферментације је било скраћено.

Садржај масних киселина је био одређен применом гасне хроматографије са масеном спектрометријом. Најзаступљеније масне киселине у свим добијеним производима су биле засићене масне киселине. Од незасићених масних киселина доминантне су биле мононезасићене масне киселине, у свим добијеним производима. Виша температура је довела до формирања мање количине засићених масних киселина у добијеним млечним производима од комбухе.

Кључне речи: температура, масне киселине, ферментисани млечни производи од комбухе, гасна хроматографија-масена спектрометрија

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