

Full Length Research Paper

Non-conventional starter kombucha cultivated in combination with probiotics for milk fermentation

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Accepted 15 September, 2017

The possibility of kombucha application cultivated on two different tea types in combination with probiotics for milk fermentation at different temperatures, as well as the physicochemical and textural properties of manufactured fermented milk products were investigated. Combination of probiotic starter culture and kombucha inoculums, cultivated on *Camellia sinensis* (black tea) and *Thymus serpyllum* (thyme tea) were used for milk fermentation. Obtained results showed that kombucha inoculums cultivated on different tea types could be used for fermented milk products in combination with probiotic starter culture. Different temperatures of milk fermentation had no significant effect on chemical characteristics of kombucha fermented dairy products. As the results of factorial ANOVA test revealed the significant influence of the interaction of factors Temperature*Tea type on L-lactic acid content. Tea type had significant influence on textural characteristics of fermented dairy products, especially after storage. After 10 day of storage, all textural characteristics except firmness had highly significant differences depending on the tea type used for kombucha inoculum cultivation. Significant effect on textural characteristics showed interaction of factors Tea Type*Day of storage.

Key words: Fermented milk product, kombucha, textural properties, physicochemical properties.

INTRODUCTION

Quality of fermented dairy products is influenced by conversion of milk components during fermentation. Metabolic activities of starter culture during the gelation process of milk are of particular importance. Type and ratio of microorganisms in starter culture contribute to different physico-chemical and sensory characteristics of fermented dairy products (Tamime and Robinson, 2004).

Fermented dairy products are manufactured using a wide range of microorganisms incorporated in starter culture. The major functions of starter cultures are: Biopreservation of the product resulting in prolonged shelf-life and enhanced safety; improvement of rheological and sensory properties; multifunctional positive effect to human health and bacteriocins production as potential food preservatives (Tamime, 2006).

Bhullar et al., 2002; De Vuyst and Vandamme, 1994). Lactic acid bacteria, yeasts, moulds or combinations of these microorganisms are applied for manufacturing of large group of highly nutritive fermented dairy products. The majority of starter cultures consist of yoghurt lactic acid bacteria: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*, and/or probiotics isolated from a human intestinal tract (prevailing *Lactobacillus* and *Bifidobacterium*) (Tamime, 2006; Parvez et al., 2006; Aso and Akazan, 1992).

In recent researches a symbiotic association of yeasts and acetic acid bacteria, known as kombucha, has been applied for milk fermentation with the aim to enlarge assortment of the fermented dairy products as a functional food (Milanović et al., 2008; Malbaša et al., 2009; Iličić et al., 2011). Kombucha is a symbiotic association of yeasts and acetic acid bacteria whose metabolic activities on sweetened tea produces a pleasant sour beverage with a number of useful compounds and positive influence on human health

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which have been investigated and confirmed by a number of authors. Kombucha has positive effect on tonsillitis, headaches, atherosclerosis, rheumatism, digestive difficulties, etc (Hartman et al., 2000; Sreeramulu et al., 2001).

In addition to sucrose, application of any other sugar (lactose, glucose, fructose) is possible (Reiss, 1994; Sreeramulu et al., 2000; Greenwalt et al., 1998; Hartmann et al., 2000; Chandan and Shandani, 1993). Kombucha is traditionally cultivated on a sweetened black and green tea. Also, it can be cultivated on some other type of tea or a dark beer, red wine, white wine, soft drink with cola, whey, lactose (Lončar et al., 2001; Bellosomora and Hernandez-Sanchez, 2003; Lončar et al., 2006). Prophylactic and therapeutic properties of kombucha are the result of metabolic activity with the production of: Acetic acid, ethanol, glycerol and other metabolites.

The formation of gel during the manufacture of certain dairy products is basically due to destabilization of the casein complex. The α -lactalbumin/ β -lactoglobulin interaction with the κ -casein (linked by —SH and —SS bridges) partially protects the micelles; however, as the pH in milk is lowered, destabilization or disruption of the micelles starts to occur. As a result, the gel network or protein matrix consists of micellar chains and/or micellar clusters and entraps within it all the other constituents of the milk base, including the water phase. The surface of set yoghurt should be smooth, shiny and free of visible whey, and exposing the inner surface with a spoon should reveal an equally homogeneous appearance (Singh et al., 1996; Tamime and Robinson, 2004; Tamime, 2007).

The texture of yoghurt is an important variable on its quality, and stirred yoghurt, which can be regarded as a concentrated dispersion of gel 'pieces' in serum, should be homogeneous and fairly viscous. Structure of the gel and its texture characteristics, are influenced by several factors, including incubation temperature, casein concentration, heat treatment of the milk, acidity and type of starter culture. The working temperature for texture measurement can also impact the values of the textural characteristics (Hui, 1993; Sodini et al., 2004)

The aim of this study was to investigate the possibility of application of non-conventional starter (kombucha cultivated on two different tea types: Thyme and black tea) in combination with probiotics for milk fermentation at different temperatures, and to examine physicochemical as well as textural properties of manufactured fermented milk products.

MATERIALS AND METHODS

Fermented milk products were manufactured in laboratory conditions, from pasteurized and homogenized milk with 1.6% fat (\gg AD IMLEK Beograd-Division Novosadska mlekarica« Novi Sad).

The following starter cultures were used for fermentation:

(i) Probiotic starter culture – ABT-7 – probiotic culture-Probio-Tek[®] contains LA-5[®], *Lactobacillus acidophilus*, BB-12[®], *Bifidobacterium*, *Streptococcus thermophilus*, CHR Hansen, Denmark.

(ii) Kombucha inoculums:

(a) Black tea kombucha inoculum – kombucha cultivated on sucrose substrate (70 g/L) with addition of 1.5 g/L black tea (oxidized *Camellia sinensis*);

(b) Thyme tea kombucha inoculum – kombucha cultivated on sucrose substrate 70 g/L with addition of 1.5 g/L thyme tea (*Thymus serpyllum*).

Samples were produced by addition of 90 mL of kombucha inoculum and 0.0935g/L of probiotic starter cultures in 900 mL of milk at three different temperatures: 37, 40 and 43°C. Fermentation was continued until pH=4.5 was reached. Then gel has cooled to 4°C, homogenized and packed in polypropylene glasses and stored in refrigerator at 4°C. Depending on the used starter culture and milk fermentation temperature, different samples were produced. The samples were labeled as BT37, BT40, BT43, TT37, TT40 and TT43. BT and TT indicate black and thyme tea, while 37, 40 and 43°C indicate temperature of fermentation. All samples were produced in triplicate.

Chemical quality was tested in fermented dairy products after production using the following methods (Carić et al., 2000): dry matter (DM) (IDF/ISO 21A:1982); milk fat (MF) according to Gerber (IDF 105:1981); total proteins (TP) (IDF 20:1962); ash (A) (IDF 90:1979); components by enzymatic tests (Megazyme, Ireland): L-lactic acid (K-DLATE 11/05), lactose and D-galactose (K-LACGAR 12/05). Water holding capacity (WHC) (Guzman-Gonzalez et al., 1999) and whey syneresis (Atamer et al., 1996) were also analyzed.

After production and 10 days of storage, textural characteristics of fermented milk products were analyzed by Texture analyzer TA.HD^{plus} (Stable Micro Systems, England) at 4°C. The compression force was measured by A/BE disc, diameter 35 mm and 5 kg extension bob. Trigger force was 10 g. The option "Return to Start" was used. The speed of disc movement before and after the test was 1.0 mm/s. The longitude of the path the disc passed was 30 mm.

Statistical analysis of results was carried out with the computer software program "Statistica 9" and results were expressed as average values with standard deviation. Factorial ANOVA test for comparisons of several average values was applied for determining differences amongst textural characteristics as well as components of different samples. The influences of the next factors and interactions were calculated: Temperature; Tea type; Day of storage; Temperature*Tea type; Tea type*Day of storage; Temperature*Day of storage; Temperature*Tea type*Day of storage.

RESULTS AND DISCUSSION

Fermentation of milk with kombucha and probiotic starter lasted from 3.5 to 4.5 h (Figure 1 a and b). Shapes of fermentation curves are sigmoidal and very similar for both groups of samples (BT-black tea, *Camellia sinensis* and TT-thyme tea, *Thymus serpyllum*). Increasing the temperature caused the decreasing of the fermentation time. Sample BT43 had the shortest fermentation time (3.5 h) that is similar with previously published research (Duraković et al., 2008). Also, these results indicate that

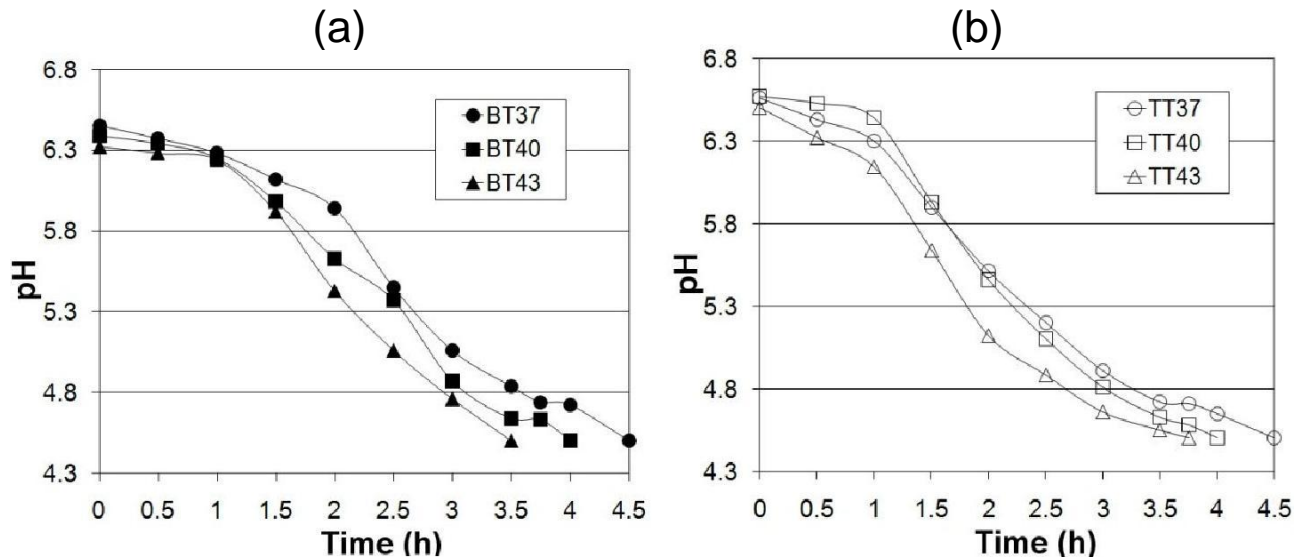


Figure 1. Changes in pH during milk fermentation.

Table 1. Chemical composition and physical characteristics of kombucha fermented dairy products.

Product	Component (% w/w)				Physical characteristics (%w/w)	
	Dry matter	Fat	Protein	Ash	Syneresis (cm ³ /50 cm ³)	WHC (% w/w)
BT37	10.12 ± 0.11	1.55 ± 0.03	3.03 ± 0.13	0.64 ± 0.10	34.00 ± 0.00	39.25 ± 0.35
BT40	10.07 ± 0.11	1.55 ± 0.03	2.92 ± 0.80	0.63 ± 0.10	32.50 ± 0.87	38.50 ± 0.71
BT43	9.93 ± 0.80	1.55 ± 0.02	2.88 ± 0.17	0.61 ± 0.03	32.20 ± 0.82	39.67 ± 1.04
TT37	10.24 ± 0.05	1.56 ± 0.02	3.03 ± 0.05	0.69 ± 0.01	31.50 ± 1.26	30.50 ± 1.02
TT40	10.22 ± 0.07	1.55 ± 0.04	3.01 ± 0.11	0.68 ± 0.14	31.75 ± 1.06	32.75 ± 0.35
TT43	10.23 ± 0.02	1.55 ± 0.02	2.85 ± 0.03	0.71 ± 0.05	33.00 ± 0.06	32.00 ± 1.52

WHC = Water holding capacity.

kombucha inoculums cultivated on different tea types could be used in combination with probiotic starter culture in fermented dairy products technology.

Chemical composition of all kombucha fermented milk products are presented in Table 1. Samples produced with kombucha cultivated on thyme tea had slightly higher level of dry matter and ash than samples produced with kombucha cultivated on black tea. All samples had similar level of milk fat and proteins content.

Samples BT40 and BT43 had much better water holding capacity than sample BT37 (Table 1). Values of water holding capacity were similar for samples produced with kombucha cultivated on thyme tea. The highest value of syneresis among samples produced with kombucha cultivated on thyme tea had sample TT43. The obtained values indicate that tea type had influence on samples WHC.

Analysis of temperature and tea type effects on lactose, galactose and L-lactic acid content showed that

temperature had significant influence on L-lactic acid content (Table 2). Also, tea type had significant impact on lactose and L-lactic acid content, and interaction of factors Temperature*Tea type had significant influence only on L-lactic acid content (Figure 2).

Tea type and temperature used for milk fermentation had significant impact on firmness of produced samples (Figure 3a). Also, very important influence on firmness had a day of storage, especially on samples fermented at 43°C, which is in accordance with previous results (Iličić et al., 2010). These results have revealed that interaction of factors Tea type*Day of storage had significant impact on firmness. Samples produced with thyme tea kombucha inoculum had lower firmness after production and after ten day of storage as well as at all temperatures, compared with samples produced with black tea kombucha inoculum. Statistical analysis of consistency showed significant influence of tea (Table 3). In the case of black tea kombucha inoculum, consistency

Table 2. Influence of temperature and tea type on lactose, D-galactose and L-lactic acid.

Parameter	Lactose			D-galactose			L-lactic acid		
	DF	MS	P	DF	MS	P	DF	MS	P
Temperature	2	0.0142	0.1624	2	0.0002	0.6726	2	0.0135	0.0001*
Tea type	1	0.0763	0.0128*	1	0.0024	0.0508	1	0.0037	0.0031*
Temperature* Tea type	2	0.0281	0.0586	2	0.0004	0.4397	2	0.0037	0.0018*

DF = Degree of freedom, MS = Mean of square, P = P value, *P<0.05.

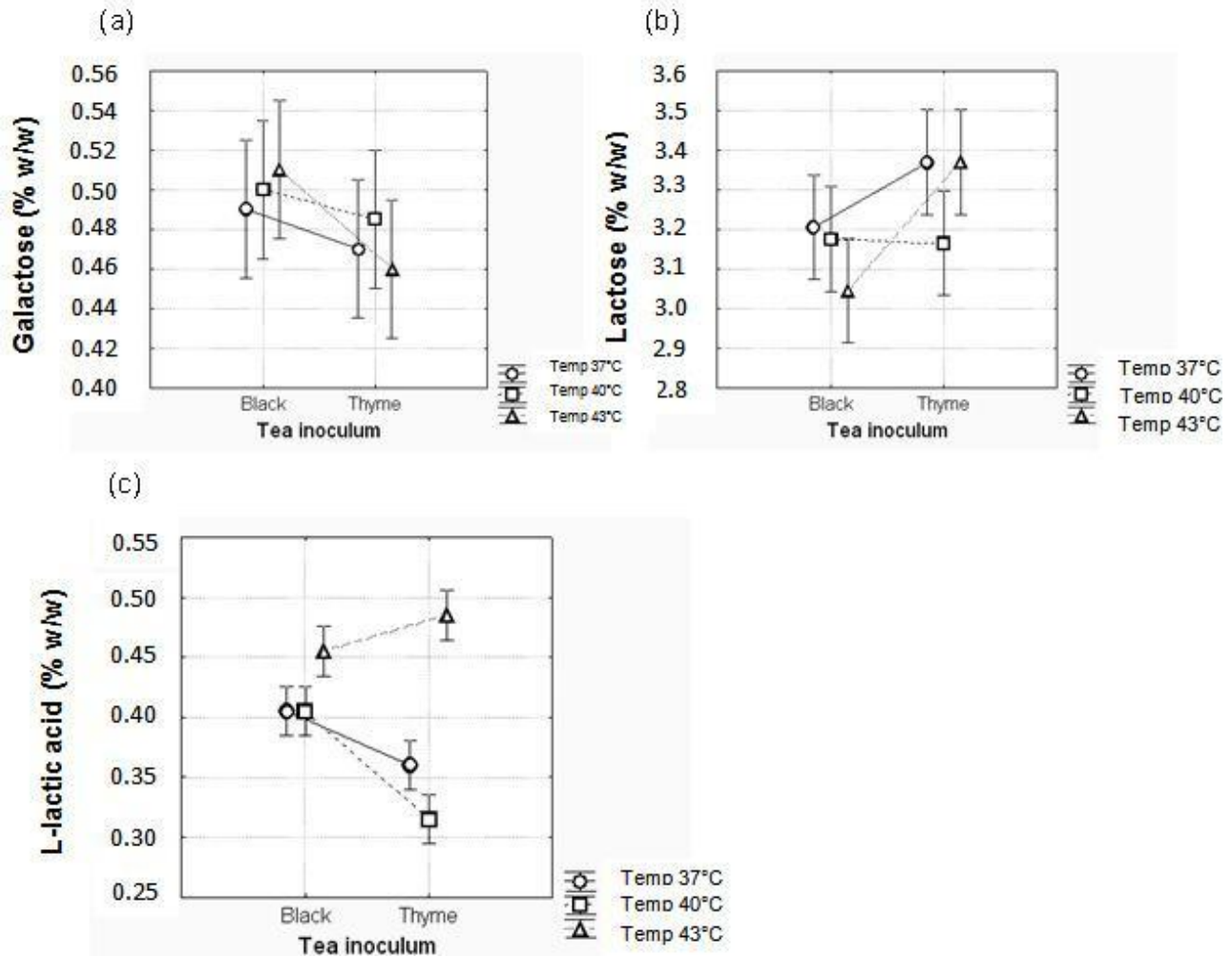


Figure 2. Influence of temperature (°C) and tea type on kombucha fermented products: (a) D-galactose (b) lactose and (c) L-lactic acid.

values significantly increased during the storage in the sample BT43. Samples produced with thyme tea kombucha inoculum didn't show significant changes in consistency during the storage (Figure 3b). Interaction of factors Temperature*Day of storage had significant influence on consistency.

Significant impact of used tea on cohesiveness was noticed (Figure 4a). These differences are very

pronounced during the storage. Namely, in all samples produced with thyme tea kombucha inoculums, cohesiveness increases during the storage. On the other hand, cohesiveness of the samples produced with black tea kombucha inoculum decreased during 10 day storage. Interaction of factors Tea*Day of storage showed high impact on cohesiveness while factor Day of storage individually had no important influence (Table 4).

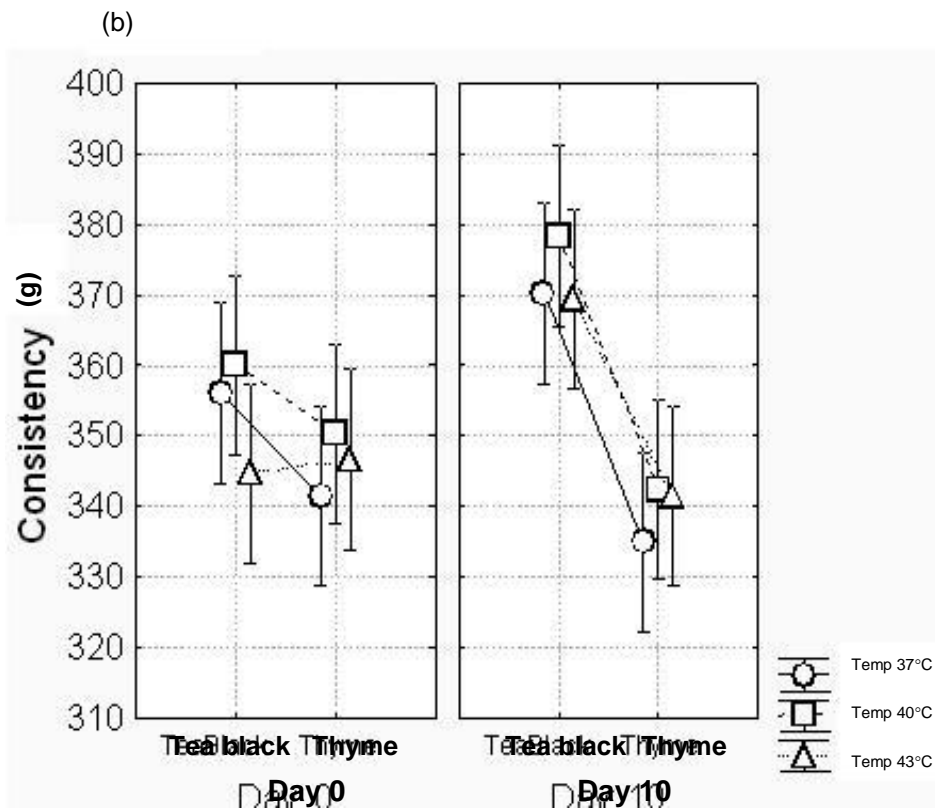
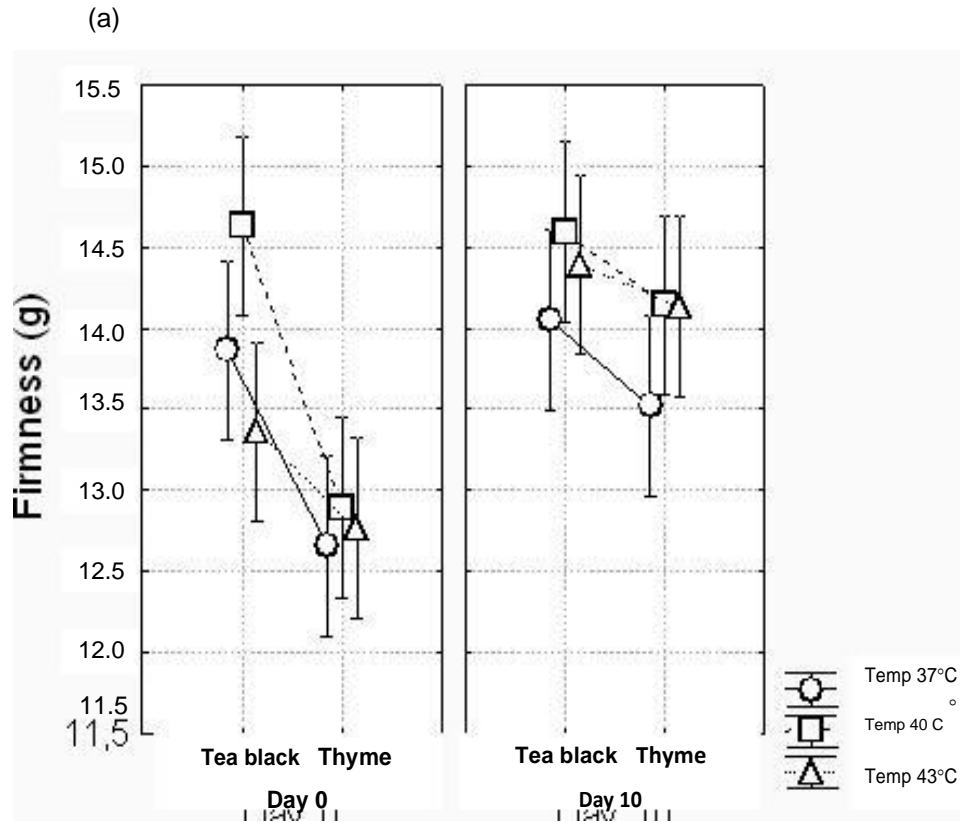


Figure 3. Influence of tea type and day of storage on kombucha fermented products: (a) firmness (b) consistency.

Table 3. Influence of different factors on firmness and consistency of kombucha fermented dairy products.

Factor	Firmness			Consistency		
	DF	MS	P	DF	MS	P
Temperature	2	0.630	0.028*	2	136	0.178
Tea type	1	3.791	0.000*	1	2457	0.000*
Day of storage	1	3.621	0.000*	1	235	0.089
Temperature*Tea type	2	0.228	0.211	2	82	0.334
Tea type*Day of storage	1	0.874	0.023*	1	983	0.003*
Temperature*Day of storage	2	0.268	0.167	2	19	0.756
Temperature*Tea type *Day of storage	2	0.113	0.437	2	11	0.850

DF = Degree of Freedom, MS = Mean of square, P = P value, *P<0.05.

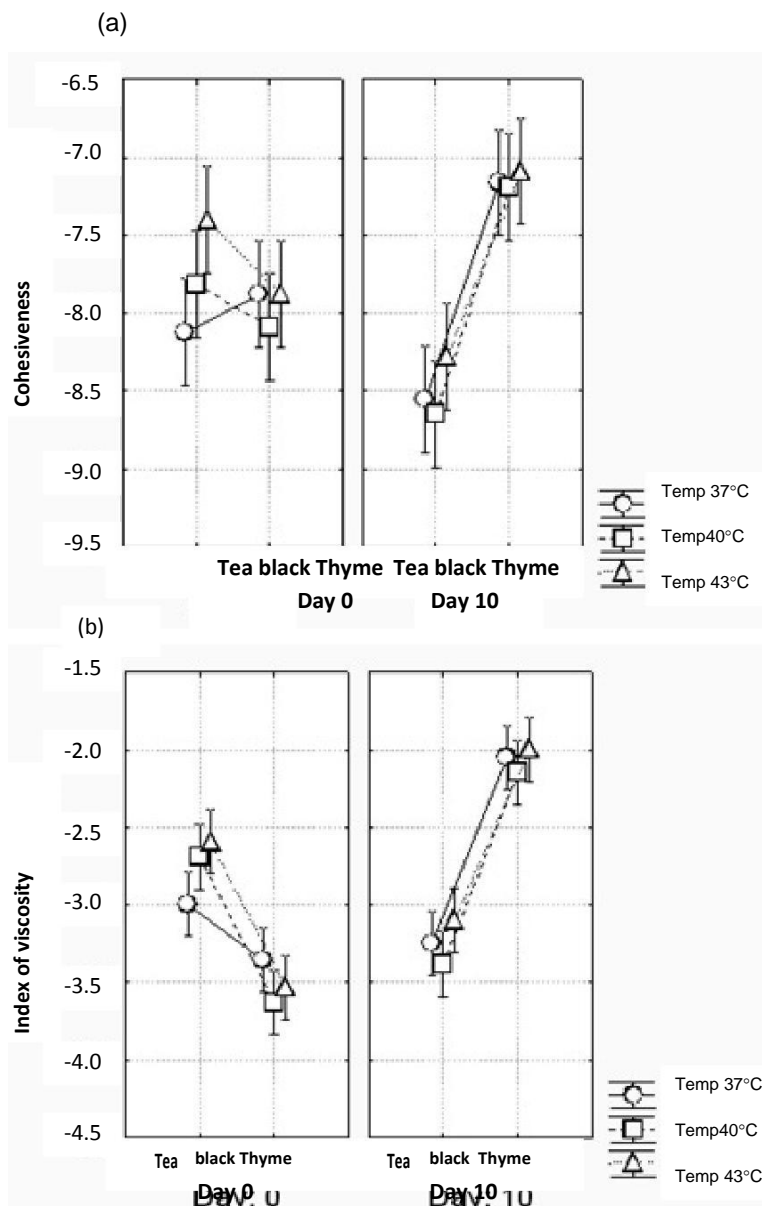


Figure 4. Influence of tea type and day of storage on products: a) cohesiveness; b) index of viscosity.

Table 4. Influence of different factors on cohesiveness and index of viscosity of kombucha fermented dairy products.

Factor	Cohesiveness			Index of viscosity		
	DF	MS	P	DF	MS	P
Temperature	2	0.197	0.048*	2	0.052	0.097
Tea type	1	2.075	0.000*	1	0.276	0.002*
Day of storage	1	0.012	0.635	1	1.384	0.000*
Temperature*Tea type	2	0.108	0.155	2	0.065	0.062
Tea type*Day of storage	1	3.449	0.000*	1	5.546	0.000*
Temperature*Day of storage	2	0.017	0.714	2	0.011	0.572
Temperature*Tea type*Day of storage	2	0.051	0.384	2	0.051	0.099

DF = Degree of Freedom, MS = Mean of square, P = P value, *P<0.05.

As well as on the cohesiveness, tea type had significant influence on index of viscosity (Table 4). Production index of viscosity was higher in samples produced with black tea kombucha inoculum compared with the samples with thyme tea kombucha inoculum. During 10 day storage, these values changed and samples produced with thyme tea kombucha inoculum had higher index of viscosity (Figure 4b).

Analysis of textural characteristics after storage revealed significantly higher textural characteristics in samples produced with black tea kombucha inoculums compared to thyme tea. These results indicate positive influence of black tea ingredients on samples gel during storage.

Conclusion

Obtained results revealed that kombucha inoculums cultivated on different tea types could be used in combination with probiotic starter culture in fermented dairy products technology. Tea type used for fermentation had significant influence on samples water holding capacity, but not on syneresis values. Different temperatures of milk fermentation had no significant effect on physico-chemical characteristics of kombucha fermented dairy products. Results of factorial ANOVA test showed that interaction of factors Temperature*Tea type had significant influence on content of L-lactic acid.

Tea types used for sample production had significant impact on textural characteristics especially after storage. Depending on tea types used for kombucha inoculum cultivation, firmness, consistency and index of viscosity had highly significant differences after 10 day storage. Significant impact on textural characteristics showed interaction of factors Tea type*Day of storage.

ACKNOWLEDGMENT

Authors want to thank Ministry of Education and Science of Republic of Serbia for the financial support of

investigations presented in this article, Project No. 46009.

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