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INVESTIGATION OF THE POTENCIAL USAGE OF TEA FUNGUS KOMBUCHA IN THE PRODUCTION OF FERMENTED DAIRY PRODUCTS
ISPITIVANJE MOGUĆNOSTI UPOTREBE ČAJNE GLJIVE KOMBUHE U PROIZVODNJI FERMENTISANIH MLEČNIH PROIZVODA

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ABSTRACT

Due to the increasing consumers' interest for 'healthy' food, it is necessary to include functional ingredients in our food. Tea kombucha mushroom is just one of them. Black tea sweetened with sucrose is most commonly used as a substrate for tea fungus microorganisms. The metabolic activity of kombucha in the milk and obtaining a new fermented diet dairy drink is the subject of these investigations. The aim is to investigate the effect of different concentrations of inoculum kombucha on the quality of the obtained fermented milk products. In the study, the standard microbiological, chemical and sensory methods were used. The low-energy fermented dairy products with 0.9% milk fat were produced by using the concentrated inoculums symbiotic kombucha culture and probiotic yogurt. In the samples produced by the fermentation of kombucha, the content of L-lactic acid was significantly higher than the content of D-lactic acid, and the highest values were recorded in the kombucha capsules (K-0, 12%),with the average of 0.958 g/100g. The metabolic activity of kombucha fermented milk lactose is slower compared with the probiotic culture, but its content is certainly acceptable, and not much higher than the probiotic yogurt. Fermented drinks contain certain amounts of D-glucose and D-fructose, which depend on the origin and amount of inoculum. The sensory quality of fully fermented milk products obtained by the kombucha fermentation indicate that the ethanol content is minute. The resulting product is characterized by high-quality sensory, microbiological and chemical properties.

***Key words**: Kombuha, probiotics, fermented milk products.*

REZIME

Zbog sve većeg interesovanja potrošača za ,,zdravom,, hranom neophdno je uključivanje funkcionalnih ingredijenata u svakodnevnu ishranu. Čajna gljiva kombuha je samo jedan od njih. Kao supstrat za mikroorganizme čajne gljive najčešće se koristi crni čaj zaslađen saharozom. Metabolička aktivnost kombuhe na mleku i dobijanje novog fermentisanog dijetalnog mlečnog napitka predmet je ovih ispitivanja. Cilj rada je ispitivanje uticaja različitih koncentracija inokuluma kombuhe na kvalitet dobijenih fermentisanih mlečnih proizvoda. U istraživanju su upotrebljene standardne mikrobioloske, hemijske i senzorne metode. To je nisko-energetski fermentisan proizvod od mleka sa 0,9% mlečne masti dobijen pomoću koncentrisanog inokulum simbiotičke kombuha kulture i probiotik jogurta. U uzorcima proizvedenim fermentacijom kombuhe, sadržaj L-mlečne kiseline bio je znatno veći od sadržaja D-mlečne kiseline, a najviša u vrednost bila je u uzorcima kombuha kapsula (K-0, 12%), u proseku 0.958 g/100g. Metabolička aktivnost kombuha fermentisanog mleka je sporija u odnosu na probiotske kulture, ali njen sadržaj je svakako prihvatljiv, a ne mnogo veći od probiotskog jogurta. Fermentisana pića sadrže određene količine D-glukoze i D-fruktozu, koje zavise od porekla i količine inokuluma. Dobijeni proizvod se karakteriše kvalitetnim senzornim, mikrobioloskim i hemijskim svojstvima.

***Ključne reči**: zdrava hrana, kombuha, fermentisani proizvodi.*

INTRODUCTION

In all branches of food and dairy industry (yogurt production in particular) , there is a tendency towards a constant improvement of product quality. The improvement in raw milk quality modernisation of technological processes, and introduction of automated equipment entailing a multiple increase in production capacity contributed to the development and application of different starter cultures (Tamime, 1985). These are natural isolates in the process of selection based on their physiological characteristics, i.e. the ability to produce a certain amount of desirable components (lactate, acetate, diacetyl, acetyl aldehyde, ethanol, carbon dioxide), on the basis of the resistance to bacteriophages, the ability to grow in combination with one another, and in recent years the emphasis has been placed on their probiotic properties (Patocka, et al., 2004; Puvanenthiran, et al., 2002). All micro-organisms that form a part of starter cultures must meet certain criteria: safety (the lack of pathogenic activities, the absence of toxic effects), technological characteristics (the dominance in relation to the spontaneous microflora, stable metabolic activity, resistance to contamination during the technological process) and economic aspects (the application must be profitable, culture should be frozen or lyophilized, the handling of the culture must be simple, basic properties cannot be changed during the period of time). Huge improvements in modern technology development of fermented dairy products were achieved by launching new types of products for special purposes, incorporating probiotic microorganisms in a fermentation starter or already fermented milk. In the production of fermented dairy drinks with probiotic microorganisms (the products of the "third generation"), the microorganisms of the genus Lactobacillus and Bifidobacterium are commonly used although other genera of this group of bacteria (Lactococcus sp., Leuconostoc sp., Some streptococci and enterococci) have a certain positive effect on human health (Biliaderis, 2008).

Due to the increasing interest of consumers for 'healthy' food, it is necessary to include functional ingredients in our food (Lazarides, 2009). Kombucha is a well known symbiotic association of yeast (Saccharomyces Ludwigia, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis sp. Zigosaccharomyces sp) and acetic acid bacteria (Acetobacter xylinum and Gluconobacter oxydans). The metabolic activity of black tea sweetened with sucrose creates a pleasant, slightly sour sparkling beverage, which contains many important nutritional and pharmacological components. The activity of sucrose kombucha is very well researched and determines the main path of conversion of sucrose into numerous products. In addition to sucrose, other sugars such as lactose, glucose and fructose are used. This leads to a lesser impact on the sensors of fermented products, whereas a significant influence on the creation of ethanol and lactic acid is recorded. Although the ability of kombucha fermentation of lactose was established, there was no additional research related to the carbon source. Loncar and colleagues have determined the metabolic activity of kombucha milk, while Bellos-Morales and Hernández-Sánchez published works on the production of kombucha beverage from whey. In addition to the established metabolic activity of kombucha in milk when inoculated in fermentative liquid from the previous fermentation, it was found that the milk can inoculate and concentrated inoculum.

METRIAL AND METHOD

During these tests, the following materials were used for inoculation: probiotic starter cultures - Delvo Yog-MY-721, (manufacturer DSM Food spécialités, Holland.), Kombucha concentrated inoculum (prepared in the laboratory), a substrate with sucrose, black tea, Kombucha capsules (Dr manufacturer. Forster, GmbH, Neu Isenburg, Germany) and homogenized pasteurized milk with 0.9% milk fat (Novi Sad Dairy producers, Novi Sad) and the control yogurt.

Method

Preparation of concentrated inoculums kombuha – The concentrated kombucha inoculum was prepared by kombucha cultivation on a substrate with sucrose (the concentration of 70 g / l) with the addition of black tea (1.5 g / l). A 10% (v / v) native inoculum fermentation liquid from a previous fermentation was added to the chilled tea . The incubation was conducted at a temperature of 29.5 ° C, for seven days.

After seven days, the fermentation liquid 6.5% of dry matter was concentrated by evaporation under vacuum at 40 ° C and dry matter content of 64%. Thus obtained concentrate was used as inoculum. Kombucha capsules were also used as inoculim (capsule contents contain dry extract of kombucha - a dietary supplement enriched with vitamins C and E and β-carotene).

Production of fermented milk drinks with starter cultures - Pasteurized, homogenized milk with 0.9% milk fat (of a dairy producer from Novi Sadwas used for manufacturing fermented dairy drinks in the laboratory.

Control yogurt, labeled Ctrl., Manufactured using 0.005% starter culture. The fermentation was performed at 42 ° C until a pH of 4.5. The gel is then cooled to a temperature of 8 o C and homogenized column.

Production of fermented milk drinks with inoculum kombucha - Three fermented milk beverage kombuchas are produced by the same procedure as the control sample and yogurt using: concentrated inoculum kombucha and kombucha capsules. The concentrated Kombucha inoculum was added in two concentrations, and to 1.5 and 3% (v / v) samples were identified as K-1, and 5% K-3%. The kombucha capsules were added at the concentration of 0:12 5 (v / v) and this pattern was designated as LC-0, 12%. The production was repeated in two series.

Method of analisis

In the laboratory-produced samples of fermented lactic acid beverage by using specific enzyme tests (Me-gazyme, Ireland) were determined: D-lactic and L-lactic acid, acetic acid, lactose and D-galactose, D-fructose and D-glucose and ethanol. Analyses were performed according to manufacturer's instructions and the content of the reaction product was measured spectrophotometrically (*Carić, et al., 2000*).

RESULTS AND DISCUSSION

Based on the grades of two qualified and four randomly selected evaluators, the produced fermented milk drinks were deemed fully acceptable sensory, with similar or slightly lower quality than the probiotic yogurt. In the samples, the contents of D-and L-lactic and acetic acid, D-glucose, D-fructose, lacto-ze, D-galactose and ethanol were determined. The results are shown in Tables 1-3.

Table 1. Chemical composition of fermented dairy drinks with Kombucha - Series 1

Sample %	Component (g/100g)							
	D-lactic acid	L-lactic acid	Acetic acid	Lactose	D-galactose	D-glucose	D-fructose	Ethanol
K - 1,5	0.025	0.604	0.004	3.42	0.47	0.119	0.046	0.0044
K – 3	0.006	0.680	0.014	3.31	0.37	0.390	0.319	0.0013
K-0,12	0.035	1.007	0.003	3.02	0.82	0.025	0.032	0.0014

According to the results (Tables 1-3), it can be observed that the reproducibility of fermentation of the two series is quite good. There are some discrepancies, such as the content of L-lactic acid in the sample K-1, 5%, the second series, which is slightly lower than expected. In addition, the content of D-fructose for the same sample is lower in the same series (Table 2).

Table 2. Results of Chemical composition of fermented milk drinks with Kombucha-SERIES 2

Sample %	Component (g/100g)							
	D-lactic acid	L-lactic acid	Acetic acid	Lactose	D-galactose	D-glucose	D-fructose	Ethanol
K-1,5	0.091	0.256	0.001	3.33	0.55	0.202	0.006	0.0040
K-3	0.005	0.650	0.013	3.24	0.41	0.420	0.202	0.0046
K-0,12	0.093	0.908	0.004	3.16	0.73	0.023	0.067	0.0020

The probiotic yogurt (Table 3) is approximately equal to the visible content of D-and L-lactic acid. In the samples produced by fermentation of kombucha (Tables 1 and 2), the content of L-lactic acid was significantly higher than the content of D-lactic acid.

Table 3. Results of Chemical composition of probiotic yogurth - SERIES 1 and 2

Sample	*Component (g/100g)*							
	D-lactic acid	L-lactic acid	Acetic acid	Lactose	D-galactose	D-glucose	D-fructose	Ethanol
Series 1	0.31	0.32	0.0076	2.40	0.82	0.55	0.056	0.0001
Series 2	0.29	0.28	0.0091	2.54	0.88	0.51	0.0063	0.0002

The sum of the contents of D-and L-lactic acid in the samples K-1, and 5% K-3%, there is a value similar to the sum of D-and L-lactic acid in yoghurt. When the sample K-0 is 12% , this sum is greater due to the higher content of L-lactic acid, which on average amounts to 0.958 g/100g. The higher content of L-lactic acid in samples of fermented Kombucha products is extremely important and very useful for the quality of such drinks produced. There are two forms of lactic acids, "good" or L-lactic, or "bad" or D-lactic acid. L-lactic acid promotes blood circulation, prevents putrefaction in the intestines and constipation, activated bowel movements. It affects the acid-base balance and supports the action of vitamin C in the body, which is reinforced by the natural resistance to infection.

The kombucha fermentation acetic acid formed from ethanol, which translate acetic acid into acetic acid bacteria. Although the symbiotic bacteria of acetic kombucha is very important, it is noted that during the fermentation milk results in a very low content of acetic acid (Tables 1 and 2). Probable reasons are that the fermentation of milk is much shorter than the kombucha fermentation of black tea with sucrose, and that acetic bacteria require a longer adaptation period. In addition, we noticed that, in terms of acid fermentation, the kombucha metabolism of lactose as the primary sugar in milk, moved in the direction of formation of L-lactic acid. The content of lactose in relation to D-galactose in samples of yoghurt is common (*Barbaros, et al., 2007*), or about three times higher (Table 3). In samples of dairy products produced kombucha is the ratio of 3.5 to 9 times higher, especially in samples K-1, and 5% K-3%. The samples of K-0, 12% of this ratio is 3.5 to 4 times higher than that of yoghurt (Tables 1 and 2). It is obvious that the kombucha fermentation of milk with 0.9% fat lactose ferments more slowly compared with the probiotic culture, hence the higher content, but this value is certainly acceptable, because it is not much higher than in the probiotic yogurt. D-glucose and D-fructose are monosaccharides that normally occur during kombucha fermentation of black tea sweetened sucrose (4-6). Also, during the fermentation of probiotic cultures milk generated a certain amount of D-glucose and minor amounts of D-fructose, which is evident from the results in Table 3. The lowest content of these monosaccharides is in the samples K-0, 12%, slightly higher in the K-1.5%, the highest in the K-3% samples.

For samples K-3%, it is clear that a much higher content of D-glucose and D-fructose is due to their presence in the inoculum, and most of the sugar and bring to a substrate, and during fermentation is not broken down completely . The lowest content in the samples of K-0, 12% can be explained with certainty. It is possible that manufacturers produce kombucha capsules without sugar because the label does not specify the composition of carbohydrates.

Microbial formation of ethanol is characterized by anaerobic fermentation. Fermentation processes and probiotic cultures in milkare typically done aerobically, so a higher content of ethanol was expected. This was much less expected for kombucha fermentation because, as already stated, yeasts produce ethanol, and the bacteria turn it into a acid. Notwithstanding the above facts, it is good to inspect the contents of this metabolite in the finished product, which both adults and children consume (Šodić, K., 2012). The results showed that in all of the samples the content of ethanol is minute and it was only detected by a very sensitive enzyme analytical method.

CONCLUSION

Low-energy fermented dairy products with 0.9% milk fat were produced by using concentrated inoculum symbiotic kombucha culture and probiotic yogurt.

In the samples produced by fermentation of kombucha, the content of L-lactic acid was significantly higher than the content of D-lactic acid, and the highest values were recorded in the samples produced by kombucha capsules (K-0, 12with th average of 0.958 g/100g.

Metabolic activity of kombucha fermented milk lactose is slower compared with the probiotic culture, but its content is certainly acceptable, and it is not much higher than the probiotic yogurt. Fermented drinks contain certain amounts of D-glucose and D-fructose. The lowest sugar content was recorded in the samples K-0, 12%, slightly higher in the K-1, 5%, and the highest in the K-3% samples, and the contents depend on the origin and quantity of inoculum. In fully fermented milk products produced by kombucha fermentation the content of ethanol was minute.

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