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

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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

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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.