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KOMBUCHA FERMENTATION ON RAW EXTRACTS OF DIFFERENT CULTIVARS OF JERUSALEM ARTICHOKE

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Kombucha is a symbiosis between yeasts and acetic bacteria. It usually grows on sweetened black tea, but cultivation is possible on many other substrates. Jerusalem artichoke tubers extract is one of them. Tubers are suitable for the dietetic nutrition because of the low monosaccharide content and presence of some polyfructan ingredients which act as prebiotic. Five different cultivars of Jerusalem artichoke were used for the preparation of substrates for kombucha fermentation. The aim of this paper was the investigation of the influence of different Jerusalem artichoke cultivars on metabolic activity of kombucha. Composition of carbohydrates was followed using thin-layer chromatography and pH, reducing sugars content and yield of biomass were measured. Most of the samples with Jerusalem artichoke tubers extract contained fructose, probably small amount of glucose, fructo-oligosaccharides with different degree of polymerization and, inulin. Considering TLC chromatograms, Jerusalem artichoke cultivar did not affect significantly the composition of oligosaccharides in the fermentative liquid, as only minor differences were observed.

KEY WORDS: Kombucha, Jerusalem artichoke, inulin, fructo-oligosaccharides, thin-layer chromatography

INTRODUCTION

Tea fungus commonly named as kombucha is a symbiotic culture of yeasts, *Acetobacter* and *Gluconobacter* species. The standard recipe for cultivation of tea fungus is black or green tea sweetened with sucrose (1-4). The metabolic activity of tea fungus has been also estimated on the other substrates: glucose, fructose, maltose, dextrin (2, 3), lactose (3), different teas (5, 6), coffee, coca-cola, beer (5), wine and vinegar (7), Jerusalem artichoke *(Helianthus tuberosus L.*) (8-10), molasses (11), milk (12, 13).

Very interesting substrate for kombucha fermentation is Jerusalem artichoke tubers extract (8-10). It is suitable for dietetic nutrition because of the low D-glucose and D-fructose content and presence of fructo-oligosaccharides (10), which act as a prebiotics (14-16).

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Namely, basic reserve carbohydrate in the roots and tubers of Jerusalem artichoke is polyfructan inulin that consists of a homologous series of linear molecules of fructose with a degree of polymerization between 3 and 50 (17). The content of fructose varies from 75-98% of dry matter, depending of the growth and storage treatment (18). In the enzymatic inulin hydrolysis, a synergic action of endo- and exo-inulinases originated from plants or microorganisms has been reported. During the fermentation on substrate with inulin kombucha synthesize either endo- and exo-inulinase (19).

This study was made to investigate the influence of different Jerusalem artichoke cultivars on the metabolic activity of kombucha, using thin-layer chromatography (TLC) as a method for monitoring composition of carbohydrates. Also, pH, reducing sugars content and yield of kombucha biomass were measured.

EXPERIMENTAL

Kombucha Culture and Substrate Preparation

Local domestic kombucha determined by Markov et al. (20) was used for the fermentation. Jerusalem artichoke tubers (JAT) were from experimental fields of the Institute of Agriculture (Bački Petrovac, Vojvodina, Serbia). The following cultivars were used: „UKR" (sample I), „BT-3" (sample II), „BT-4" (sample III), „Violet" (sample IV) and „Bela" (sample V).

Sliced Jerusalem artichoke tubers and water in ratio 1:1 (w/w) were mixed and heated for 15 minutes at 80^{o}C. The mixture was filtered through cheesecloth. The filtrate with addition of 1.5 g per litre of black tea was sterilized at 121^{o}C for 30 minutes. After sterilization, the sediment was removed by centrifugation at 3000 rpm for 10 minutes and the extract was used for the further step. This substrate with a final volume of 2 L was placed in a glass jar with a diameter of 11 cm. Five different substrates were obtained using the described procedure. After cooling to room temperature, the mixtures were inoculated with kombucha pellicles in a mass range from 33.2 to 45 g. The jars were covered with a cotton cloth. The incubation was carried out at 28^{o}C. Samples were taken periodically after 3, 7, 10 and 14 days. JAT extracts with tea were labelled as zero samples.

Methods of Analysis

pH values were measured on an electronic pH meter.

Reducing sugars content was determined using method by Miller (21).

Yield of biomass was determined by mass measurement; the cellulose floating net was removed from the surface of fermentative liquid, rinsed with distilled water and dried with filter paper.

TLC of sugars was performed on silica gel plates. Mass of 30 g of silica gel G (Merck, Germany) was suspended in 60 mL of distilled water and the suspension was coated onto five glass plates (20x20 cm) with Desaga (Germany) equipment. The layers were dried naturally at room temperature and activated by standard method, drying at 120^{o}C for 1 hour.

Standard substance solutions were as follows: 1% aqueous solution of fructose, sucrose and inulin.

The chromatograms were developed twice using the solvent system chloroform-acetic acid-water (3:6:1, v/v). The dried chromatograms were sprayed with 50% sulphuric acid in ethanol and heated in a dryer at 100-110^{o}C for 10-15 minutes.

RESULTS AND DISCUSSION

Kombucha was metabolized intensively on all substrates with different Jerusalem artichoke tubers extract, with fast formation of kombucha floating net. Fermentation was followed with a decrease of the pH by about 2 pH units after 3 days of fermentation (Table 1), similar to the fermentation of traditional substrate with sweetened black tea (4). From the third to fourteenth day of fermentation the changes were significantly slower, being in a range of 0.43 (sample I) to 0.66 pH units (sample IV).

Table 1. Changes in pH during the fermentation of kombucha on different Jerusalem artichoke tubers extracts

Fermentation time (days)	pH				
	Jerusalem artichoke cultivar				
	I („UKR")	II („BT-3")	III („BT-4")	IV („Violet")	V („Bela")
0	6.20	6.30	6.40	6.64	6.42
3	4.62	4.68	4.53	4.79	4.50
7	4.50	4.40	4.25	4.70	4.45
10	4.34	4.25	4.09	4.64	3.97
14	4.19	4.16	4.02	4.13	3.91

Intensive metabolic activity is certainly related to the reducing sugars content in JAT extracts (0 day of fermentation, Table 2).

Table 2. Reducing sugars content during the kombucha fermentation on different Jerusalem artichoke tubers extracts

Fermentation time (days)	Reducing sugars (g/L)				
	Jerusalem artichoke cultivar				
	I („UKR")	II („BT-3")	III („BT-4")	IV („Violet")	V („Bela")
0	14.12	12.75	7.87	6.50	8.75
3	19.25	14.87	20.25	14.62	21.00
7	2.90	4.75	3.12	4.00	3.00
10	5.50	5.37	4.37	5.50	2.63
14	6.25	5.75	8.62	6.50	6.00

The dominant monosaccharide is fructose (10) and the highest initial concentration of fructose was in substrates I and II. It is well known that kombucha metabolizes fructose (2, 3, 9, 22) and, depending of the origin, can utilize fructose prior to glucose (22). It was possible to observe three different periods of fermentation: period of significant production of reducing sugars (0-3 days of fermentation), period of intensive utilization (3-7

days) and with substrates III and V new period of production of reducing sugars (10-14 days). The most intensive production of reducing sugars of 7.87-20.25 g/L and 8.75-21.00 g/L (after 3 days) and utilization of 20.25-3.12 g/L and 21.00-3.00 g/L (after 7 days) was observed for substrates III and V, respectively. Of course, these values were affected also by the other reducing substances in the fermentative liquids. Changes of the mass of kombucha biomass (Table 3) and pH fermentative profiles (Table 1) are in accordance with these results.

Table 3. Biomass of kombucha floating net after the fermentation on different Jerusalem artichoke tubers extracts

Jerusalem artichoke cultivar	Initial biomass (g)	Final biomass (g)
I („UKR")	43.2	122.3
II („BT-3")	45.0	111.8
III („BT-4")	33.2	122.9
IV („Violet")	38.8	123.0
V („Bela")	39.6	130.3

TLC analysis of sugars indicated complex composition of JAT extract and obtained fermentation liquids of kombucha. Representative chromatograms are shown in Figs. 1-5.

JAT extracts (spot 5 in Figs. 1-5) contain fructose, probably small amount of glucose (10) (R_f values of fructose and glucose are very similar), fructo-oligosaccharides with different degree of polymerization and, inulin.

Fermentation liquids of tea fungus with JAT extracts (Figs. 1-5) showed that the cultivar of Jerusalem artichoke did not affect the composition of oligosaccharides in the fermentation liquid during first three days of fermentation.

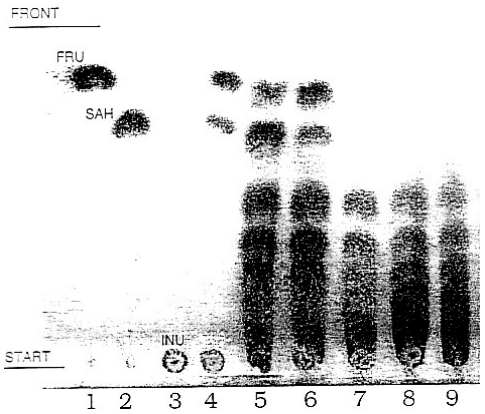

Fig. 1. Chromatogram of the fermentation liquids of tea fungus on substrate I (cultivar „UKR")
1-fructose; 2-sucrose; 3-inulin; 4-mixture 1-3; 5 - Jerusalem artichoke extract; 6-9 - fermentative liquids of tea fungus after 3, 7, 10 and 14 days of fermentation.

During kombucha fermentation, different fructo-oligosaccharides and fructose are produced as a result of polyfructan hydrolysis caused by the activity of inulinases. Beside metabolites, the fermentation liquids contain the non-fermented inulin (spots 6-9). Also, there is the confirmation of the periods of production (spots 6, 9) and utilization (spots 7, 8) of reducing sugars, i.e. fructose.

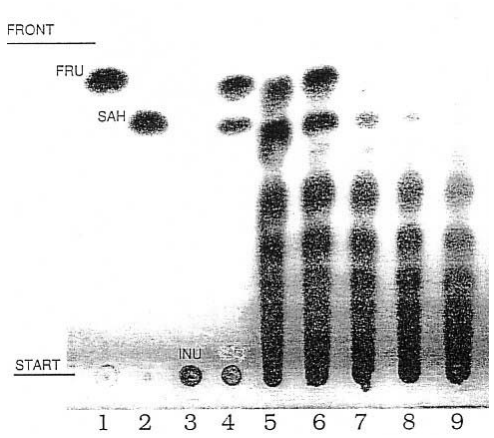

Fig. 2. Chromatogram of the fermentation liquids of tea fungus on substrate II (cultivar „BT-3"). Designation of spots is as in Fig. 1.

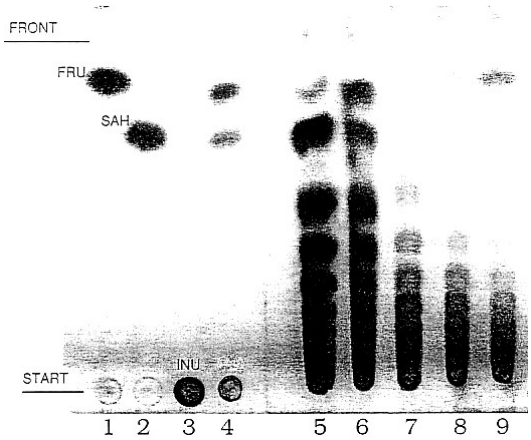

Fig. 3. Chromatogram of the fermentation liquids of tea fungus on substrate III (cultivar „BT-4"). Designation of spots is as in Fig. 1.

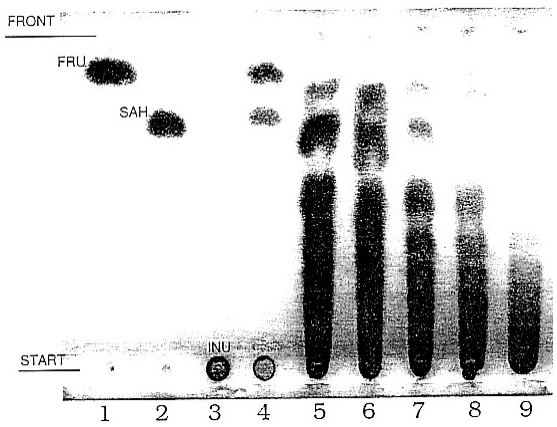

Fig. 4. Chromatogram of the fermentation liquids of tea fungus on substrate IV (cultivar „Violet"). Designation of spots is as in Fig. 1.

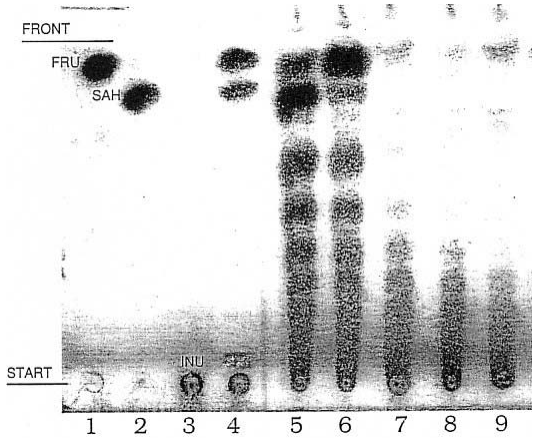

Fig. 5. Chromatogram of the fermentation liquids of tea fungus on substrate V (cultivar „Bela"). Designation of spots is as in Fig. 1.

Considering the fact that fermentative liquids with JAT extract contain almost the same metabolites as the beverage with sucrose (19), additional ingredients, fructo-oligo-saccharides and inulin, which are prebiotics, contribute to the quality of final product. The presence of fructose, small amounts of glucose (10) and the absence of sucrose are very important characteristics which recommend this beverage for the dietetic nutrition.

CONCLUSSION

Kombucha is metabolized intensively on the substrates with extract of Jerusalem artichoke.

Changes in pH were similar to those observed in the fermentation on traditional substrate, black tea sweetened with sucrose.

The most intensive production and utilization of reducing sugars is noticed on substrates III („BT-4") and V („BELA").

As a result of polyfructan hydrolysis during kombucha fermentation, fructo-oligosaccharides and fructose are produced.

Prebiotics, fructo-oligosaccharides and inulin, contribute the final quality of the product.

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ФЕРМЕНТАЦИЈА КОМБУХЕ НА ЕКСТРАКТИМА РАЗЛИЧИТИХ СОРТИ ТОПИНАМБУРА

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

Комбуха је симбиоза различитих квасаца и сирћетних бактерија. Обично расте на заслађеном црном чају, мада је могућа култивација и на разним другим супстратима. Један од њих је и екстракт топинамбура. Топинамбур је погодан за дијететску исхрану због ниског садржаја моносахарида и присуства неких полифруктанских састојака који имају пребиотско дејство. За припрему супстрата за ферментацију комбухе је коришћено пет различитих сорти топинамбура. Циљ рада је био испитивање утицаја сорте топинамбура на метаболичку активност комбухе. Састав угљених хидрата је праћен хроматографијом на танком слоју, а осим тога су мерени и pH, садржај редукујућих шећера и принос биомасе комбухе. Узорци са екстрактом топинамбура су садржали фруктозу, мале количине глукозе, фруктоолигосахариде различитог степена полимеризације и инулин. Сорта топинамбура није имала утицај на састав олигосахарида у ферментационој течности.

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