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NUTRITIVE AND FUNCTIONAL CHARACTERISTICS OF KOMBUCHA FERMENTED MILK BEVERAGE

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Abstract: *The effect of an addition of 10% non-convectional starter culture-kombucha on nutritive and functional characteristics of fermented milk beverage produced from milk with 0.9% fat was investigated in this paper. Based on this research, it can be concluded that this kind of drink can be classified as a high valuable functional food intended for all consumers categories.*

Key Words: ***Kombucha, fermented milk beverage, nutritive and functional characteristics, microstructure***

1. INTRODUCTION

Fermented milk beverages are a large group of highly nutritive products. These beverages are obtained by usage of a starter cultures. Convectional starter cultures can be yoghurt (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*) or probiotic starter culture (*Lactobacillus* and *Bifidobacterium*) which are isolated from a human intestinal tract. Latest forms of fermented milk beverages contain prebiotics (inulin, oligofructose). They are digestible food ingredients, which stimulate the growth and the viability of present probiotic starter cultures. Products that contain both probiotics and prebiotics can be call a functional food [1]. Functional food, beside nutritive values, contains a components which have a positive effect on the health, physical and psychological state of a human organisms.

In this study kombucha was used as a non-convectional starter culture for a production of fermented milk beverage. Kombucha is a symbiotic association of yeast (*Pichia, Zygosaccharomyces, Saccharomyces, Schizosaccharomyces,Saccharomycodes, Brettanomyces, Torulaspora* and *Candida*) and acetic acid bacteria (*Acetobacter* and *Gluconobacter*). Kombucha is traditionally cultivated on a sweetened black and green tea [2, 3]. Also, it can be cultivated on a dark beer, red vine, white vine, whey, lactose or some other type of a tea [4, 5]. As a result of kombucha's metabolic activity, a refreshing and midly sour beverage can be obtained.

The traditional carbon source for kombucha fermentation is saccharose. Therefore, the main pathways of conversion of saccharose into numerous compounds have been investigated [3, 6-8]. Glucose liberated from saccharose is metabolized for the synthesis of cellulose and gluconic acid by *Acetobacter* strains. Fructose is metabolized into ethanol and carbon dioxide by yeasts. Ethanol is oxidized to acetic acid by *Acetobacter* strains. Besides monosaccharides, metabolite contains organic acids, proteins (enzymes) produced during fermentation, the tannins originally present in the tea broth as well as a number of other useful compounds [9-12].

Most properties of Kombucha, beneficial to human health, are attributed to the acidic composition of the beverage [13-16]. The following acids can be mentioned: glucuronic, gluconic, acetic, lactic, succinic, mannonic, propionic, ascorbic, etc. Detoxifying property of the metabolite is presumably due to the capacity of glucuronic acid to bind to toxin molecules and increase their excretion from organism by the kidneys or the intestines [8, 15, 17]. Glucuronic acid is normally produced by a healthy liver and can readily be converted into glucosamine, the foundations of our skeletal system [18]. Kombucha may also produce vitamins B_1, B_2, B_3, B_6 and B_{12} and folic acid [19]. The unique antimicrobial activities are developed during the fermentation by kombucha. It was suggested that these activities come from kombucha metabolism and not from the originally present catechins or polyphenols of tea leaves, as the activities increase with the fermentation time. Also, the antimicrobial compounds are unlikely large proteins or enzymes, because they are inactivated during sample extraction with hot water. Acetic acid is considered to be responsible for the inhibitory effect towards a number of microbes tested [13, 16, 20].

Milk is a very complex physical and chemical system, because it contains a significant amount of proteins, fat and lactose. Inoculation of a kombucha in milk is a specific type of fermentation [21].

The aim of this study was to investigate the effect of a addition of 10% non-convectional starter culture-kombucha on nutritive and functional characteristics of fermented milk beverage produced from milk with 0.9% fat. Nutritive characteristics of a kombucha fermented milk beverage contents were analysed after the production: lactose, galactose, glucose, fructose, lactic and acetic acid, ethanol, vitamins (B_1, B_2, B_6 and C), minerals (Ca, Mg, K, Na, Pb, Cd, Cu, Zn) and total fatty acids, as well as microstructure.

2. MATERIALS AND METHODS

2.1. Milk

Homogenized and pasteurized cow milk with 0.9% fat was taken from AD Imlek, Division Novi Sad Dairy, Serbia for the production of a kombucha fermented milk beverage. Milk chemical and physico-chemical composition are shown in Table 1.

Table 1. *Milk quality*

Chemical composition (g/100g)	**Milk**
Milk fat	0.9
Dry matter	9.85
Proteins	3.15
Lactose	4.74
Ash	0.78
Physico-chemical composition	
pH	6.63
Eh (mV)	16.0
Acidity (°SH)	6.6

2.2. Kombucha inoculum

Preparation of kombucha, before its application as a starter culture, consisted of the following steps: 1 L of boiled tap water with 70 g of saccharose and 1.5 g of black tea leaves ("Adonis", Jagodina, Serbia) were heated at 100^{o}C for 5 min. The obtained solution was cooled to room temperature and the leaves were removed by filtration. Such a solution was inoculated with 10% v/v fermentation broth from previous kombucha fermentation, covered with cheesecloth and incubated (at constant temperature of 29.5 ± 1^{o}C) for seven days.

2.3. Kombucha fermented milk beverage production

Milk with 0.9% fat content was inoculated at 42°C, with 10% of kombucha starter. Fermentation lasted until pH reached 4.5 and then was cooled on 8°C. After cooling, gel was homogenized with electric mixer and stored in refrigerator on 4°C. Kombucha fermented milk beverage production is represented on Figure 1.

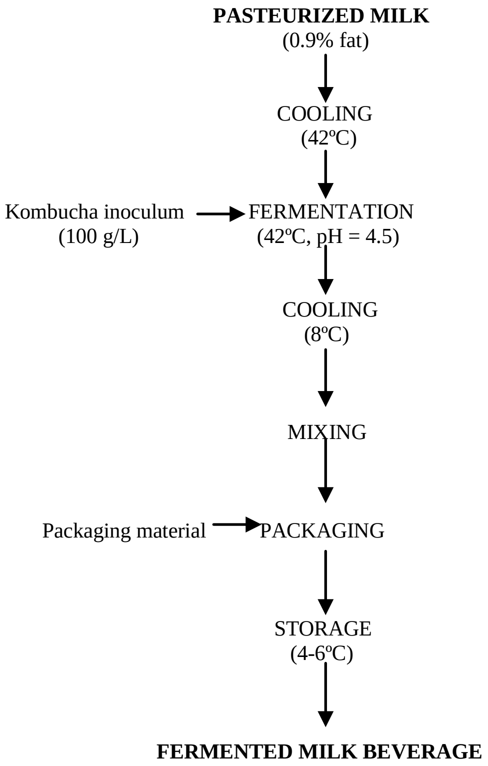

Figure 1. *Kombucha fermented milk beverage manufacturing*

2.4. Methods of Analysis

Content of lactose, galactose, glucose, fructose, lactic and acetic acid were determined by using enzymatic tests (Megazyme, Ireland). Products of the reactions were monitored using a spectrophotometer (T80+ UV/VIS Spectrometer, PG Instruments Ltd.).

Minerals were analyzed with atomic absorption spectroscopy, atomic emission spectroscopy and spectrophotometric methods (GBC 932 plus) [21].

The content of vitamins B_1, B_2 and B_6 was analyzed with reversed-phase liquid chromatography with a fluorescence detector-method HCTM-01, HCTM-02, HCTM-03. Vitamin C was analyzed by HPLC system “Agilent 1100” U.S., with a loop injector of 20µL, C-18 column, 5µm diameter particles, and UV-DAD detection.

Examination of the fatty acids content was performed using gas chromatography (VARIAN, model 1400), with a flame-ionization detector [21].

Microstructure analyse was done by scaning electron microscopy (SEM) technique, using *Joel, JSM-6460LV* Scanning Electrone Microscope (Oxford, Instruments). The sample preparation included: samples fixation in 2.8% gluteraldehyde, sample dehydratation in different percentage of ethanol solutions, sample extraction in chloroform, sample dehydratation in absolute ethanol for 24 hours, drying of samples using CPD 030 “Critical Point Dryer“, BAL-TEC, Germany, and coating of samples with gold using BAL-TEC, SCD 005, Sputter coater [22-24]. Voltage used for SEM analysis was 25kV.

3. RESULTS AND DISCUSSION

3.1. Nutritive characteristics

The fermentation of milk with kombucha lasted beetwen 9.5 and 10 hours. The fermentation time for milk with kombucha as starter lasted longer than with traditional or probiotic starter culture, because kombucha needs time to adapt to milk as a substrate [25, 26].

One of the main pathways of a milk fermentation (Embden-Meyerhof-Parnas pathway) starts with hydrolysis of lactose into glucose and galactose, which is associated with the activity of enzyme β-D-galactosydase. Under the influence of lactic acid bacteria, glucose converts into lactic acid, while in the presence of yeasts it transforms into ethanol and CO_2 (Figure 2).

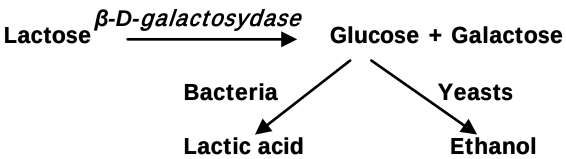

Figure 2. *Pathway of the milk fermentation by kombucha*

Kombucha fermented milk beverage contained 4.10 g/100g lactose and that was 13.5% less lactose than in a milk used for production of this beverage.

Galactose content in a milk is very low and it's usual value is approximatly 0.018 g/100g. Content of galactose in samples was 0.28 g/100g and it is in direct dependence with amount of lactose transformed.

Other products of a kombucha in milk are monosaccharides: glucose and fructose. During milk fermentation glucose was produced in quantity of 0.06 g/100g, but a very small amount of fructose was detected, too. The difference between the amount of glucose and fructose in samples can be explained by the fact that glucose is derived from two sugars: lactose and saccharose, and fructose from saccharose only.

Content of L-lactic acid, as a result of lactose fermentation, was 0.42 g/100g. In comparison with probiotic yoghurt amount of L-lactic acid is 50% lower in kombucha fermented milk beverage. Content of D-lactic acid was very small - 0.03 g/100g [21].

Acetic acid is produced by conversion of ethanol with acetic acid bacteria presented in a kombucha. Amount of acetic acid was 0.095 g/100g. Content of ethanol was very small - 0.032 g/100g, and it is produced by fermentation of glucose and fructose due to the metabolic activity of a yeast.

Many minerals were detected in kombucha fermented milk beverage (Table 2). The highest content of minerals had calcium – 1210 mg/kg. Sodium content was 721 mg/kg, and value for magnesium was 125 mg/kg. Other detected minerals in beverage were Pb, Cd, Zn and Cu.

Table 2. *Minerals content in kombucha fermented milk beverage*

Minerals	Mg	Ca	K	Na	Pb	Cd	Zn	Cu
Content (mg/kg)	125	1210	794	721	3.80	0.31	4.25	0.06

Content of vitamin C was 11.06 mg/100g. This is very high amount compared to traditional yoghurt that has in average 1.0 mg/100g [1]. On the other hand, content of vitamins B complex was significantly smaller in kombucha fermented milk beverage compared to traditional yoghurt. Content of vitamins B complex in milk with 0.9% fat and in the kombucha fermented milk beverage is represented in Table 3.

Table 3. *Vitamins of B complex in milk with 0.9% fat and in kombucha fermented milk beverage*

Vitamins of B complex	**Milk 0.9% fat (μg/100g)**	**Kombucha fermented milk beverage (μg/100g)**
B_1	35.43	37.42
B_2	104.47	108.39
B_6	79.34	81.36

Fatty acids content was analysed in this study and it is represented in Table 4.

Table 4. *Fatty acids in kombucha fermented milk beverage*

Fatty acids	**Kombucha fermented milk beverage (%)**
4:0 (butyric)	4.2
6:0 (capric)	3.1
8:0 (caprylic)	1.7
10:0 (caproic)	3.3
unidentified	-
12:0 (lauric)	3.3
unidentified	0.8
unidentified	6.6
14:0 (myristic)	11.7
14:1	0.6
15:0	0.6
16:0 (palmitic)	29.9
16:1 (cis+trans)	2.4
17:0	-
18:0 (stearic)	8.5
18:1 (cis+trans) (oleic)	20.1
18:2 (linoleic) (cis+trans+conug.)	2.1
18:3 (linolenic)	0.6
20:0 (arachidonic)	0.4

Palmitic acid had the highest content with a rate of 29.9%. With a rate of 20.1% comes oleic acid and then myristic acid with a rate of 11.7%. Stearic, butyric, caproic, lauric, capric and linoleic acids were also detected in fermented milk beverage produced by the addition of 10% kombucha inoculum.

3.2. Microstructure

Ability of a milk proteins to form gel is the basis for the manufacture of fermented milk products. The structure of gel consists of casein micelles interconnected to form protein chains and clusters. The basic characteristics of a gel are hard and homogeneous consistency, good syneresis (water holding capacity) and characteristic microstructure.

The electron microscopy (SEM technique) was used to examine the microstructure of milk components, changes and interactions among them, and with other ingredients during processing. Generally, a microstructure of dairy products depends on their chemical composition, technological procces, starter culture applied, etc. A microstructure of dairy products is characterized by fatty globules, membranes, colloidal aggregates, and crystals. These elements in the technological process can react with each other and form a variety of textural characteristics of dairy products [27-29]. The least visible particles in milk are casein submicels, with a diameter about 10 nm. Casein micelles are protein globule size from 50 - 500 nm and in addition to fat globules (3-5 µm) can be seen under the electron microscope [30, 31]. Whey proteins and lactose cannot be seen under the electron microscope, as they are dissolved in the aqueous phase. However, denaturated whey protein and lactose crystals are visible, because the molecules of these substances form a part of whey protein coagulum, or have been properly arranged in the form of solid crystals consisting of a large number of molecules [28, 32].

Tamime et al. (1984) [33] reported that the SEM analysis of yoghurts made from low-fat cow's milk fortified by the addition of skimmed milk powder or concentrated by evaporation or reverse osmosis showed similar microstructure. The densest matrix protein was produced by skimmed milk powder, followed by ultrafiltrated samples and samples with sodium caseinate with medium dense matrices.

The microstructure of kombucha fermented milk beverage is shown in Figure 3.

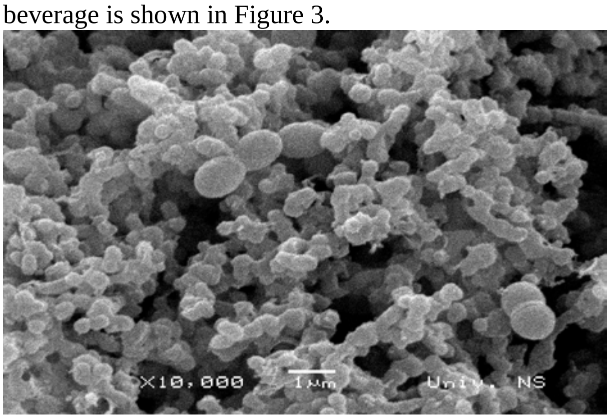

Figure 3. *Microstructure of kombucha fermented milk beverage*

The microstructure of the sample form interwined casein micells with loser distributed and less interconnected casein micelles in comparison with a probiotic yoghurt [24]. The presence of bacteria *Streptococci* from milk is noticable, too. The microstructure of kombucha fermented milk beverage is homogenous, with a regular arrangement of casein micelles and hollow spaces between them.

4. CONCLUSION

Kombucha fermented milk beverage was produced from low-fat milk inoculated at 42°C and by addition of 10% kombucha starter. Milk fermentation with kombucha starter lasted longer than with a traditional or probiotic starter culture, because kombucha needed time to adapt to milk.

The fermented milk beverage produced from milk of 0.9% fat with an addition of local kombucha inoculum showed good nutritive and functional characteristics.

This kind of drink can be classified as high valuable functional food intended for all consumers categories.

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