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Lactose fermentation by Kombucha – a process to obtain new milk–based beverages

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Abstract

This paper focuses on fermentation of lactose from a model system (black tea) and from two types of milk (0.9% w/w and 2.2% w/w of fat) by application of Kombucha. Quantities of the applied Kombucha starter were 10% v/v and 15% v/v. All fermentations were performed at 42°C. The process to achieve a desirable pH=4.5 was slower in the model system (16 h) than in milks (9 - 10 h). Regarding starter quantity, 10% v/v proved the optimal. Regarding types of milk, higher fat content guarantees shorter fermentation and higher yield of metabolites. Utilization of lactose was found at a level of ≈20% and ≈30% in milks with 0.9% w/w and 2.2% w/w of fat, respectively. This was correlated with an appearance of intermediates and/or products. Glucose underwent further transformations almost entirely, while galactose showed much lower reactivity. Seven to twelve times higher contents of lactic acid were found compared to acetic acid. Milk-based beverage from the reduced fat sample, inoculated with 10% v/v of Kombucha starter, has the best physical characteristics (syneresis and water holding capacity). It also developed a good texture (especially cohesiveness and index of viscosity). Milk lactose fermentation was a process that could have been used for obtaining new milk-based products.

Key words: Kombucha, lactose fermentation, new milk products

Introduction

Lactose is a unique disaccharide that has a role of bioactive ingredient in human nutrition [1]. When used as feedstock for the chemical, enzymatic or microbiological conversion, lactose can be transformed to even more valuable compounds [2]. In dairy industry, lactose is extensively utilized by a great number of the commercially available starter cultures, which hydrolyse lactose (to glucose and galactose) thanks to the β-D-galactosydase activity. The main pathway of milk fermentation (Embden-Meyerhof-Parnas pathway) starts with such a hydrolysis [3]. Further, glucose converts into lactic acid, under the influence of lactic acid bacteria, and, alternatively, into ethanol and CO_2 if yeasts are present. When bifidobacteria exist, lactose can be converted into acetic acid as well.

Application of Kombucha in lactose fermentation is still under investigations, because the traditional carbon source for Kombucha is saccharose. Kombucha is a unique symbiotic association of yeasts and bacteria that has been applied for fermentation of sweetened black and green tea for centuries. Microbiological analysis has shown that Kombucha bacteria belong to the strains of the genus *Acetobacter* [4 – 6], while yeast strains are *Saccharomycodes ludwigii, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis* sp. and *Zygosaccharomyces* sp. [7]. Total number of viable cells was as follows: approximately $5x10^4$ of yeast cells per cm^3 of the reaction mixture and approximately $2x10^5$ of bacteria cells per cm^3 of the mentioned mixture. The main pathway of saccharose conversion is known to a certain extent [4 – 6, 8]. Glucose, liberated from saccharose, is metabolized for the synthesis of cellulose and gluconic acid by *Acetobacter* strains. It is also metabolized into ethanol and carbon dioxide by yeasts. Ethanol is oxidized to acetic acid by *Acetobacter* strains. Apart from the mentioned compounds, the reaction mixture contains organic acids (glucuronic, lactic, succinic, mannonic, propionic, ascorbic, etc), proteins (enzymes), the tannins, as well as a number of other useful compounds [9 – 13]. Most properties of Kombucha, beneficial to human health, are attributed to the acidic composition of the beverage [14 – 16]. Acetic acid is considered to be responsible for the inhibitory effect towards a number of microbes tested [17, 18]. Detoxifying property of the metabolites is due to the capacity of glucuronic acid to bind to toxin molecules [8, 15, 19]. Kombucha may also produce vitamins B and folic acid [20].

Apart from saccharose, other sugars might be of interest as substrates of fermentation by Kombucha [21 – 23]. As far as lactose is concerned, only a few investigations were reported [4, 24, 25]. In 1994, Reiss [4] performed a comparative study on fermentation of saccharose, glucose, fructose and lactose by Kombucha at room temperature. In 2003, Belloso-Morales and Hernández-Sánchez [24] manufactured the Kombucha beverages from three types of whey (fresh sweet, fresh acid and reconstituted sweet). Our recent work [25] suggests production of milk-based beverages from cow milk using several Kombucha starters, which have been concentrated before application. Yet another investigation is reported on similar system - the Caucasian yoghurt [26], which is interesting due to unique activities of *Lactococcus lactis* ssp. *cremoris* and *Acetobacter orientalis* that produce lactobionic acid.

The aim of this work is to analyze fermentation of lactose from two types of milk, low fat (0.9% w/w) and reduced fat (2.2% w/w), inoculated with two quantities of plain Kombucha starter (which is not concentrated before application). The work also, presents characteristics of the obtained metabolites - potentially new products.

Materials and Methods

Cultures

Preparation of Kombucha, before its application as a starter culture, consisted of following steps: 1 dm^3 of boiled tap water with 70 g of saccharose and 1.5 g of black tea leaves (Indian tea, "Vitamin", Horgoš, Serbia) were heated at 100^oC 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^oC) for 6 days. So obtained system (pH=3.21, lactic acid 0.040% w/w and total acids 0.29% w/w) was used as a starter culture for producing Kombucha containing milk-based beverages.

Model System - Black Tea with Lactose

Black tea was prepared with 46 g dm^{-3} of lactose, instead of saccharose, according to already presented procedure. Lactose concentration was adjusted to a value typical of cow milk.

Milk

Two types of homogenized and pasteurized cow milk were taken from AD Imlek, Ogranak Novosadska mlekara, Novi Sad, Serbia, for the production of all milk-based beverages. The first type of milk contained 0.9% w/w of fat (also, 9.80% w/w of dry matter, 3.20% w/w of proteins, 4.74% w/w of lactose, 0.82% w/w of ash, pH=6.58), while the second one contained 2.2% w/w of fat (also, 11.1% w/w of dry matter, 3.20% w/w of proteins, 4.54% w/w of lactose, 0.85% w/w of ash, pH=6.59). The milk containing 2.2% w/w of fat was chosen as a common version of semi-skimmed milk in Serbia, while the milk with 0.9% w/w of fat was selected due to its low fat content.

Methods of Analysis

pH values were determined using a pH-meter (pH Spear, Eutech Instruments Oakton, England), while content of total acids in a model system was determined by the volumetric method with sodium hydroxide and phenolphthalein as an indicator.

Macro-components, in all samples of milk and milk-based beverage, were determined using standard methods: a) dry matter content after drying at 105^{o}C, b) fat according to Gerber and Van Gulik, c) total nitrogen according to Kjeldahl and d) ash after mineralization at 550^{o}C. All the methods applied are described in the handbook [27].

Primary and secondary products of fermentation and lactose were detected in all fermented products from milk using specific enzyme-tests, such as K-FRUGL 11/05 (D-glucose and D-fructose), K-LACGAR 12/05 (lactose and D-galactose), K-DLATE 11/05 (L-lactic acid and D-lactic acid), K-ACET 11/05 (acetic acid) and K-ETOH 03/06 (ethanol). The analysis was performed according to the guidelines supplied by the producer (Megazyme, Ireland). Products of the reactions were monitored using spectrophotometer (T80+ UV/VIS Spectrometer PG Instruments Ltd.).

The measured physical properties are: a) syneresis (in cm^{3} of whey separated by filtration of the 50 cm^{3} sample at room temperature for 3 h) [28], b) water holding capacity (WHC) [29] and c) acidity (in ^{o}SH) according to Soxhlet-Henkel method [27].

Textural properties of Kombucha beverages (firmness, consistency, cohesiveness and index of viscosity) were measured by Texture Analyser TA XP (Stable Micro System, Godalming, England) within a single compression test at 4^{o}C using a disc (A/BE, diameter 35 mm, distance 30 mm, speed 10 mm/s) and an extension bar with a load of 5 kg.

Results and Discussions

Fermentation of Lactose in the Model System

Due to comparison of fermentation of black tea sweetened with lactose and fermentation of milk, the processes were performed in both systems, according to the plan of experiments presented in Table 1. Black tea containing 46 g dm^{-3} of lactose was inoculated with 10% v/v of Kombucha inoculum and than exposed to the fermentation at 42^{o}C. Lactose concentration was selected to correspond to the quantity of lactose in milk, while the temperature was chosen as the one typical of yoghurt production, especially due to the fact that no information about the optimal temperature of lactose fermentation by Kombucha was avalibale. The efficiency of this fermentation was monitored by the rate of both a decrease of pH and an increase of total acids content expressed as a quantity of lactic acid (Figure 1). Fermentation was carried out for 96 h although the desired acidity (pH=4.5) was reached after 16 h. As it is obvious from Figure 1, pH value decreased exponentially (to pH=3.8) and the corresponding content of total acids increased also exponentially (to 0.79 g dm^{-3}). This augmented acidity most likely suppresses the metabolic activity of the existing cultures, causing attenuation of the reaction in further phases of fermentation.

Table 1. Experimental design

PRODUCT	Raw material	Culture concentration
Model system	Black tea	10% v/v
M1K10	Milk with 0.9 % w/w fat	10% v/v
M1K15	Milk with 0.9 % w/w fat	15% v/v
M2K10	Milk with 2.2 % w/w fat	10% v/v
M2K15	Milk with 2.2 % w/w fat	15% v/v

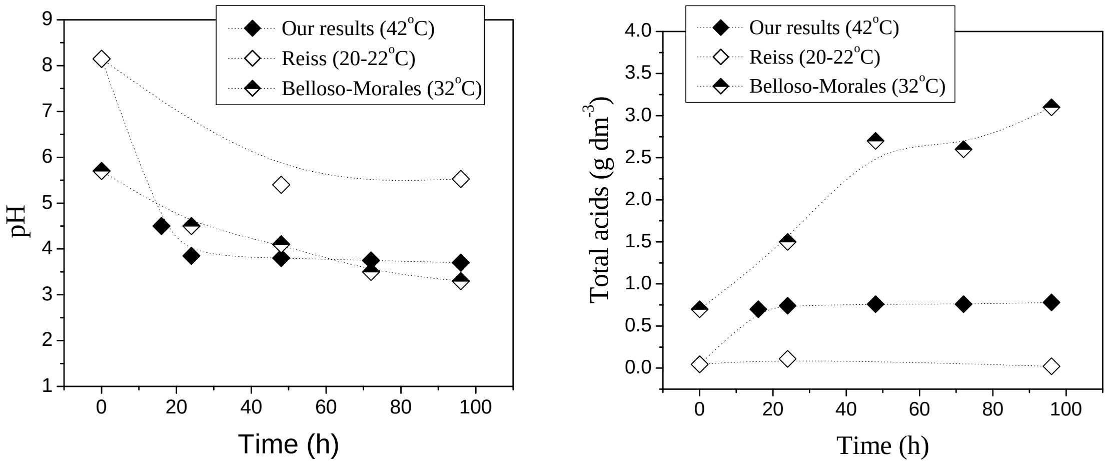

Figure 1. Changes in both pH and total acids content during fermentation of model system

Results of our experiments in the model system (Figure 1) were compared to the results obtained by Reiss [4] (black tea, 50 g dm^{-3} of lactose, 10% v/v of Kombucha inoculum, 20-22^{o}C) and Belloso-Morales and Hernández-Sánchez [24] (fresh sweet whey, 47 g dm^{-3} of lactose, 8% w/v of Kombucha inoculum, 32^{o}C). Apart from quantities of ethanol that were found negligible in ours and Reiss' systems, his results differed significantly from ours regarding kinetics of fermentation. In Reiss's experiments, a decrease of pH and related increase of total acids occur slowly, mainly because of much lower fermentation temperature, since all the other factors (experimental conditions) and systems (black teas) are very similar. In the contrary, Belloso-Morales and Hernández-Sánchez investigated fermentation of fresh sweet whey, which is very different from our model system. At the beginning, its pH is low and total acids are high as a result of previous fermentation. Lactic acid prevails; its amount is 7 times higher than acetic acid quantity. After the fermentation by Kombucha, the difference became even higher (3 g dm^{-3} of lactic acid against 0.1 g dm^{-3} of acetic acid). Typical of whey is a buffering effect of the present proteins (0.67%); although quantity of acids increased significantly, pH values changed slowly. After the 96 h fermentation of fresh sweet whey, 5 g dm^{-3} of ethanol was found.

Fermentation of Lactose in Milk

All the results acquired on our model system suggest that plain Kombucha inoculum can be used for lactose fermentation. In the next step of this investigation, two milks (with 0.9% w/w and 2.2% w/w of fat), containing 4.74% w/w and 4.54% w/w of lactose, were inoculated with Kombucha culture and fermented at 42^{o}C. Changes in pH during fermentation in low fat milk samples are presented in Figure 2a, while the pH changes in reduced fat milk systems are shown in Figure 2b. Shapes of fermentation curves are very similar for both milks, indicating similar occurrences in these systems. Appearance of lag phase is evident in all the samples at approximately pH=6.2 during the first 6-7 h of fermentation. After that, the fermentation became intensive in all the investigated Kombucha samples until the end of the process (Figure 2). Fermentation is completed (pH=4.5) after 9 - 10 h, when the gels were cooled to 8^{o}C, homogenized by mixing and packed in plastic containers for further examination. Each of the fermentation was repeated three times.

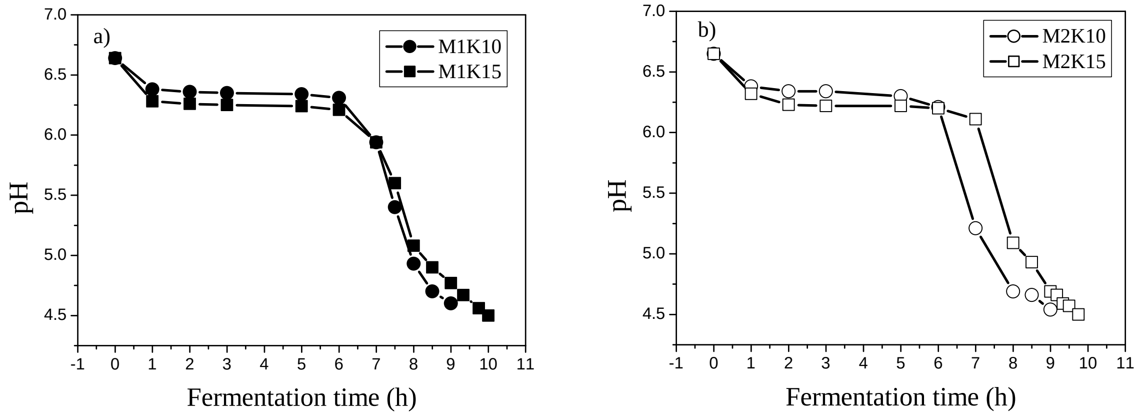

Figure 2. Changes in pH during fermentation of both milks: a) Low fat milk, b) Reduced fat milk

Analysis of the fermentation curves shows that inoculum concentration (10% v/v or 15% v/v) did not significantly affect the rate of fermentation in the investigated Kombucha systems. So, this investigation proves that not only plain Kombucha inoculum can be used, but the starter concentration can be the smaller of the two concentrations applied in experiments. The same conclusion was drawn on saccharose fermentation [30]. The fermentation time for the samples with 2.2% w/w of fat is a little bit shorter than the time of fermentation for low fat samples in all investigated cases. The reduced fat milk is slightly better substrate than the low fat milk. When comparing duration of the processes to achieve pH=4.5 in black tea samples and in milk samples, it can be concluded that the process is faster on milk (9 - 10 h) than on the model system (16 h).

Characteristics of the Obtained Milk-based Beverages

Content of Macro-components

Content of macro-components in all milk products was determined and presented in Table 2. Low fat Kombucha samples had slightly lower level of dry matter (10.55% w/w in average) than the reduced fat samples (10.7% w/w in average) and lower level of proteins (2.75% w/w in average) than the reduced fat samples (2.85% w/w in average). Concerning fat content, it decreased by ≈10% in low fat products and by ≈5% in reduced fat samples. Slightly higher amounts of minerals remained in low fat metabolites compared to the reduced fat Kombucha products. At the end of fermentation, higher quantity of lactose remained in the low fat samples (3.83% w/w, in average) than in the reduced fat samples (3.16% w/w in average). Accordingly, Kombucha utilized ≈20% of lactose from low fat milk, but ≈30% of lactose from reduced fat milk. Higher content of fat in raw milk most likely contributes to more efficient fermentation of lactose by Kombucha.

Table 2. Macro-components of the fermented beverages

PRODUCT	**Macro-components (% w/w)[b]**				
	Dry	**Fat**	**Proteins**	**Ash**	**Lactose**
M1K10[a]	10.9±0.33	0.800±0.026	2.56±0.022	0.680±0.020	3.96±0.010
M1K15	10.2±0.27	0.800±0.019	2.93±0.010	0.570±0.020	3.70±0.020
M2K10	10.9±0.14	2.10±0.040	2.71±0.026	0.590±0.020	3.10±0.010
M2K15	10.6±0.13	2.10±0.035	2.98±0.021	0.570±0.016	3.22±0.015

a) Labels of products according to experimental design from Table 1

b) Mean value (n=3), three significant digits± standard dev., two significant digits

It might be interesting to know, are the two types of milk products (low fat and reduced fat) significantly different, regarding their macro composition? So, the differences in dry matter, proteins and ash content were analyzed by Duncan's multiple range tests [31]. The tests have shown that the compared quantities differed significantly in most of the cases.

Primary and Secondary Products of Fermentation

For better understanding of fermentation itself, quantities of main primary and secondary products of fermentation were measured in all fermented milk samples (Table 3). Glucose and galactose appeared as the primary products of lactose hydrolysis [3]. Based on lactose utilization (≈20% and ≈30%, as mentioned), average quantity of glucose/galactose released through hydrolysis can be estimated (4.5 g dm^{-3} in low fat metabolites and 6.9 g dm^{-3} in reduced fat metabolites). Analysis of the measured glucose quantities (Table 3) shows that approximately 85% of glucose in low fat samples and 90% in reduced fat samples underwent further transformations. Quite opposite, only ≈25% and ≈10% of galactose reacted further, respectively. The main secondary product appeared to be lactic acid along with acetic acid amount of which is much smaller. Very probably, glucose together with small quantity of galactose converts into L-lactic acid (4.9 g dm^{-3} in low fat samples and 7.6 g dm^{-3} in reduced fat samples) through a homolactic fermentation. Therefore, a minimal quantity of ethanol was found in our samples; it ranges from 0.08 g dm^{-3} to 0.3 g dm^{-3}. Other reason for small quantity of ethanol might be its conversion to acetic acid by acetic acid bacteria of the genus *Acetobacter* present in Kombucha.

Table 3. Primary and secondary products of fermentation

PRODUCT	**Micro-components (% w/w)[b]**			
	D-glucose	**D-galactose**	**L-lactic acid**	**Acetic acid**
M1K10[a]	0.065±0.001	0.30±0.01	0.42±0.009	0.030±0.004
M1K15	0.060±0.001	0.35±0.008	0.56±0.007	0.046±0.005
M2K10	0.067±0.007	0.67±0.009	0.82±0.01	0.041±0.007
M2K15	0.062±0.008	0.65±0.008	0.71±0.005	0.042±0.005

a) Labels of products according to experimental design from Table 1
b) Mean value (n=3), two significant digits± standard dev., one significant digit

The main difference between the metabolites from model system and from milk with lactose is quantity of acids. At pH=4.5, fermented black tea contained 0.71 g dm^{-3} of total acids, while low fat fermented milk had 7 times higher content of acids and reduced fat milk contained even 12 times higher quantity of acids. Also, acetic acid prevails in tea samples, while great part of total acids in our milk-based beverages belongs to lactic acid. Comparable results were obtained in our previous investigations (milk with 2.2% w/w of fat, 46.5 g dm^{-3} of lactose, 10% v/v of concentrated Kombucha inoculum, at 42^{o}C) [25]. We found that content of all acids was 5.2 g dm^{-3}, from which 4.5 g dm^{-3} belong to lactic acid. Similar results were found on fresh sweet whey [24]. At pH=4.5, products of its fermentation contained 1.5 g dm^{-3} of lactic acid, but only 0.1 g dm^{-3} of acetic acid.

Physical and Textural Characteristics

Physical characteristics of all milk products, whose labels are shown in Table 1, are presented in Table 4. Among syneresis values, not great differences are noticed; the best value (30.0 cm^3/50 cm^3) is typical of reduced fat sample inoculated with 10% v/v of Kombucha starter. Water holding capacities are also similar. Low fat Kombucha beverages have smaller water holding capacities (43.2% w/w in average) than the reduced fat samples (45.2% w/w in average). According to the degrees of acidity (34.8 ^{o}SH – 37.4 ^{o}SH), Kombucha beverages are less acid than the traditional fermented products as yoghurts.

Table 4. Physical characteristics of the fermented beverages

PRODUCT	**Physical characteristic**[b]		
	Syneresis (cm^3/50 cm^3)	**WHC**[c] **(% w/w)**	**Acidity (^{o}SH)**
M1K10[a]	32.0±1.2	45.5±1.6	37.4±1.7
M1K15	33.0±1.3	41.0±1.9	37.0±1.8
M2K10	30.0±1.0	46.0±0.92	37.2±1.7
M2K15	31.0±1.6	44.5±1.1	34.8±0.95

a) Labels of products according to experimental design from Table 1
b) Mean value (n=3), three significant digits± standard dev., two significant digits
c) WHC-Water holding capacity

Textural characteristics of the fermented beverages are presented in Figure 3. Following characteristics were measured: firmness, consistency, cohesiveness and index of viscosity. According to the methods applied, the last two characteristics are expressed as negative numbers (the lower the better). Quite expectedly, products M2K10 and M2K15 that are high in both fat and proteins developed better textural characteristics than low fat products M1K10 and M1K15. It seems that optimal texture has the product labelled as M2K10, with the best cohesiveness (-8 g) and index of viscosity (-2.7 g s) and with the consistency 410 (g s) equal to the consistency of the M2K15 beverage. Once again, it was proved that concentration of the applied Kombucha starter has negligible effect on the texture of fermented products, when compared to the influence of other factors.

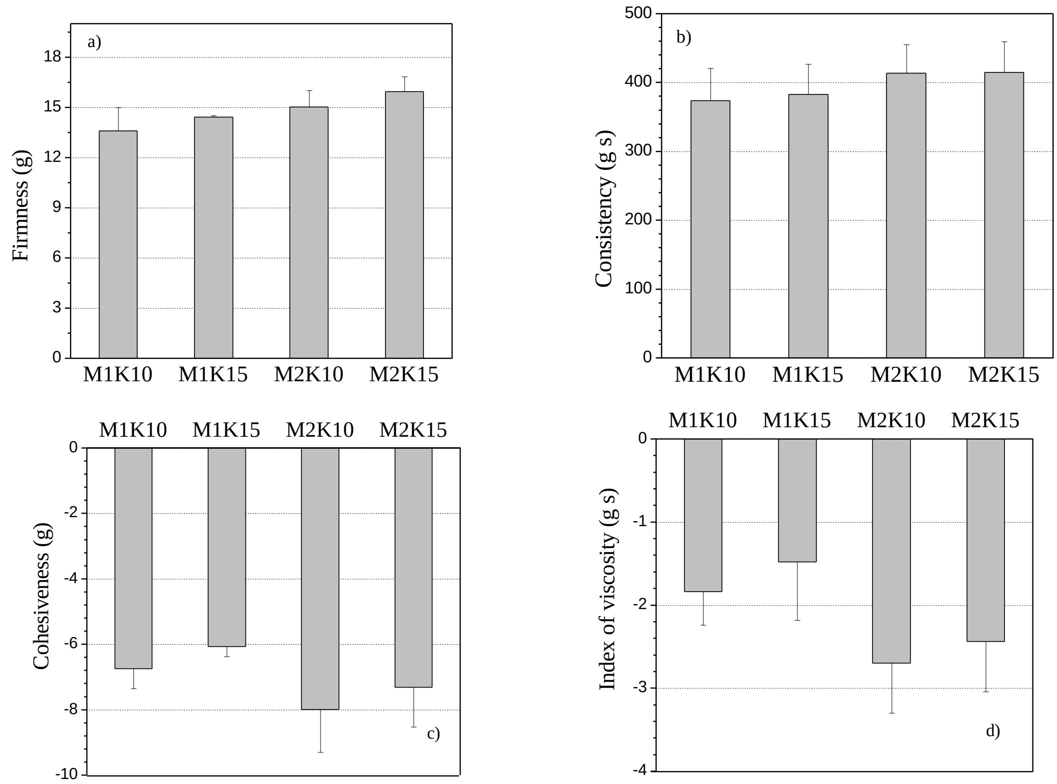

Figure 3. Textural characteristics of fermented beverages: a) Firmness, b) Consistency, c) Cohesiveness and d) Index of viscosity

Conclusions

This investigation gave us an opportunity to acquire facts related to the milk lactose fermentation by Kombucha. The most important are:

- Plain traditional Kombucha can be used as a starter culture to initiate and to lead milk lactose fermentation. There is no need to concentrate starter before its application;
- Starter quantity of 10% v/v proved the optimal;
- Type of milk significantly influences fermentation itself and affects the characteristics of the obtained metabolites;
- Properties of the metabolites (composition, physical characteristics and texture) are acceptable;
- Primary products of fermentation go through further transformations, glucose much faster than galactose;
- The main secondary products are acetic acid and lactic acid, whose amount is unexpectedly high.

According to the obtained results, milk fermentation by plain Kombucha was a process that could have been used for obtaining new milk-based products in practice.

What is not known about lactose fermentation by Kombucha, and should be investigated in the later phases? Several points deserve the attention:

- Microbiological changes during and after the fermentation should be monitored;
- Issues related to storage of the obtained products are particularly significant;
- Facts related to potential benefits in using Kombucha products should be scientifically approved.

Acknowledgment

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References

1. G. SCHAAFSMA, Lactose and lactose derivatives as bioactive ingredients in human nutrition. *Int. Dairy J.*, 18, 458–465 (2008).
2. M. G. GÄNZLE, G. HAASE, P. JELEN, Lactose: Crystallization, hydrolysis and value-added derivatives. *Int. Dairy J.*, 18, 685– 694 (2008).
3. A. Y. TAMIME, R. K. ROBINSON, *Yoghurt-Science and Technology*, Woodhead Publishing Ltd., England, 2004.
4. J. REISS, Influence of different sugars on the metabolism of the tea fungus. *Z. Lebensm. Unters. For.*, 198, 258–261 (1994).
5. M. C. SIEVERS, A. LANINI, U. WEBER, M. SCHULER–SCHMID, M. TEUBER, Microbiology and fermentation balance in kombucha beverage obtained from a tea fungus fermentation. *Syst. Appl. Microbiol.*, 18, 590–594 (1995).
6. A. L. TEOH, G. HEARD, J. COX, Yeast ecology of kombucha fermentation. *Int. J. Food Microbiol.*, 95, 119–126 (2004).
7. S. MARKOV, R. MALBAŠA, M. HAUK, D. CVETKOVIĆ, Investigation of Tea Fungus Microbe Associations. I. The Yeasts. *Acta Periodica Technologica*, 32, 133–138 (2001).
8. C. DUFRESNE, E. FARNWORTH, Tea, kombucha and health: A Review. *Food Res. Int.*, 33, 409–421 (2000).
9. C. W. HESSELTINE, A millenium of fungi. Food and fermentation. *Mycologia*, 57, 148–167 (1965).
10. T. KAPPEL, R. H. ANKEN, The tea mushroom. *The Mycologist*, 7, 12–13 (1993).

11. S. PETROVIĆ, E. LONČAR, Content of water-soluble vitamins in fermentative liquids of tea fungus. *Mikrobiologija*, 33, 101–106 (1996).
12. D. A. BALENTINE, S. A. WISEMAN, L. C. BOUWENS, The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr.*, 37, 693–704 (1997).
13. C. PASHA, G. REDDY, Nutritional and medicinal improvement of black tea by yeast fermentation. *Food Chem.*, 89, 449–453 (2005).
14. R. MALBAŠA, E. LONČAR, LJ. KOLAROV, L-lactic, L-ascorbic, total and volatile acids contents in dietetic kombucha beverage. *Roum. Biotechnol. Lett.*, 7, 891–895 (2002).
15. R. JAYABALAN, S. MARIMUTHU, K. SWAMINATHAN, Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chem.*, 102, 392–398 (2007).
16. H. MO, Y. ZHUB, Z. CHEN, Microbial fermented tea–a potential source of natural food preservatives. *Trends Food Sci. Technol.*, 19, 124–130 (2008).
17. C. J. GREENWALT, R. A. LEDFORD, K. H. STEINKRAUS, Determination and characterization of the antimicrobial activity of the fermented tea kombucha. *Lebensm.–Wiss. Technol.*, 31, 291–296 (1998).
18. G. SREERAMULU, Y. ZHU, W. KNOL, Characterization of antimicrobial activity in Kombucha fermentation. *Acta Biotechnol.*, 21, 49–56 (2001).
19. E. LONČAR, S. PETROVIĆ, R. MALBAŠA, R. VERAC, Biosynthesis of glucuronic acid by means of tea fungus. *Nahrung*, 44, 138–139 (2000).
20. P. J. BLANC, Characterization of the tea fungus metabolites. *Biotechnol. Lett.*, 18, 139–142 (1996).
21. S. PETROVIĆ, E. LONČAR, LJ. KOLAROV, R. MALBAŠA, Vitamin C biosynthesis by tea fungus using Artichoke tubers extract. *Acta Periodica Technologica*, 28, 67–72 (1997).
22. R. MALBAŠA, Investigation of antioxidant activity of beverage from tea fungus fermentation. Ph. D. Thesis, University of Novi Sad, Faculty of Technology, Novi Sad, 2004.
23. R. MALBAŠA, E. LONČAR, M. DJURIĆ, Comparison of the products of Kombucha fermentation on sucrose and molasses. *Food Chem.*, 106, 1039–1045 (2008).
24. G. BELLOSO-MORALES, H. HERNÁNDEZ-SÁNCHEZ, Manufacture of a beverage from cheese whey using “tea fungus” fermentation. *Rev. Latinoam. Microbiol.*, 45, 5–11 (2003).
25. R. MALBAŠA, S. MILANOVIĆ, E. LONČAR, M. DJURIĆ, M. CARIĆ, M. ILIČIĆ, LJ. KOLAROV, Milk–based beverages obtained by Kombucha application. *Food Chem.*, 112, 178–184 (2009).
26. T. KIRYU, T. KISO, H. NAKANO, K. OOE, T. KIMURA, H. MURAKAMI, Involvement of Acetobacter orientalis in the production of lactobionic acid in Caucasian yogurt (“Caspian Sea yogurt”) in Japan. *J. Dairy Sci.*, 92, 25–34 (2009).
27. M. CARIĆ, S. MILANOVIĆ, D. VUCELJA, *Standard Methods of Analysing Milk and Dairy Products*, University of Novi Sad, Faculty of Technology, Novi Sad, 2000.
28. M. ATAMER, M. CARIĆ, S. MILANOVIĆ, D. GAVARIĆ, Quality of the yoghurt produced from UF milk. *Zbornik Matice srpske za prirodne nauke*, 91, 27-35 (1996).
29. M. GUZMAN–GONZALEZ, F. MORAIS, M. RAMOS, L. AMIGO, Influence of skimmed milk concentrate replacement by dry dairy products in a low fat set-type yoghurt model system: I: Use of whey protein concentrates. Milk protein concentrates and skimmed milk powder. *J. Sci. Food Agric.*, 79, 1117-1122 (1999).
30. E. LONČAR, M. DJURIĆ, R. MALBAŠA, LJ. KOLAROV, M. KLAŠNJA, Influence of working conditions upon Kombucha conducted fermentation of black tea. *Food Bioprod. Process.*, 84, 186–192 (2006).
31. S. AKHNAZAROVA, V. KAFAROV, *Experiment Optimization in Chemistry and Chemical Engineering*, Mir Pub, Moscow, 1982.