Available online at www.sciencedirect.com

ScienceDirect

Food Chemistry 108 (2008) 926–932

Food Chemistry

www.elsevier.com/locate/foodchem

Effect of sucrose concentration on the products of Kombucha fermentation on molasses

R. Malbaša *, E. Lončar, M. Djurić, I. Došenović

Faculty of Technology, University of Novi Sad, Bul. Cara Lazara 1, 21000 Novi Sad, Serbia

Received 5 October 2007; received in revised form 22 October 2007; accepted 28 November 2007

Abstract

Fermentation of 1.5 g/l of Indian black tea, sweetened with adequate quantities of molasses (containing approx. 70 g/l, 50 g/l and 35 g/l of sucrose), was conducted using domestic Kombucha. Inoculation was performed with 10% of fermentation broth from a previous process. The fermentation in cylindrical vessels containing 2 l of liquid phase, was carried out at 22 ± 1 °C for 14 days, with periodical sampling, to measure pH, content of acids (total, acetic and L-lactic), content of remaining sucrose, and the yield of biomass at the end of fermentation. A product with 70 g/l sucrose from molasses corresponds to an optimal concentration of carbon source, which provided metabolites with high pH, a low content of less desired acetic acid, a high content of highly desired L-lactic acid, an acceptable content of total acids and the highest possible level of utilisation of sucrose.

Keywords: Kombucha; Molasses; Sucrose concentration; Black tea; L-Lactic acid; Acetic acid

1. Introduction

A pleasantly sour beverage is produced as a result of Kombucha fermentation on sweetened black tea. The main metabolites of this fermentation are monosaccharides, several organic acids, some vitamins and a great number of other compounds, appearing as a result of numerous reactions (Balentine, Wiseman, & Bouwens, 1997; Danielova, 1957; Hesseltine, 1965; Kappel & Anken, 1993; Pasha & Reddy, 2005; Petrović & Lončar, 1996; Steiger & Steinegger, 1957). Especially important are organic acids – active ingredients of fermented tea that may exert beneficial effects on human health (Greenwalt, Ledford, & Steinkraus, 1998; Jayabalan, Marimuthu, & Swaminathan, 2007; Malbaša, Lončar, & Kolarov, 2002).

Kombucha is a symbiosis of osmophilic yeasts and acetic acid bacteria (Dufresne & Farnworth, 2000; Reiss, 1994; Teoh, Heard, & Cox, 2004). A traditional carbon source for its fermentation is sucrose. Yeasts and bacteria make use of this substrate in complementary ways; yeast cells hydrolyse sucrose into glucose and fructose, producing ethanol, with a preference for fructose as a substrate, while acetic acid bacteria utilise glucose to produce gluconic acid, and ethanol to produce acetic acid (Dufresne & Farnworth, 2000; Reiss, 1994; Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995). The influence of different sugars (sucrose, lactose, glucose and fructose), as well as different sugar concentrations (50–150 g/l) on the metabolism of the tea fungus was investigated by Reiss, in 1994. He found distinct effects of the mentioned carbon sources on the formation of ethanol and lactic acid. In 1996, Blanc also investigated influence of sucrose concentration (50, 70 and 100 g/l) on the content of ethanol, lactic, acetic, gluconic and glucuronic acids in the metabolites. His results were slightly different from the results reported by Reiss (1994) very probably because of the differences in the tea fungus used. After reviewing a great number of papers dealing with the Kombucha fermentation, it can be

---

* Corresponding author. Tel.: +381 21 4853645; fax: +381 21 450413.
E-mail address: bingula@yahoo.com (R. Malbaša).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.11.069

concluded that many scientists consider 70 g/l sucrose as the optimal concentration.

Besides pure sucrose, various complex systems containing sucrose are worth considering. Particularly interesting are some agricultural and industrial by-products, including molasses from sugar beet processing. It is attractive, not only because of its low price but also because of the presence of a number of other components, such as minerals, organic compounds and vitamins, which are very useful for the fermentation process (Rodrigues, Teixeira, & Oliveira, 2006). Molasses is also a commonly-used carbon source in the industrial production of lactic acid (Kanwar, Tewari, Chadha, Punj, & Sharma, 1995; Shukla et al., 2004; Wee, Kim, Yun, & Ryu, 2004).

Our first results on sugar beet molasses application were published in 2001 (Lončar, Malbaša, & Kolarov, 2001). We have found that molasses is a suitable carbon source for Kombucha fermentation. Recently, we extended the investigations, to include three molasses of different characteristics (Malbaša, Lončar, & Djurić, 2008). We concluded that the products obtained from all samples of molasses were richer in lactic acid than the product from sucrose, which was related to the presence of invert sugar, biotin and amino nitrogen in the molasses. To our knowledge, no other reports on this matter have appeared in the literature so far.

The aim of this paper was to investigate the effect of sucrose concentration (approx. 70 g/l, 50 g/l and 35 g/l) on the content of the main metabolites of the Kombucha fermentation of sugar beet molasses.

2. Materials and methods

2.1. Kombucha culture

Local domestic Kombucha was used for fermentation. Primary Kombucha bacterium belongs to the strains of the genus *Acetobacter* (Reiss, 1994; Sievers et al., 1995; Teoh et al., 2004). Yeasts from liquid Kombucha samples were isolated on OGYA medium. The plates were incubated at 28 °C for 3 days. Yeast colonies were purified by repeated streak cultures on the same medium. Yeast isolates were identified by the standard morphological and biochemical tests described by Kreger-van Rij (1984). The presence of yeasts *Saccharomycodes ludwigii, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis* sp. and *Zygosaccharomyces* sp. were established (Markov, Malbaša, Hauk, & Cvetković, 2001).

2.2. Molasses characteristics

Molasses was taken from a sugar factory located near the city of Sremska Mitrovica in the Pannonian basin. In the factory, the sugar beet from the mentioned locality was processed. The main characteristics of the molasses were as follows: 84.2% dry matter, 50.4% sucrose, 0.83% invert sugar, 6.7 pH, 1.8% total nitrogen, 0.29% amino nitrogen and 5.5 μg/100 g biotin.

2.3. Fermentation systems

The fermentation systems, labelled MC1, MC2 and MC3, were prepared, using boiled tap water and an adequate quantity of previously sterilised molasses. In this way, 2 l of each system was obtained, containing sucrose at a level of approx.: 70 g/l (MC1), 50 g/l (MC2) and 35 g/l (MC3). To each system, 3 g of black tea (Indian tea, "Vitamin", Horgoš, Serbia) was added. The tea was heated for 5 min at 100 °C, and the leaves were removed by filtration.

The prepared liquids were poured into cylindrical vessels of equal size and geometry and, after cooling to room temperature, inoculated with 10% of fermentation broth from a previous Kombucha fermentation. The vessels were covered with cheesecloth and the contents left to incubate at a constant temperature of 22 ± 1 °C for 14 days. Samples of the obtained beverages were taken after 0, 3, 7, 10 and 14 days, to measure the following parameters: pH, total acids, L-lactic and acetic acid, as well as the quantity of the remaining sucrose. Also, the yield of biomass at the end of the 14th day was determined.

All experiments were repeated three times.

2.4. Methods of analysis

Values of pH were measured using an electronic pH meter (Iskra, Kranj, Slovenia).

Total acidity was determined by titration with a standard solution of sodium hydroxide and phenolphthalein as indicator.

Acetic acid was determined by a UV method for its determination in foodstuffs and other materials (Böhringer, Mannheim, Cat. No. 148261). The method is based on several enzymatic reactions, resulting in the formation of NADH. Absorbance was measured at 340 nm.

L-Lactic acid was also determined using a Böhringer–Mannheim test kit (Cat. No. 139084). L-Lactic acid (L-lactate) was oxidised with NAD, in the presence of L-lactate dehydrogenase, to pyruvate. By trapping the pyruvate in a subsequent reaction catalysed by the enzyme glutamate–pyruvate transaminase in the presence of L-glutamate, the equilibrium can be displaced in favour of pyruvate and NADH. The amount of NADH formed is stoichiometric with the amount of L-lactic acid.

Sucrose content in the molasses was determined by a standard polarimetric method described in the sugar industry manual (Milić, Karadžić, & Obradović, 1992). The sucrose remaining after fermentation was determined using a Böhringer–Mannheim test kit (Cat. No. 716260).

The yield of the obtained biomass was determined by mass measurement; the cellulose floating pellicle layer was removed from the fermented liquid surface, rinsed with distilled water and dried with filter paper.

3. Results and discussion

3.1. pH values

As a result of acids formation, the pH values of the fermentation products decreased during the process (Fig. 1). However, there is no simple relation between pH value of metabolites and the content of acids. The decrease of pH values in all systems followed a kind of exponential law, with the most rapid decay at the beginning of fermentation (to the 3rd day). After the 3rd day, the decrease became much slower, turning into pH stagnation, especially in the systems containing molasses. This phenomenon may be associated with the buffer effect arising from the reactions between the synthesised organic acids and minerals from the substrate (Lončar, Petrović, Malbaša, & Verac, 2000).

The highest pH values were detected in the system MC1 (70 g/l sucrose), while the lowest pH values were measured in the systems MC2 (50 g/l sucrose) and MC3 (35 g/l sucrose). All of them contained sucrose from molasses as a carbon source. According to the Duncan's multiple range test there was no significant difference between the last two systems. In this way, a previous finding was confirmed in systems MC2 and MC3, i.e., the lower the sucrose concentration, the lower the pH value. On the other hand, a decrease of pH values, in all cases, follows an increase of acetic acid content in the metabolites, making a sense of the previous conclusions.

3.2. Acetic acid

As already mentioned, acetic acid is one of the main metabolites of the acetic acid bacteria from Kombucha association. Our results related to its generation in the systems with molasses (MC1–MC3) are shown in Fig. 2. An almost linear increase of acetic acid content was noticed, within 14 days of fermentation, in all investigated systems. The increase was approximately three times slower in the system MC1 with 70 g/l sucrose from molasses. The final concentration of acetic acid amounted to 0.59 g/l, on average, for sucrose, MC2 and MC3, but reached only 0.19 g/l for MC1 (see Table 1). All samples were significantly different from each other; the sample MC1 is extremely different from the others. These results are in agreement with our

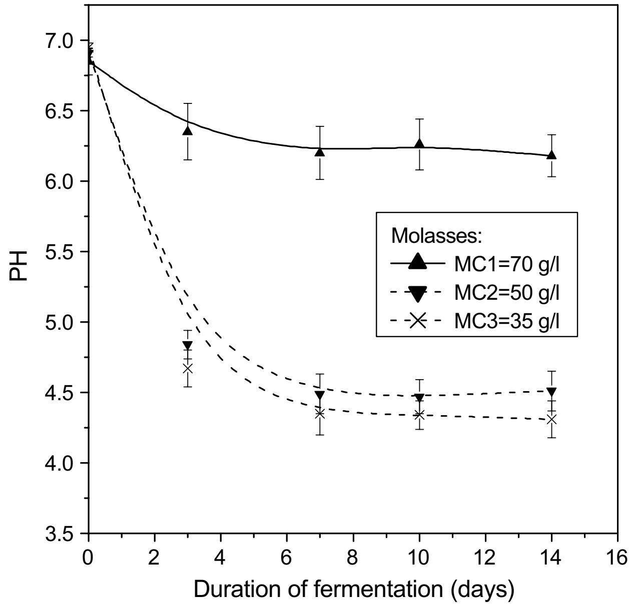

Fig. 1. Values of pH of the liquid phase of Kombucha as a function of time.

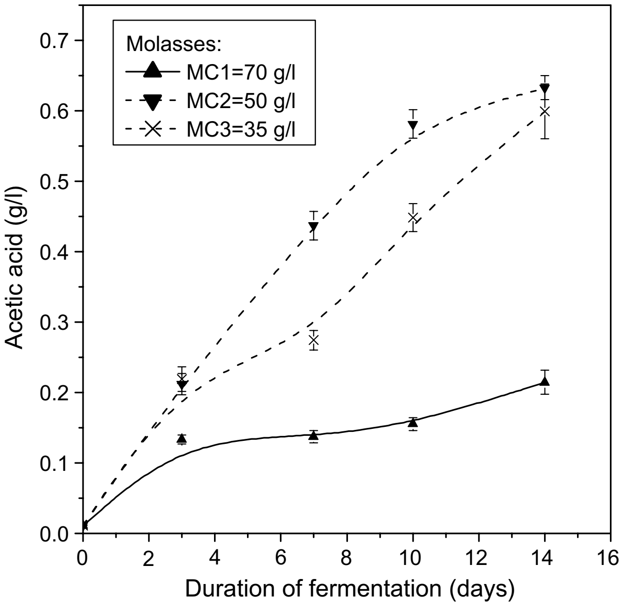

Fig. 2. Content of acetic acid in the liquid phase of Kombucha as a function of time.

Table 1
Comparison of published results on molasses fermentation by Kombucha

Reference	Ferment. conditions		Products on the 14th day of fermentation				
	Sucrose (g/l)	Day	pH	Acids in the metabolites (g/l)			
				Acetic	L-Lactic	Total	Biomass, g
Lončar et al. (2001)	70	14	5.5	1.3[a]	–[b]	3	
Malbaša et al. (2008)							
Sucrose[c]	70	14	5.3	0.53	0.05	1.15	17
Molasses M1[d]	70	14	6.1	0.28	0.4	2.5	155
Molasses M2[e]	70	14	4.8	0.24	0.16	4.2	166
Molasses M3[f]	70	14	6.2	0.21	0.32	2.5	271
This paper							
System MC1	70	14	6.3	0.19	0.33	2.3	260
System MC2	50	14	4.5	0.63	0.15	2.9	100
System MC3	35	14	4.3	0.60	0.12	2.7	85

[a] Volatile.
[b] Not tested for the presence of lactic acid.
[c] System with pure sucrose due to comparison.
[d–f] Characteristics of fermentation on molasses presented in the paper Malbaša et al. (2008).

earlier results (Malbaša et al., 2008). Namely, a significantly higher content of acetic acid was found in metabolites of 70 g/l pure sucrose, compared to the metabolites of three kinds of molasses with 70 g/l sucrose (see Table 1).

When the concentration of sucrose in samples with and without molasses is correlated to the content of acetic acid, no simple relation was found. So, acetic acid appeared in system MC1 (70 g/l sucrose) at low level, in system MC3 (35 g/l sucrose) at higher level and in system MC2 (50 g/l sucrose) at the highest level. While investigating systems with pure sucrose, Blanc (1996) obtained the following results: in a system with 70 g/l sucrose a low level of acetic acid, in a system with 100 g/l sucrose a higher level of acetic acid, and in a system with 50 g/l sucrose the highest level of acetic acid. Even a superficial analysis of Blanc's results shows the absence of any regularity. Also, the absolute values of the generated quantities of acetic acid in Blanc's systems were much higher compared to our systems. This might be caused by the higher metabolic activity of the Kombucha used in Blanc's experiments.

As mentioned earlier, an increase of the acetic acid concentration corresponds to a certain extent to the decrease of the pH of the metabolites. It can be concluded that sample MC1 gives the product with the lowest concentration of acetic acid and therefore a high pH value, thus being highly acceptable as a substrate for Kombucha fermentation.

3.3. L-Lactic acid

An increase of the content of L-lactic acid with the duration of fermentation, on systems with two carbon sources as well as different concentrations of sucrose, is presented in Fig. 3. As can be seen, the smallest increase was obtained in the fermentation of pure sucrose (from 0 g/l to 0.053 g/l). On the contrary, metabolites obtained from molasses contained much larger amounts of L-lactic acid, the descending sequence being MC1 > MC2 > MC3. The increase was more significant in the early phase of fermentation (up to the 3rd day), which was followed by a phase of stagnation. The values at the end of fermentation were 0.33 g/l for MC1, 0.15 g/l for MC2 and 0.11 g/l for MC3. These differences were confirmed as significant by Duncan's test. They are also in high agreement with our earlier results (Malbaša et al., 2008); namely, significantly lower content of L-lactic acid was found in metabolites of 70 g/l pure sucrose, compared to the metabolites of three kinds of molasses with 70 g/l sucrose (see Table 1).

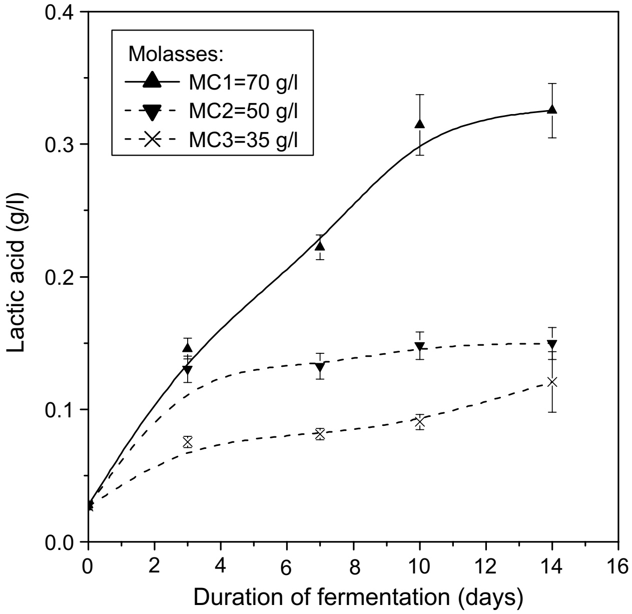

Fig. 3. Content of L-lactic acid in the liquid phase of Kombucha as a function of time.

Having in mind that formation of lactic acid is highly desired, it is obvious that molasses is a better substrate than pure sucrose. Molasses contained the growth factor biotin (5.5 μg/100 g) and amino nitrogen (29%), which could be the reasons for a more intensive Kombucha metabolism, resulting in higher contents of L-lactic acid. In lactic acid formation pyruvate and lactate appear as the end products of the first anaerobic step in the glycolysis. Due to a decline in the availability of oxygen, pyruvate is accumulated, as well as converted to lactate. The conversion of glucose to pyruvate requires NAD^+ as a substrate. This is produced from the oxidation of NADH by the electron transport chain. During oxygen deficiency, the regeneration of NAD^+ ceases, and glycolysis cannot proceed because of the lack of substrate (Handy, 2006). As Kombucha is an association of aerobic bacteria and facultative anaerobic yeasts, the system itself ferments without forced oxygenation, so that the produced lactic acid could possibly serve as an electron sink.

Concerning an effect of sucrose concentration on lactic acid production, a concentration of 70 g/l sucrose proved the best, because 50 g/l and 35 g/l gave a substantially lower content of lactic acid. From this point of view, the sample MC1 is highly acceptable as a substrate for Kombucha fermentation.

3.4. Total acids

Although not simply related to the pH value, content of total acids (as a function of fermentation time) is an important characteristic of the metabolites (Fig. 4). The initial concentration of acids was higher in all systems with molasses (0.75 g/l on average), compared to the system with sucrose (0.15 g/l). The reason might be the presence of certain groups of compounds in molasses (such as free fatty acids, non-volatile organic acids and a small quantity of lactic acid from the degradation of invert sugar). As fermentation proceeded, the actual concentration of acids increased in all systems. On average, the increase in acidity in the systems with the molasses ranged from 0.75 g/l to 2.7 g/l. According to Duncan's test, the acidity of each system differs from the acidity of the others significantly, although these differences are much smaller between the

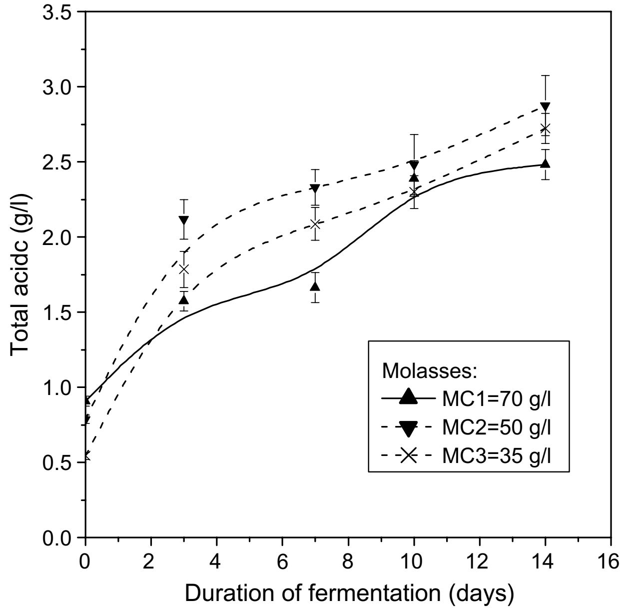

Fig. 4. Content of total acids in the liquid phase of Kombucha as a function of time.

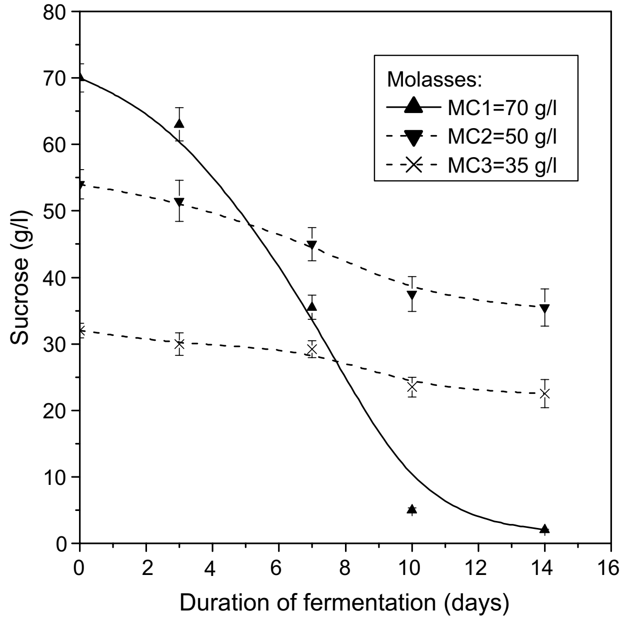

Fig. 5. Content of sucrose in the liquid phase of Kombucha as a function of time.

samples with molasses, compared to the samples with sucrose (see Table 1). A similar result was obtained in our previous investigation performed on three kinds of molasses (Malbaša et al., 2008). It has been concluded that the higher content of total acids in the molasses fermentation samples is due to the chemical composition of the molasses, which make a very convenient substrate for the industrial manufacturing of different organic acids, such as lactic, butyric, propionic, acetic, citric, fumaric and gluconic (Došenović, 2004).

As far as the concentration of sucrose in molasses and no-molasses samples is concerned, it seems that some other factors affect the metabolic activity of Kombucha as well. Otherwise, it woud be difficult to explain why the difference between samples with pure sucrose and MC1, with 70 g/l sucrose, was greater than the difference among the three samples (MC1, MC2 and MC3) with approx. 70 g/l, 50 g/l and 35 g/l sucrose (see Table 1).

Finally, the MC1 system, although having a high content of total acids, has a high pH value, meaning that fermented tea with this molasses will have a reduced sour taste, despite a high content of acids. A similar conclusion (see Table 1) has been drawn by Malbaša et al. (2008); it was found that the tea sweetened with 70 g/l sucrose from molasses had a higher pH than the tea with the same concentration of pure sucrose, although it had a higher content of total acids.

3.5. Utilisation of sucrose

Kombucha converts sucrose to glucose and fructose, and further to ethanol, acetic acid, lactic acid, and a large number of other compounds. In a way, the time decrease of sucrose concentration (Fig. 5) may be used as a measure of the rate of fermentation. As it is obvious, utilisation of 70 g/l sucrose, available from the system MC1 with molasses, after the 14th day of fermentation, is almost complete. It reached 97%, which was not the case for the rest of the systems. Levels of utilisation were 35% for MC2 and 25% for MC3. In our previous investigation (Malbaša et al., 2008) on three kinds of molasses, containing 70 g/l sucrose, it was found that the decay of sucrose concentration in the system with molasses was much faster than in the systems with pure sucrose. Results presented here confirmed this conclusion but only if the concentration of sucrose is optimal (70 g/l). If it is lower, utilisation in the samples with molasses is slow, regardless of the presence of numerous compounds acting positively on the fermentation.

3.6. Yield of biomass

The yield of biomass during the fermentation is closely related to the nature of the substrate. The amounts of biomass in particular systems differed so significantly, the differences could be seen even with the naked eye. Concerning the systems with molasses, the following yield were: MC1 – 260 g, MC2 – 100 g and MC3 – 85 g. This sequence agreed with the sequence of sucrose concentration (70 g/l, 50 g/l and 35 g/l), proving the importance of the carbon source to the fermentation process. However, other factors, particularly nitrogen source, might affect the yield of biomass. During the fermentation with sucrose the only source of nitrogen is tea, while the fermentation with molasses occurred in the presence of non-sucrose organic matter at a level of about 20% of the total mass. Here, the content of total nitrogen (from free amino acids, pyrrolidone carboxylic acid, peptides, nucleic acid components, etc.) can be up to 2% of the total mass (Došenović, 2004). As the content of nitrogen-containing compounds in the systems with molasses was much higher, compared to the systems with sucrose, the observed differences in the biomass yield must be ascribed to these components. The same conclusion was drawn in our previous investigations (Malbaša et al., 2008); molasses proved a better substrate for Kombucha generation than a sucrose solution.

3.6. Sensory characteristics of fermented tea

Black tea fermented by Kombucha was light brown coloured, sparkling, sour and a refreshing beverage. Kombucha beverages obtained from molasses were dark, slightly carbonated and sweet, with caramelized taste and a smell typical of molasses. Beverages obtained from MC2 and MC3 were tasteless, in comparison to the beverage obtained from MC1.

4. Conclusion

While comparing the composition of metabolites obtained from pure sucrose and from molasses, with an equal concentration of sucrose (70 g/l), a great difference is noticed. Quantities of some components in both systems' metabolites differ even more than the quantities of these components in systems with molasses, regardless of the concentration of sucrose. This implicates that the Kombucha fermentation processes on pure sucrose and molasses differ significantly, which is related to the higher quantity of invert sugar, biotin and amino nitrogen in the molasses.

As far as systems with molasses are concerned, a primary task is to determine an effect of sucrose concentration on the production of particular components in the metabolites. The conclusions are as follows:

- There is no simple relation between the concentration of sucrose and the content of acetic acid.
- High levels of sucrose guarantee high levels of L-lactic acid.
- Total acids are slightly dependent on the concentration of sucrose.
- A high level of sucrose guarantees a high level of its utilisation and also provides a high yield of biomass.

The chemical composition of the substrate with molasses is considerably richer, in comparison to the substrate with pure sucrose but it was proved that 70 g/l sucrose from molasses corresponded to an optimal concentration, which produced metabolites with high pH, a low content of less desired acetic acid, a high content of highly desired L-lactic acid, an acceptable content of total acids and the highest possible level of utilisation of sucrose. Sensory characteristics confirm the quality of that beverage.

It is possible to perform a successful fermentation on a substrate with molasses containing sucrose below 70 g/l, but the final product is of lesser quality.

Acknowledgement

Financial support from the Ministry of Science and Environmental Protection of Serbia is highly acknowledged (Grant BTN-371002B).

References

Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Critical Review in Food Science and Nutrition, 37*, 693–704.

Blanc, Ph. (1996). Characterization of the tea fungus metabolites. *Biotechnology Letters, 18*, 139–142.

Danielova, L. T. (1957). K khimicheskomu sostavu i fiziko-khemicheskim svoistvam kulturalnoi zhidkosti chainogo gryba. *Trudy Erevanskogo zooveterinarnogo Instituta, 22*, 111–121.

Došenović, I. (2004). Development of methods for biotin determination in molasses. Ph.D. Thesis, University of Novi Sad, Faculty of Technology, Novi Sad.

Dufresne, C., & Farnworth, E. (2000). Tea, kombucha and health: A review. *Food Research International, 33*, 409–421.

Greenwalt, C. J., Ledford, R. A., & Steinkraus, K. H. (1998). Determination and characterization of the antimicrobial activity of the fermented tea kombucha. *Lebensmittel Wissenschaft und Technologie, 31*, 291–296.

Handy, J. (2006). Lactate – The bad boy of metabolism or simply misunderstood? *Anaesthesia & Critical Care, 17*, 71–76.

Hesseltine, C. W. (1965). A millenium of fungi. *Food and Fermentation, Mycologia, 57*(2), 148–167.

Jayabalan, R., Marimuthu, S., & Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chemistry, 102*, 392–398.

Kanwar, S. S., Tewari, H. K., Chadha, B. S., Punj, V., & Sharma, V. K. (1995). Lactic acid production from molasses by *Sporolactobacillus cellulosolvens*. *Acta Microbiologica and Immunologica Hungarica, 42*, 331–338.

Kappel, T., & Anken, R. H. (1993). The tea mushroom. *The Mycologist, 7*, 12–13.

Kreger-van Rij, N. J. W. (1984). *The yeasts – A taxonomic study*. Amsterdam: Elsevier.

Lončar, E. S., Malbaša, R. V., & Kolarov, Lj. A. (2001). Metabolic activity of tea fungus on molasses as a source of carbon. *Acta Periodica Technologica, 32*, 21–26.

Lončar, E. S., Petrović, S., Malbaša, R. V., & Verac, R. M. (2000). Biosynthesis of glucuronic acid by means of tea fungus. *Nahrung, 44*, 138–139.

Malbaša, R., Lončar, E., & Djurić, M. (2008). Comparison of the products of kombucha fermentation on sucrose and molasses. *Food Chemistry, 106*, 1039–1045.

Malbaša, R., Lončar, E., & Kolarov, Lj. (2002). L-Lactic, L-ascorbic, total and volatile acids contents in dietetic kombucha beverage. *Roumanian Biotechnological Letters, 7*(5), 891–895.

Markov, S. L., Malbaša, R. V., Hauk, M. J., & Cvetković, D. D. (2001). Investigation of tea fungus associations. I. The yeasts. *Acta Periodica Technologica, 32*, 133–138.

Milić, M., Karadžić, V., & Obradović, S. (1992). Methods for laboratory control of sugar production in a factory. University of Novi Sad, Institute for sugar, Novi Sad.

Pasha, C., & Reddy, G. (2005). Nutritional and medicinal improvement of black tea by yeast fermentation. *Food Chemistry, 89*, 449–453.

Petrović, S., & Lončar, E. (1996). Content of water-soluble vitamins in fermentative liquids of tea fungus. *Mikrobiologija, 33*, 101–106.

Reiss, J. (1994). Influence of different sugars on the metabolism of the tea fungus. *Zeitschrift fur Lebensmittel Untersuchung und Forschung, 198*, 258–261.

Rodrigues, L. R., Teixeira, J. A., & Oliveira, R. (2006). Low-cost fermentative medium for biosurfactant production by probiotic bacteria. *Biochemical Engineering Journal, 32*, 135–142.

Shukla, V. B., Zhou, S., Yomano, L. P., Shanmugam, K. T., Preston, J. F., & Ingram, L. O. (2004). Production of D(−)-lactate from sucrose and molasses. *Biotechnology Letters, 26*, 689–693.

Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., & Teuber, M. (1995). Microbiology and fermentation balance in kombucha beverage obtained from a tea fungus fermentation. *Systems Applied Microbiology, 18*, 590–594.

Steiger, K. E., & Steinegger, E. (1957). Uber den Teepilz. *Pharmaceutica Acta Helvetica, 32*, 133–154.

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of kombucha fermentation. *International Journal of Food and Microbiology, 95*, 119–126.

Wee, Y., Kim, J., Yun, J., & Ryu, H. (2004). Utilization of sugar molasses for economical L(+)-lactic acid production by batch fermentation of *Enterococcus faecalis*. *Enzyme and Microbial Technology, 35*, 568–573.