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INFLUENCE OF BLACK TEA CONCENTRATE ON KOMBUCHA FERMENTATION

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Kombucha is cultivated on substrates prepared with different concentrations of black tea and substrate obtained by diluting a substrate with highest concentration of black tea with cold tap water. Quality of produced beverages is compared with the beverage obtained in traditional fermentation of 1.5 g/L of black tea, sweetened with 70 g/L of sucrose. Inoculation was performed with 10% (v/v) of fermentation liquid from previous process, and the fermentation was carried out at 28°C under aerobic conditions, for ten days. Process of fermentation was monitored by following pH, total acids, D-gluconic acid and caffeine content. Beverages obtained in fermentation with diluted black tea concentrate had similar amounts of investigated metabolites compared with traditional one. Use of diluted black tea concentrate as a substrate needs the shorter time for the substrate preparation, which significantly saves energy.

KEYWORDS: Kombucha; fermentation; beverage; black tea concentrates; metabolites

INTRODUCTION

Kombucha and tea fungus are the most common names for the symbiosis of acetic bacteria and yeast species. Microbial composition of kombucha culture depends on the origin and growing conditions (1). Traditional substrate for kombucha cultivation is black tea sweetened with 5 to 10% of sucrose. During 6 to 10 days of fermentation under aerobic conditions, at temperatures from 20 to 30° C, slightly carbonated, refreshing, non-alcoholic beverage is obtained (2-4). Kombucha metabolism produces glucose, fructose, small amount of ethanol, carbon-dioxide, vitamins C, B_1, B_2, B_3, B_6, B_{12}, folic acid, different organic acids, mainly acetic, gluconic, L-lactic, glucuronic, enzymes and some antibioti-

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cally active compounds, and many others (5-8). Beverage also contains most of tea ingredients like tea catechines and caffeine (7). A widespread interest in kombucha products derives from its purported therapeutic benefits, which range from wrinkles and hair loss to curing cancer and AIDS (9, 10). It has been proven that the beverage does exert antimicrobial activity against a range of microorganisms (8-11).

Many people consume kombucha beverage prepared at home. World market offers different commercial kombucha products. Kombucha beverage processing on a large scale needs reasonably bigger substrate volumes which causes a problem of substrate preparation. However, tap water for tea extraction must be boiled and extract cooled to fermentation temperature. It causes the problem of time efficiency and high energy costs.

The aim of this article was the investigation of influence of black tea concentration on kombucha fermentation and possibility of application of black tea solution diluted with cold tap water as a useful substrate. Quality of beverages is compared with one fermented on traditional substrate, 1.5 g/L of black tea and 70 g/L of sucrose. Fermentation was monitored by following pH, total acids, gluconic acid and caffeine content.

EXPERIMENTAL

Kombucha Culture and Substrate Preparation

Local domestic kombucha determined by Markov et al. (12) was used for the fermentation.

Tea infusion was prepared by decoction of appropriate mass of black tea (Indian tea, "Adonis", Jagodina, Serbia) using boiled tap water sweetened with approximately 70 g/L of sucrose. After 15 minutes tea was removed by filtration. Four substrates were used for kombucha fermentation:

- Traditional substrate, named Standard, prepared with 1 L of boiled water, 70 g of sucrose and 1.5 g of black tea;
- Concentrated substrate, named Concentrate 1, prepared with 1 L of boiled water, app. 322 g of sucrose and 7.5 g of black tea;
- Concentrated substrate, named Concentrate 2, prepared with 1 L of boiled water, app. 381 g of sucrose and 15 g of black tea; and
- Diluted concentrate, prepared by dilution of Concentrate 2 with cold tap water, wherethe concentration of sucrose was adjusted to the one in Standard.

It is clear that Concentrates 1 and 2 are named so, because the concentration of black tea was significantly higher than in traditional substrate. Masses of added sucrose in Concentrates 1 and 2 were calculated considering content of extracted nonvolatile matter, dry matter content, which is determined using evaporation at 105°C to a constant mass. Dry matter content was not a linear function of mass of tea.

After cooling to room temperature, substrates were put in plastic glasses, inoculated with 10% of fermentation liquid from previous process, and fermentation at constant room temperature of 28°C w as performed for ten days. Samples were taken after 3, 7 and 10 days to monitor pH, total acidity, gluconic acid and caffeine content.

Methods of Analysis

pH values were measured using an electric pH meter.

Total acids content was determined using titration with standard solution of sodium-hydroxide with phenolphthalein as indicator (13). The result is expressed as mass of acetic acid, g, per liter of solution.

Gluconic acid content was measured in accordance with the procedure of Boehringer Mannheim (Cat. No. 428191).

Caffeine content was determined by non-aqueous titration with perchloric acid using crystal violet as indicator (14).

RESULTS AND DISSCUSSION

It was possible to observe metabolic activity of kombucha visually. During fermentation substrate was lightening and kombucha pellicle growing. After 3 days of fermentation stable pellicle was formed on Standard and substrate with diluted concentrate, while on Concentrate 1 the pellicle was not stable and on Concentrate 2 was not formed. Stable pellicle was noticed after 7 days of fermentation.

Changes in pH during kombucha fermentation in different substrates are shown in Table 1.

Table 1. Changes in pH during kombucha fermentation

Fermentation time [days]	**pH**			
	Standard	Concentrate 1	Concentrate 2	Diluted concentrate
0	7.96	7.35	6.88	7.17
3	3.73	3.28	3.70	3.60
7	3.05	2.87	3.24	3.06
10	2.72	2.74	2.80	2.79

pH value decreases during entire period of fermentation in all substrates. The most significant change was after 3 days, with average value of 3.76 pH units. From the 3rd day to the end of kombucha fermentation, the changes in pH followed the trend of slight continual decrease which is usual for the traditional process (15, 16). In samples with Concentrate 1, after 3 and 7 days are noticed lower pH values in comparison with the other fermentation series in the same period; however final pH values in all substrates were very similar (Table 1).

Different organic acids are the metabolic products of kombucha fermentation, as was mentioned before. Quantity of biosynthesized organic acids in fermentative samples of kombucha is presented in Fig. 1.

During fermentation, the content of organic acids increases in all substrates. The highest total increase is registered in fermentations on tea Concentrates 1 and 2 (Fig. 1). It is

obvious that the higher content of tea and sugar in Concentrates 1 and 2 affected the content of organic acids, but the most important fact is that measured values are not too high to damage the taste of the beverage after 7 days of fermentation. Taste of standard and beverage obtained with diluted concentrate was similar, slightly acidic and pleasant.

D-gluconic acid is one of the main metabolites of kombucha (2). Gluconates are very important for the absorption and transport of zinc, manganese(II) and chromium(III) in organism, which affect utilization of glucose, proteins and lipids, as well as insulin secretion (17-19). The highest degree of conversion of sucrose into gluconic acid was reported by Blanc (3). D-gluconic acid in fermentative liquids of kombucha is presented in Fig. 2.

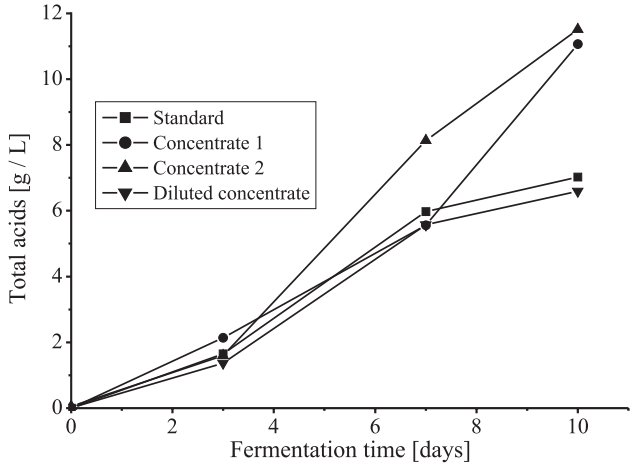

Fig. 1. Changes of total acids content during kombucha fermentation

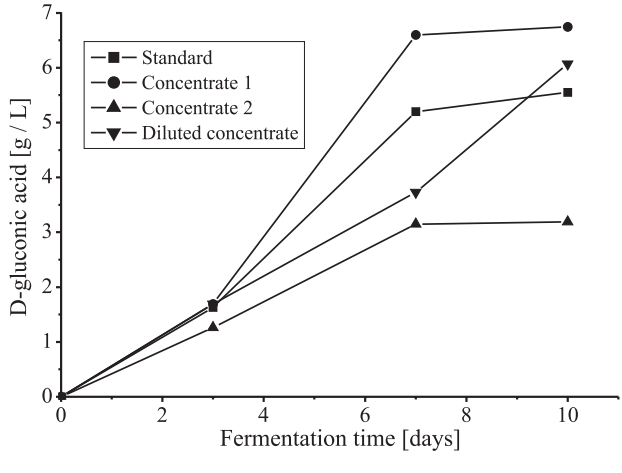

Fig. 2. Changes of D-gluconic acid content during kombucha fermentation

It is very important that D-gluconic acid content increases to the seventh day of fermentation. At the end of the process, the increasing trend is observed just in the substrate with diluted concentrate, while in the others saturation was observed (Fig. 2).

Caffeine is one of the major constituents of black tea and as a source of nitrogen, in addition to other xanthine derivatives, is essential for the microbial growth. The following effects on behavior of adult humans may occur when individuals consume moderate amounts of caffeine. It increases alertness and reduces fatigue, improves performance on vigilance tasks and simple tasks that require sustained response. Regular caffeine usage appears to be beneficial, with higher users having better mental functioning (20). Monitored caffeine content in fermentative liquids during kombucha fermentation is presented in Fig. 3.

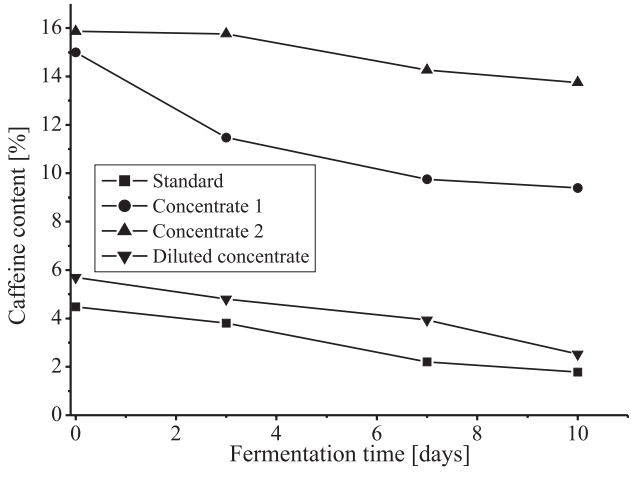

Fig. 3. Changes of caffeine content during kombucha fermentation

Caffeine content during kombucha fermentation decreases continually, and such dynamics is independent of tea concentration. As expected, Concentrates 1 and 2 contain much more caffeine than Standard and diluted substrate. Even in Concentrate 2 prepared with ten times higher mass of black tea than Standard and two times higher than Concentrate 1, measured caffeine content was not proportionally higher. The reason is the method of preparation of tea infusion using decoction. These results indicate that there is no need to take too high masses of black tea using mentioned method of preparation of tea infusion. Standard contains approximately 1.2 times lower caffeine contents in comparison with diluted tea, which means that concentrate dilution did properly.

CONCLUSSION

Presented results indicate that it is possible to perform kombucha fermentation in substrates with higher black tea concentration than is the traditional one, but metabolites content in fermentative liquids is not proportional to the amount of used tea and sucrose.

When solution with tea concentration of 15 g/L is diluted with cold tap water in such ratio that final solution is corresponding to standard substrate for fermentation with 1.5 g/L of black tea and 70 g/L of sucrose, the compared metabolites contents in fermentative samples of standard and diluted concentrate are very similar.

Use of the substrate with diluted black tea concentrate influences energy saves because the time for substrate preparation is significantly shorter.

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УТИЦАЈ КОНЦЕНТРАТА ЦРНОГ ЧАЈА НА ФЕРМЕНТАЦИЈУ КОМБУХЕ

Радомир В. Малбаша, Ева С. Лончар и Љиљана А. Коларов

Локална домаћа комбуха је култивисана на супстратима припремљеним са различитим концентрацијама црног чаја, као и супстрату припремљеном разблаживањем најконцентрованијег црног чаја хладном чесменском водом. Квалитет произведених напитака је упоређен са напитком добијеним традиционалном ферментацијом супстрата са 1,5 g/L црног чаја и 70 g/L сахарозе. Инокулација је урађена са 10% ферментационе течности из претходне ферментације, рачунато на запремину супстрата, и ферментација изведена на 28°C, у трајању од 10 дана. Ферментација је праћена мерењем pH, укупних киселина, Д-глуконске киселине и садржаја кофеина. Показало се да узорци припремљени са разблаженим концентратом чаја имају сличан садржај испитиваних метаболита у поређењу са традиционалним супстратом. Употребом разблаженог концентрата црног чаја скраћује се време потребно за припрему супстрата и значајно штеди енергија.

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зависи од односа запремина суд: подлога. У сукцесивним ферментацијама не мења се метаболичка активност ћелија квасаца и бактерија сирћетног врења. На брзину процеса, у запреминама подлоге већим од 3 литра не утиче физиолошко стање ћелија у навлаци.

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