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Milk-based beverages obtained by Kombucha application

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ABSTRACT

This paper investigates the manufacture of milk-based beverages by application of several Kombucha starters. Local Kombucha culture was grown up on three substrates: sweetened black and green tea, and topinambur. Their concentrates were obtained by vacuum-evaporation and amounts of 10% and 15% (v/v) were applied to milk (2.2% fat). The traditional yoghurt starter (B3) was applied for producing control samples. All fermentations were stopped when the pH reached 4.4. Fermentation curves were registered, linear for yoghurt and sigmoidal for Kombucha. Two times faster process was achieved with yoghurt starter. Influence of inoculum concentration on the rate of fermentation was insignificant. Viscosities were higher for Kombucha beverages at lower speeds of spindle, but lower at higher speeds of spindle. Very high sensory scores were achieved for all beverages, after production and after 5-days' storage.

1. Introduction

A great number of scientists have confirmed the importance of milk and dairy products in satisfying the recommended daily intake of nutritionally important components. Particularly valuable is yoghurt, manufactured by application of starters with prevailing cultures of *Streptococcus* and *Lactobacillus*. Also, well-known is symbiotic association of yeasts and bacteria, known as Kombucha (Dufresne & Farnworth, 2000; Teoh, Heard, & Cox, 2004), whose metabolic activity on sweetened tea produces a pleasant sour beverage containing, in addition to ethanol and acetic acid, a number of useful compounds (Balentine, Wiseman, & Bouwens, 1997; Danielova, 1957; Hesseltine, 1965; Kappel & Anken, 1993; Pasha & Reddy, 2005; Petrović & Lončar, 1996; Steiger & Steinegger, 1957). Their positive influence on human health has been investigated and confirmed by a number of authors (Greenwalt, Ledford, & Steinkraus, 1998; Jayabalan, Marimuthu, & Swaminathan, 2007; Malbaša, Lončar, & Kolarov, 2002).

The activity of Kombucha on sucrose has been investigated by several authors (Dufresne & Farnworth, 2000; Reiss, 1994; Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995; Teoh et al., 2004) and the main pathways of conversion of sucrose into numerous products have been determined. In addition to sucrose, application of any other sugar (lactose, glucose or fructose) is possible. This will have minor effect on the flavour of the fermented beverage, but may have major effect on the formation of ethanol and lactic acid as products (Reiss, 1994). For example, it was proved that fermentation on lactose gave insignificant quantities of ethanol, in comparison with the fermentation on sucrose (Reiss, 1994). However, very little evidence on applying Kombucha to carbon sources different from sucrose exists. Petrović, Lončar, Kolarov, and Malbaša (1997) investigated the biosynthesis of vitamin C during tea fungus fermentation on polysaccharide inulin obtained from the tubers of the Jerusalem artichoke (*Helianthus tuberosus*). Malbaša (2004) reviewed some attempts in applying atypical nutrients such as Coca-Cola, red wine, white wine, vinegar, extract of *Echinacea*, *Mentha*, etc. Also, some agricultural and industrial by-products, including molasses from sugar beet processing, were considered as potential sources of carbon and nitrogen (Malbaša, Lončar, & Djurić, 2008).

As far as lactose fermentation by tea fungus is concerned, only a few investigations have been reported; Lončar, Milanović, Carić, Malbaša, and Panić (2001) reported investigations of metabolic activity of tea fungus on milk, whilst, Belloso-Morales and Hernández-Sánchez (2003) published a paper on the manufacture of a beverage from cheese whey. A great number of milk-based beverages are known, in different parts of the world, often as home-made products, obtained by application of various combinations of native microorganisms (mixtures of bacteria and sometimes yeasts), whose overall metabolic activities have not yet been completely explained. These fermented milks include busa (Turkestan), cieddu (Italy), dadhi (India), kefir (Balkans), kumiss (Steppes), laban Zabadi (Egypt), mazun (Armenia), taette (N. Europe), skyr (Iceland), masl (Iran), crowdies (Scotland), kuban, etc. It might be expected that kefir, commercially available in many countries, including Serbia, has a certain degree of similarity with the Kombucha metabolites of milk. Some data on kefir microbiology

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can be found in recently published papers (Abraham & de Antoni, 1999; Lopitz-Otsoa, Rementeria, Elguezabal, & Garaizar, 2006).

This paper describes the manufacture of milk-based beverages by application of several Kombucha starters. It also compares chemical compositions, physical characteristics and sensory characteristics of new products to traditional yoghurt and commercially available kefir.

2. Materials and methods

2.1. Milk

Homogenised and pasteurised cow's milk with 2.2% fat, 3.0% protein and 4.65% lactose, produced in AD Novosadska mlekara, Novi Sad, Serbia, was used for the production of milk-based beverages. Physical, chemical and microbiological characteristics of milk were entirely in accordance with the accepted standards.

2.2. Cultures

2.2.1. Kombucha culture

Local Kombucha culture was applied for production of all milk-based beverages except yoghurt. Previous investigations (Markov, Malbaša, Hauk, & Cvetković, 2001) showed that the applied fungus contained at least five yeast strains (*Saccharomycodes ludwigii, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis* sp. and *Zygosaccharomyces* sp.). Primary Kombucha bacterium belongs to the strains of the genus *Acetobacter* (Reiss, 1994; Sievers et al., 1995; Teoh et al., 2004).

2.2.2. Yoghurt culture

Yoghurt was obtained by application of standard culture B3, produced by Chr. Hansen, Copenhagen, Denmark, containing *Streptococcus thermophilus and Lactobacillus delbruecki* spp.bulgaricus.

2.3. Kombucha inoculum concentrates

2.3.1. Concentrates from black and green tea

The concentrate from black tea was prepared from 1 l of boiled tap water with 70 g of sucrose (forming 7% solution of sucrose) and 1.5 g of black tea (Indian tea, "Vitamin", Horgoš, Serbia). The tea was heated for 5 min at 100 °C, tea leaves were removed by filtration and the obtained solution was cooled to room temperature. Cold tea was inoculated with 10% of fermentation broth from previous Kombucha fermentation, covered with cheesecloth and incubated (at constant temperature of 29.5 ± 1 °C) for six days. Finally, the solution was exposed to vacuum-evaporation, so that 60 ml of concentrate was obtained from 300 ml of the original solution.

The other concentrate was prepared from green tea (Green tea, "Adonis", Jagodina, Serbia), under the same conditions as for the concentrates from black tea.

2.3.2. Concentrate from topinambur

This concentrate was made from an extract of topinambur (*Helianthus tuberosus* L.), according to the procedure completely described in the paper by Petrović et al. (1997). The obtained solution was exposed to vacuum-evaporation, under the same conditions as those described previously.

2.4. Milk-based beverages

Traditional yoghurt and six fermented beverages were manufactured from milk inoculated at 42 °C (Table 1). The fermentations lasted 3–6.5 h to reach pH 4.4. Afterwards, the obtained milk gels were cooled to 8 °C, homogenised by mixing, and packed in plastic containers. Each fermentation was repeated three times.

Table 1
Fermented beverage composition

No.	Fermented beverage	Inoculum concentration (%)	Inoculum type
1.	Yoghurt	3	Yoghurt culture B3
2.	BT10	10	Concentrate from black tea
3.	BT15	15	Concentrate from black tea
4.	GT10	10	Concentrate from green tea
5.	GT15	15	Concentrate from green tea
6.	TA10	10	Concentrate from topinambur
7.	TA15	15	Concentrate from topinambur

Also, commercially available kefir, produced from milk with 2.5% fat, was bought from a local store.

2.5. Methods of analysis

2.5.1. Chemical analysis

Chemical analysis of milk, yoghurt and fermented beverages was performed by employing standard methods. Dry matter content (D) was measured after drying at 105 °C (IDF, 1982). Fat content in milk (F) was analysed according to Gerber (IDF, 1981). Total nitrogen content was determined according to the Kjeldahl method (IDF, 1962), whilst total proteins (P) were calculated by multiplying total nitrogen result by 6.38%. Lactose content in milk (L) was measured by titrimetry (IDF, 1964), whilst quantity of lactose in the fermented beverages was calculated. Ash content (A) was measured after mineralisation at 550 °C (Carić, Milanović, & Vucelja, 2000). Based on the chemical composition, energy value of each beverage was calculated ($EV = 4.186\ (9.3\ F + 4.4\ P + 4.1\ L)$ in kJ/100 g. Total acidity was determined by titration with a standard solution of sodium hydroxide and phenolphthalein as indicator. L-Lactic acid was determined by spectrophotometer (Steinsholt & Calbert, 1960).

Duncan's multiple range test, for comparison of several means (Akhnazarova & Kafarov, 1982), was applied for determining differences amongst chemical compositions of fermented beverages.

2.5.2. pH, viscosity and sensory characteristics

pH values were measured by an electric pH-meter (Iskra, MA 5713, Kranj, Slovenia).

Viscosity was determined on 100 g samples, by using a rotational viscometer (Digital DV-E, Brookfield, UK), with spindle S4, code 04, at 20 °C.

Sensory characterisation of yoghurt, kefir and remaining fermented beverages was performed by qualified evaluators together with untrained consumers, who assessed each particular element of quality as follows: appearance (0–1), colour (0–1), consistency (0–4), odour (0–2) and taste (0–12).

3. Results and discussion

3.1. Development of fermentation

In accordance with the plan of experiments (Table 1), seven kinds of fermented beverages were produced: the traditional yoghurt and six Kombucha-containing beverages. The concentrated Kombucha tea liquors were used as an inoculum, rather than tea fungus culture itself, because of the quality of products obtained in preliminary experiments and also better reproducibility of fermentations. Development of all fermentation processes was followed by measuring pH values with time and the results in Fig. 1 were obtained. As the end of fermentation, a value typical of yoghurt (pH 4.4) was obtained. The fermentation curves in Fig. 1 shows two times faster process in yoghurt than in systems inoculated by the Kombucha concentrates. pH in yoghurt decreased quickly and almost linearly, whilst pH in Kombucha beverages decreased very slowly, at the beginning (to the 3rd hour), then dropped exponentially in the middle of the process, and stagnated at the end. Different shapes of fermentation curves (linear for yoghurt and sigmoidal for Kombucha fermentation) can be related to the differences in metabolic activities of the applied starters. In order to explain the noticed differences more precisely, further investigations are required. When the original Kombucha starters (without pre-concentration) had been applied, more than three times slower activity, compared to the yoghurt starter, was noticed (Lončar et al., 2001). So, our preliminary investigations suggested concentration of Kombucha-based starters lead to an improvement of their activity.

Once obtained, concentrates of Kombucha starters were applied at two levels, 10% and 15% (v/v). It seemed that these amounts did not affect the fermentation duration in a regular way, i.e., application of the 10% starter did not always mean slower process, whilst the 15% starter did not always guarantee faster fermentation. So, the inoculation at a level of 10% was followed by slower fermentation in the case of concentrate from black tea (Fig. 1a) but it was followed by faster fermentation in the case of concentrate from topinambur (Fig. 1c). When concentrate from green tea was applied, similar metabolic activities were noticed for both inoculum concentrations (Fig. 1b). These findings led to the conclusion that influence of inoculation concentration (10% or 15%) on the rate of fermentation is not significant, which is in agreement with our earlier results on sucrose fermentation (Lončar, Djurić, Malbaša, Kolarov, & Klašnja, 2006). Namely, when investigating influence of several independent variables (time, temperature and inoculum concentration) on the concentration of Kombucha metabolites, it has been statistically proven that process duration was the most significant variable; temperature was the second important variable, whilst the inoculum concentration was the least important.

3.2. Chemical composition

Chemical composition of all the fermented beverages, including yoghurt, is presented in Table 2. Also, composition of kefir, bought from a local supermarket, was determined. Chemical analysis showed that Kombucha beverages contained higher levels of dry matter (11.2% on average) compared to the dry matter of yoghurt (10.1%). Kefir also contained a relatively high value of dry matter (11.3%). Fat content of all beverages produced from milk with 2.2% fat was almost constant; a slightly higher value was obtained for kefir produced from milk with 2.5% fat. Protein content varied from 2.8% to 3.9% in all samples. Level of total acids in yoghurt and kefir (0.7%) was higher than the level in the new beverages

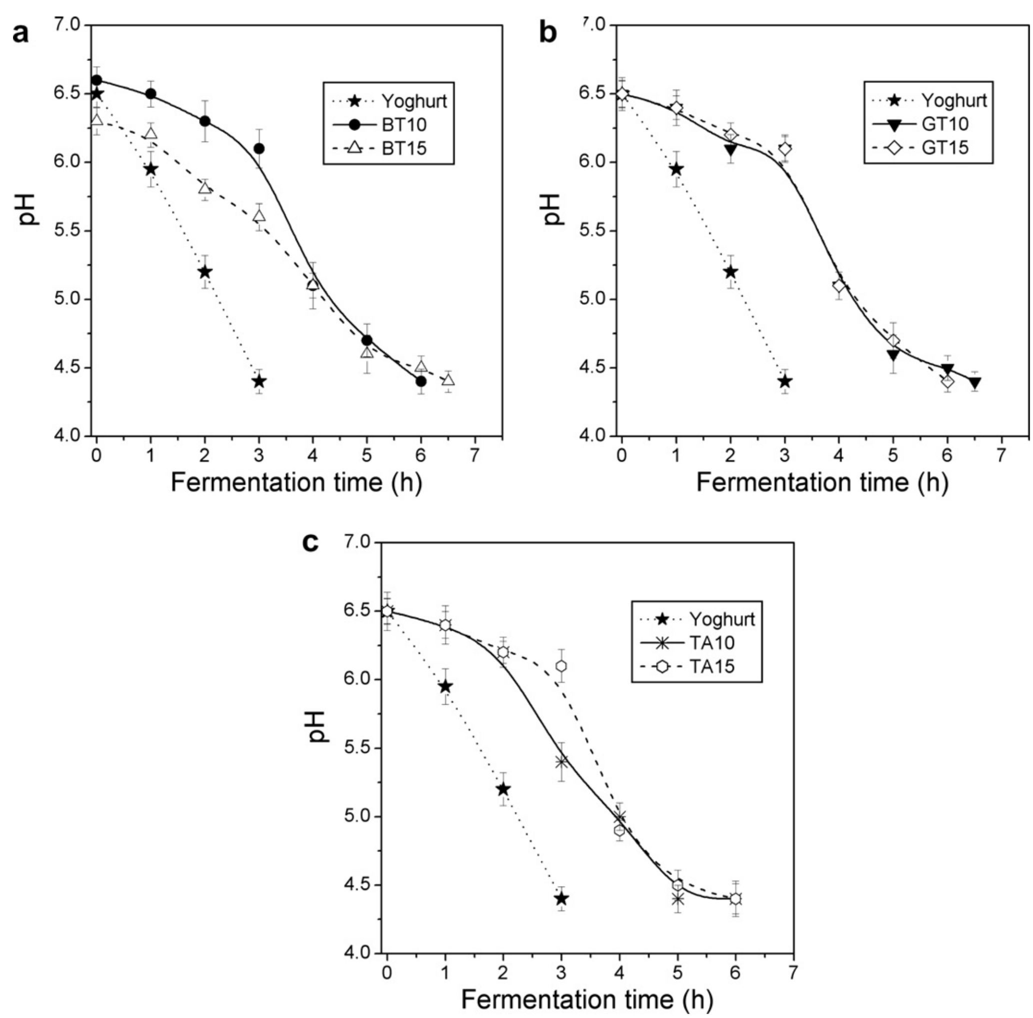

Fig. 1. pH changing during milk fermentation process with different inoculums: (a) black tea, (b) green tea,(c) topinambur.

Table 2
Chemical composition of the fermented beverages

Product	Component (%)						EV[a]
	Dry matter	Fat	Proteins	Ash	Total acids	Lactose[a]	(kJ/100 g)
Yoghurt	10.1 ± 0.19	2.4 ± 0.071	3.0 ± 0.021	0.88 ± 0.029	0.70 ± 0.25	3.82	215
Kefir[b]	11.3	2.6	3.2	0.77	0.70	4.73	243
BT10	11.5 ± 0.15	2.4 ± 0.072	3.8 ± 0.024	0.84 ± 0.022	0.52 ± 0.21	4.46	240
BT15	11.2 ± 0.17	2.3 ± 0.070	3.7 ± 0.028	0.79 ± 0.023	0.55 ± 0.21	4.41	233
GT10	10.5 ± 0.17	2.3 ± 0.071	2.8 ± 0.019	0.83 ± 0.019	0.54 ± 0.23	3.71	219
GT15	11.7 ± 0.16	2.3 ± 0.072	3.9 ± 0.029	0.83 ± 0.022	0.59 ± 0.16	4.67	242
TA10	11.1 ± 0.17	2.2 ± 0.066	3.5 ± 0.021	0.88 ± 0.031	0.55 ± 0.15	4.52	227
TA15	11.3 ± 0.13	2.4 ± 0.072	3.6 ± 0.021	0.88 ± 0.026	0.62 ± 0.20	4.42	235

[a] Calculated values.
[b] Standard deviations not known.

(0.56% on average). The calculated content of lactose in yoghurt (3.8%) was lower than the lactose content in the Kombucha beverages (4.4% on average) and in kefir (4.7%). The obtained results for total acid level were in accordance with lactose content in fermented beverages. Both findings (more lactose and less total acids) are very probably related to the slower metabolic activity of Kombucha starters.

In order to compare the differences amongst chemical compositions of products, Duncan's multiple range test (Akhnazarova & Kafarov, 1982) was performed and the results in Table 3 were obtained. An analysis of values in *D*-columns shows that yoghurt has a dry matter content significantly lower than all the beverages being developed. What is more, differences are also evident amongst new products. However, dry matter of BT10 does not differ significantly from the dry matter of BT15, GT15 and TA15, whilst dry matter of BT15 does not differ significantly from the dry matter of TA10 and TA15. The labels for the fermented beverages, are in Table 1. Conclusions drawn after an analysis of *P*-columns in Table 3 are as follows; yoghurt has protein content significantly lower than all the other Kombucha beverages. Also, Kombucha beverages differ amongst themselves where protein content is concerned. When analysing ash content, approximately one half of the compared systems are not significantly different. Finally, few differences in fat content were observed.

Not only the differences in gross chemical compositions of yoghurt and new beverages but also the differences in some metabolites (such as essential amino acids, essential fatty acids, valuable micro-elements and vitamins, etc.) can be expected. Therefore, further investigations should be oriented towards detailed characterisation of new developed fermented products obtained by Kombucha application.

3.2.1. Lactic acid

Lactic acid, one of the metabolites associated with nutritive and functional characteristics of fermented beverages, was determined. The values for yoghurt and six Kombucha-based beverages, measured immediately after production and after 5-day storage at 4 °C, are shown in Fig. 2. As for kefir, the content of lactic acid was measured after being a few days exposed to the market.

The greatest content of lactic acid was found in fresh yoghurt (0.62%), whilst in Kombucha beverages the highest level was 0.58% for system TA15 inoculated with 15% of Kombuha concentrate on topinambur. As expected, during storage the acidity increased. So, the increase of lactic acid content within five days was extreme for yoghurt (45%) and lower for new products. Extremely low content was found in kefir samples (0.37%).

It also should be emphasised that pH value of all beverages remained unchanged during storage (pH 4.4), except yoghurt that

Table 3
Comparison of differences in dry matter (*D*), fat (*F*), proteins (*P*) and ash (*A*) amongst fermented beverages by Duncan's multiple range test

Sample	BT10				BT15				GT10				GT15				TA10				TA15			
	D	*F*	*P*	*A*	*D*	*F*	*P*	*A*	*D*	*F*	*P*	*A*	*D*	*F*	*P*	*A*	*D*	*F*	*P*	*A*	*D*	*F*	*P*	*A*
Yoghurt	+[a]	-[b]	+	-	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	-
TA15	-	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+	-	+	+	+				
TA10	+	+	+	-	-	-	+	+	+	-	+	+	+	-	+	+								
GT15	-	-	+	-	+	-	+	-	+	-	+	-												
GT10	+	-	+	-	+	-	+	-																
BT15	-	-	+	-																				

a) + significant difference
b) – not significant difference

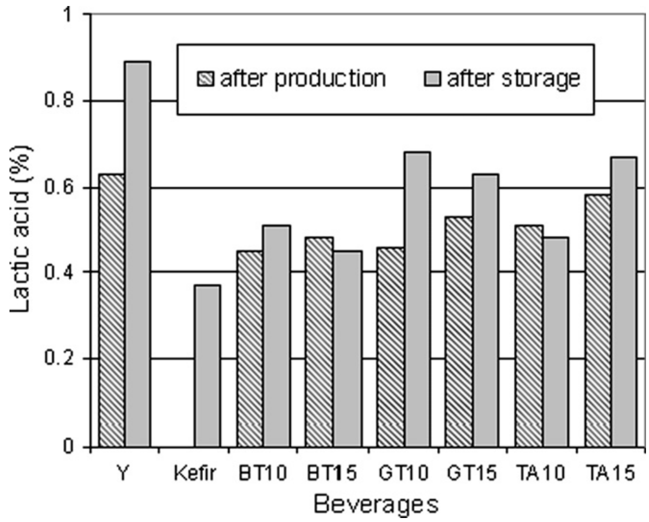

Fig. 2. Content of lactic acid in fermented beverages.

became rather sour (pH 4.1). On the other hand, pH value for kefir was 4.45.

3.3. Viscosity

An important characteristic of fermented milk beverages, as non-Newtonian fluids, is viscosity. Its value is a relevant element of product quality when measured after production and also after storage. The results obtained in these investigations are presented in Figs. 3–5. An analysis of the presented values showed that all Kombucha-based beverages possessed higher viscosities than the traditional yoghurt at lower speeds of spindle. The differences diminished with an increase in speed; e.g., at a speed of 50 rpm, all Kombucha beverages had lower viscosities than yoghurt. Similar conclusions could be drawn for viscosity after the products were stored for five days. In most cases, slightly lower viscosity values were obtained after storage of all investigated systems.

3.4. Sensory characteristics

A certain difference between sensory characteristics of yoghurt, on one hand, and the other investigated beverages, on the other, might be expected. It, also, can be expected that there will be a decline of quality during storage of products. Therefore, sensory characterisation of yoghurt, kefir and the new fermented beverages was performed and the results in Figs. 6 and 7 were obtained. Very

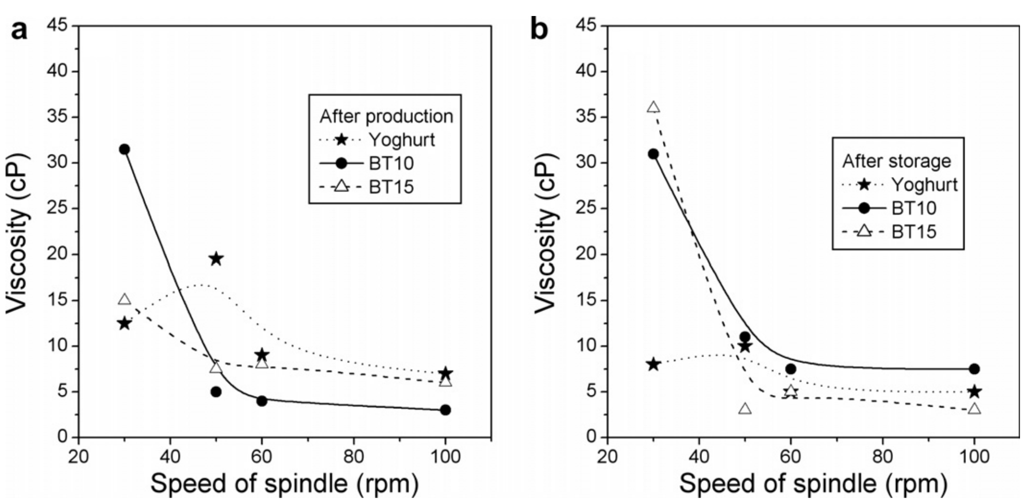

Fig. 3. Viscosity of milk-based beverages inoculated with Kombuha-on-black-tea starters: (a) after production, (b) after storage.

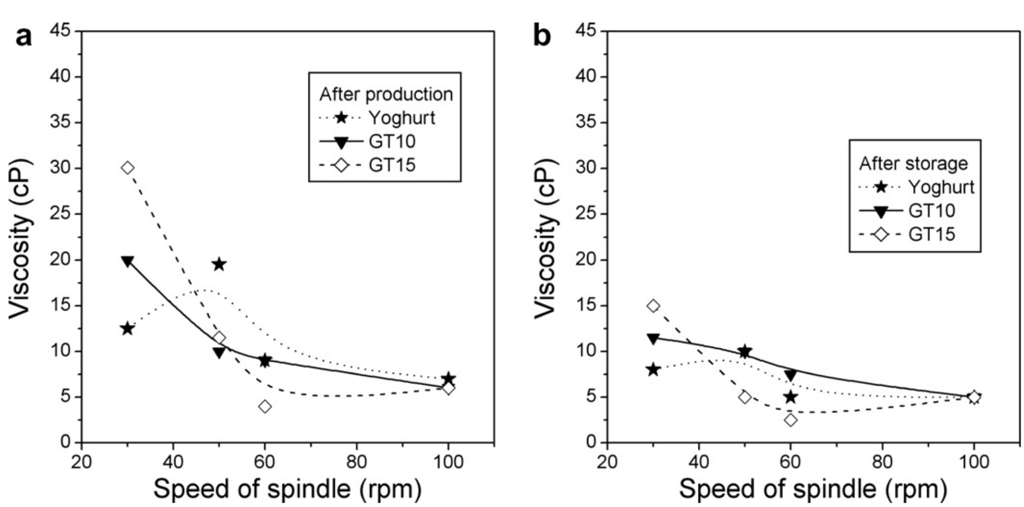

Fig. 4. Viscosity of milk-based beverages inoculated with Kombuha-on-green-tea starters: (a) after production, (b) after storage.

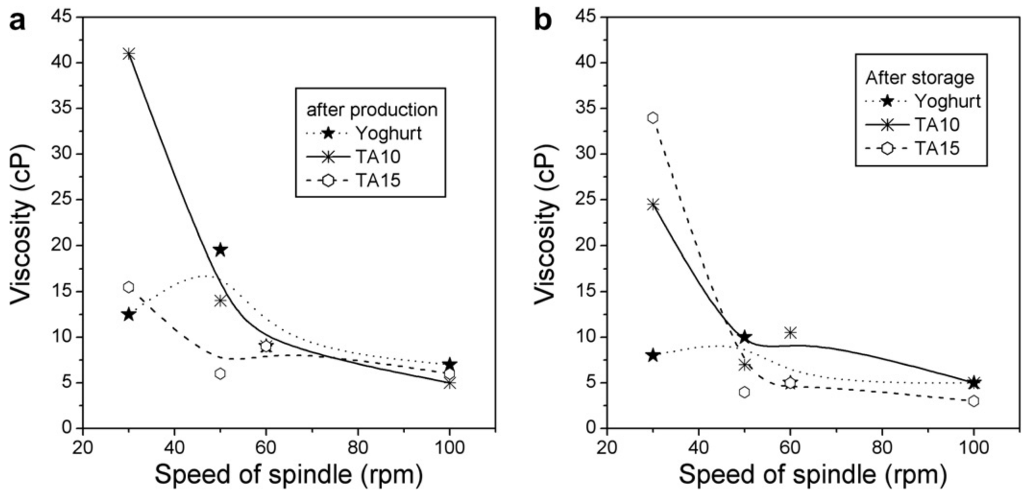

Fig. 5. Viscosity of milk-based beverages inoculated with Kombuha-on-topinambur starters: (a) after production, (b) after storage.

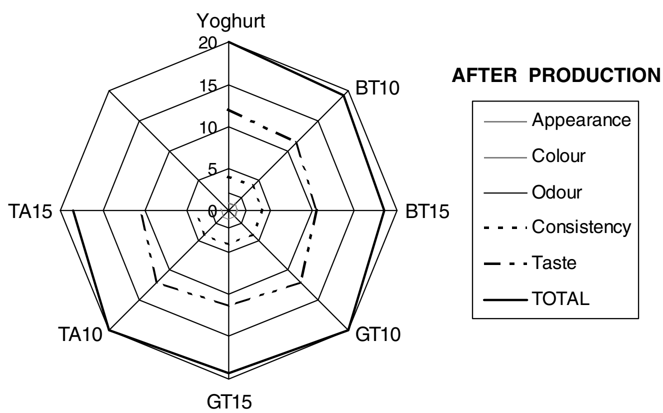

Fig. 6. Sensory characteristics of all fermented beverages after production.

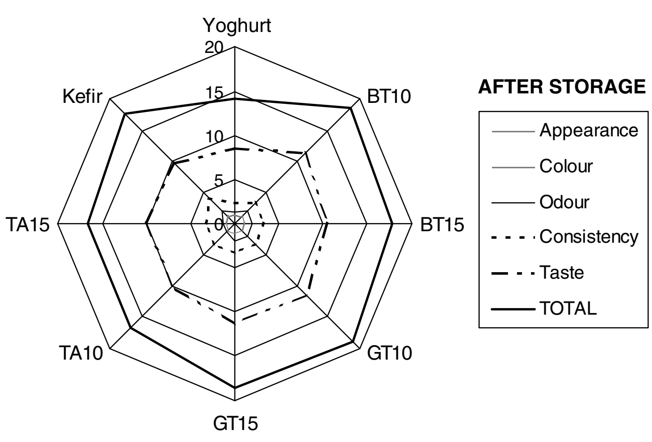

Fig. 7. Sensory characteristics of all fermented beverages after storage.

high marks were given to all products after the fermentation. Only the taste of some Kombucha-based beverages (BT15 and TA15) was less acceptable. However, during storage yoghurt sensory characteristics declined the most, especially its taste, which became inferior compared to the taste of all the other beverages. Also, yoghurt odour became unpleasant and its consistency weakened. It seems that the low rate fermentation continues in yoghurt samples but not in the new beverages.

4. Conclusion

These investigations proved that fermented beverages can be produced by application of local Kombucha starters to cow's milk.

The metabolic activities of all three Kombucha cultures (grown-up on black tea, green tea and topinambur) were similar but different from the activity of yoghurt starter. Also, the metabolic activity of Kombucha starters on lactose was significantly different from the activity on sucrose. The chemical compositions of the new beverages differed significantly from the composition of yoghurt. The differences were large for dry matter and protein content. Less significant differences were observed in ash contents, whilst non-significant differences were found in fat contents. The chemical compositions of the new beverages varied, particularly in the case of protein content. Many of the new products had significantly different contents of dry matter, whilst the least significant differences were found in fat contents.

The viscosities of new products showed higher degree of variation (related to the speed of spindle and storage duration) compared to yoghurt and according to their sensory characteristics the new products are competitive to yoghurt. During storage these products behave even better than yoghurt; their acidity remains unchanged and most of the quality parameters were better.

Before suggesting production of new beverages on a larger scale, further investigations have to be performed, oriented towards characterisation of the functional properties of the developed milk-based beverages obtained by Kombucha application.

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