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Influence of starter cultures on the antioxidant activity of kombucha beverage

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ABSTRACT

This paper investigates the influence of starter cultures, obtained from kombucha isolates, on the antioxidant activity of kombucha beverages. Three starter cultures were used as follows: (1) mixed culture of acetic bacteria and *Zygosaccharomyces* sp. (SC1); (2) mixed culture of acetic bacteria and *Saccharomyces cerevisiae* (SC2); as well as (3) native local kombucha. The starter cultures were added to black and green tea sweetened with 7% of sucrose. Fermentation was carried out at 28 °C for 10 days. Antioxidant activity to hydroxyl and DPPH radicals was monitored. Kombucha beverage on black tea has shown the highest antioxidant activity to both types of radicals with starter SC1, while the green tea beverage has shown the highest activity with native kombucha. The main reason for the different antioxidant activities, beside tea composition, was ascribed to differing production of both vitamin C and total organic acids in the investigated systems.

1. Introduction

Kombucha is a symbiosis of several yeast strains and acetic acid bacteria. It is known under a number of trivial names, such as red tea fungus, champignon de longue vie, ling zhi, kocha kinoko, chainii grib, chainii kvass and many others (Hartman, Burleson, Holmes, & Geist, 2000). The dominant bacterium is *Acetobacter xylinum*, while the yeasts belong to the genera *Zygosaccharomyces, Schizosaccharomyces, Saccharomyces, Saccharomycodes, Candida, Pichia, Brettanomyces* and *Torulopsis* (Dufresne & Farnworth, 2000; Teoh, Heard, & Cox, 2004). The microbiological composition depends on the culture origin. During fermentation, *A. xylinum* produces a thin cellulose film where a cell mass of bacteria and yeasts is attached. It is a fungus-like mixture of cellulose and microorganisms (Sreeramulu, Zhu, & Knol, 2000).

Under aerobic conditions, kombucha symbiosis is capable of converting a very simple substrate (sucrose and black or green tea), over a period of 7–10 days, into a slightly carbonated, mildly sour and refreshing beverage. This beverage is composed of sugars, gluconic, glucuronic, L-lactic, acetic, malic, tartaric, malonic, citric, and oxalic acid, as well as ethanol, 14 amino acids, water soluble vitamins, antibiotically active matters and some hydrolytic enzymes (Balentine, Wiseman, & Bouwens, 1997; Bauer-Petrovska & Petrushevska-Tozi, 2000; Chen & Liu, 2000; Danielova, 1957; Hesseltine, 1965; Kappel & Anken, 1993; Pasha & Reddy, 2005; Steiger & Steinegger, 1957).

It has been reported that the kombucha beverage helps digestion, gives relief from arthritis, acts as a laxative, prevents microbial infections, helps in combating stress and cancer and vitalizes the physical body, etc. It is believed that this beverage enhances immunity (Dufresne & Farnworth, 2000). Prevention of microbial infections has been demonstrated against broad spectra of microorganisms, such as *Escherichia coli, Helicobacter pylori, Staphylococcus aureus, Salmonela cholerasius* serotype *typhymurium and Bacillus cereus* (Greenwalt, Steinkraus, & Ledford, 2000; Sreeramulu et al., 2000).

Activity of kombucha on the traditional carbon source sucrose was investigated by several authors (Dufresne & Farnworth, 2000; Reiss, 1994; Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995; Teoh et al., 2004) and main pathways of conversion of sucrose into numerous products were determined. In addition to sucrose, the application of any other sugar (lactose, glucose or fructose) is possible.

Many beneficial effects to the human body can be achieved using substances with antioxidative properties. Substrates for kombucha fermentation contain antioxidants which originated from tea leaves. These are mainly polyphenols, especially catechins, which belong to the flavanols group (Graham, 1992; Mukhtar & Ahmad, 2000). Beside polyphenols, kombucha beverage contain metabolites, like vitamin C, B_2, B_6, and catalase, which have a free-radicals trapping ability (Djilas, Čanadanović-Brunet, & Ćetković, 2002) or can act synergistically with antioxidants like citric acid (Rižner Hraš, Hadolin, Knez, & Bauman, 2000).

The study of Jayabalan, Subathradevi, Marimuthu, Satishkumar, and Swaminathan (2008) demonstrated that kombucha tea, prepared from green tea, black tea and tea waste material developed excellent antioxidant activities. The authors pointed out a great potential usage of waste tea material for the preparation of the kombucha beverage with a high antioxidant capacity. However,

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many practical aspects, related to the working conditions, must be solved before the production of this kombucha beverage, at a large scale, could succeed. Having an optimal and stable (controllable) starter culture is one of the most important conditions. Recent investigations suggested the scaling up of kombucha beverage production using native kombucha as a starter culture (Malbaša et al., 2006). It can be assumed that kombucha fermentation on a larger scale could be easier to control when using starter cultures which contain a smaller number of microorganism species.

This article considers the influence of kombucha starter cultures on the antioxidant activity of kombucha beverages to certain free radicals. The starter cultures were created using kombucha isolates, while native kombucha was used for comparison.

2. Material and methods

2.1. Microorganisms

The local kombucha culture contains at least five yeast strains (*Saccharomycodes ludwigii, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis* sp. and *Zygosaccharomyces* sp.), which were determined in previous investigations (Markov, Malbaša, Hauk, & Cvetković, 2001). Primary kombucha bacterium belongs to the strains of the genus *Acetobacter* (Reiss, 1994; Sievers et al., 1995; Teoh et al., 2004).

Three different inoculums are used as starter cultures. These are:

– Fermentation liquid of native local kombucha culture (Control) after 7 days of fermentation.

– Starter culture (SC1) composed of local kombucha isolates: *S. cerevisiae* (approx. 5.8×10^4 cells/ml of substrate) and mixed culture of acetic acid bacteria (approx. 10^5 cells/ml of substrate).
– Starter culture (SC2) composed of local kombucha isolates: *Zygosaccharomyces* sp. (approx. 5.8×10^4 cells/ml of substrate) and mixed culture of acetic acid bacteria (approx. 10^5 cells/ml of substrate).

The number of yeasts and bacteria in SC1 and SC2 correlates to the number of microorganisms in the control.

The yeast isolates *Zygosaccharomyces* sp. and *S. cerevisiae* were chosen from five yeast isolates because they showed the highest and the lowest production of acids, respectively, in preliminary investigations. Thus the extremes were chosen.

2.2. Substrates

The kombucha culture (Control), SC1 and SC2 were all cultivated on two different substrates.

The substrate 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 a temperature of 100 °C, then tea leaves were removed by filtration and the obtained solution was cooled to room temperature.

The substrate from green tea (Grüner Tee, Milford, Austria) was prepared under the same conditions as the substrate from black tea.

2.3. Fermentation

Fermentation was carried out at 28 °C for 10 days. Samples were taken periodically for further analysis. Fermentation was repeated three times.

2.4. Methods of analysis

2.4.1. Hydroxyl and DPPH radical antioxidant activity

Generation of hydroxyl radicals was conducted by the mixing of 0.2 ml 0.3 M DMPO (5,5-dimethyl-1-pyrroline-N-oxide), 0.2 ml 10 mM H_2O_2, 0.2 ml 10 mM Fe^{2+} and 0.2 ml of black or green tea. The reaction mixture was then transferred to a quartz ESR flat cell ER-160-FC for ESR analyses. ESR spectra were recorded after 5 min on a Bruker ESR-300 E spectrometer (Rheinstetten, Karlsruhe, Germany). To establish the influence of kombucha beverages on the formation and stabilization of hydroxyl radicals, 0.2 ml of fermentation samples were added to the appropriate mixture of reagents instead of black or green tea. The antioxidant activity AA_{OH} was calculated as the percentage of reduction of hydroxyl radical concentration.

Solutions with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were prepared by mixing of 0.1 ml methanol, 0.6 ml 0.4 M DPPH and 0.1 ml of black or green tea. To establish the influence of kombucha beverages on the stabilization of DPPH radicals, 0.1 ml of the fermentation samples were added to the appropriate DPPH solution instead of black or green tea. The antioxidant activity AA_{DPPH} was calculated as the percentage of reduction of DPPH radical concentration.

All chemicals for ESR analysis were purchased from Sigma Chemicals Co. and used without further purification.

All analyses were performed in triplicate.

2.5. Determination of pH, total acid, citric acid, vitamin B_2 and vitamin C content

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

Total acid content was determined by titration with sodium hydroxide (0.09551 M) using phenolphthalein as indicator.

Citric acid content was quantified with Megazyme (Ireland) kit (Cat. No. K-CITR).

Vitamin B_2 was determined using the HPLC technique. A NOVA-PAK™ column (Waters, USA), having dimensions 100×8 mm, diameter 10 μm and with cartridge RP-Radial PAK™, was used. As a mobile phase, ammonium acetate (5 mM) and methanol 7.7:3 (v/v), at pH 3, were applied. All chemicals were HPLC purity grade. The flow rate was 2 ml/min and the loop volume was 20 μl. Vitamin B_2 was detected by a fluorescence detector; excitation and emission were recorded at both 450 and 530 nm. Shimadzu-Japan software was used. The samples for HPLC analyses were prepared by filtering through a hydrophilic membrane filter, which had a pore diameter of 22 μm.

Vitamin C content was quantified with Megazyme (Ireland) kit (Cat. No. K-ASCO).

All analyses were performed in triplicate.

3. Results and discussion

3.1. Development of fermentation

Kombucha fermentation was monitored over a period of 10 days by measuring pH (Fig. 1). The same pattern in changes of pH, in addition very similar pH values were noticed for the Control, SC1 and SC2 series of samples. Although the initial pH value of green tea was lower compared to black tea, the pH values in samples with green tea were higher during the fermentation process (Fig. 1b). This difference between the pH values of the substrates could be the consequence of differences in chemical composition of black and green tea (Mukhtar & Ahmad, 2000). The pH pattern during fermentation was as expected and it had the same shape