Article 2 ▪ L-Lactic, L-Ascorbic, Total and Volatile Acids Contents in Dietetic Kombucha Beverage

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Abstract

Kombucha or tea fungus is a symbiosis of osmophilic yeasts and acetic acid bacteria. After about 7 days, this symbiosis converts a very simple substrate, sucrose and black tea, in a slightly carbonated, acidic, refreshing beverage with high pharmaceutical and nutritional value. Inulin is a reserve carbohydrate polymer (fructan of inulin type) present in some plants such as Liliaceae, Iridaceae, Graminaceae and Compositae. An interesting source of inulin is the Jerusalem Artichoke tuber (J.A.T.) that belongs to genera Compositae. Using J.A.T. extract for kombucha fermentation, it is possible to obtain the healthy beverage with dietetic characteristics. The aim of this paper was quantification of some tea fungus metabolites in dietetic beverage and also comparison between the same in usual kombucha beverage. We followed L-lactic, L-ascorbic, total and volatile acids contents and they were higher on the J.A.T. substrate.

Keywords: kombucha, tea fungus, fermentation, Jerusalem Artichoke, L-lactic acid, L-ascorbic acid, organic acids

Introduction

Kombucha and tea fungus are the most common used names for a symbiotic relationship between acetic acid bacteria and yeasts [1, 2]. *Acetobacter xylinum* is the primary bacterium in tea fungus culture. In different tea fungus cultures from different origins were identified yeast species belonging to the genera *Zygosaccharomyces*, *Pichia*, *Brettanomyces*, *Schizosaccharomyces*, *Saccharomycodes*, *Saccharomyces*, *Torulaspora* and *Candida*[3, 4]. This symbiosis is capable to converting a very simple substrate (sucrose and black tea) in a slightly carbonated, acidic, refreshing beverage comprised of sugars, gluconic, glucuronic, Llactic, acetic, malic, tartaric, malonic, citric, oxalic acid, ethanol, 14 amino acids, water soluble vitamins, antibiotically active substances, some hydrolytic enzymes [1, 5-8]. The cultivation period for beverage preparing is usually of 7 to 10 days, under aerobic conditions. The usual name for the final product is kombucha or tea fungus beverage.

Besides the refreshing action and significant nutritive value of the kombucha beverage, there were different data about its curing action [4]. From either personal accounts or doctor-patient accounts, headaches, hemoroids, athersclerosis, metabolic disorders, gout,

arthritis, diabetes, sluggishness of the bowels, fatigue, stress old age and cancer were all documented to be cured by regular consumption of kombucha beverage [4]. These data can be controversial and nonadequate because there were not seriuos clinical investigations with humans.

It was proved that kombucha shows antibiotic effects against different microorganisms. These are *Staphylococcus aureus, Shigela sonnei, Escherichia coli, Aeromonas hydrophylia, Yersinia enterolitica, pseudomonas aeruginosa, Enterobacter cloacae, Staphylococcus epidermis, Salmonella enteritidis, Salmonella typhimurium, Bacillus cereus, Listeria monocytogenes, Candida albicans, Helicobacter pylori i Campylobacter jejuni* [9].

Inulin is a polymeric βDfructofuranoside, whose general structure is GFF_n where G and F represent glucose and fructose respectively, and GF a sucrosyl group. The subscript *n* indicates the different degree of polymerization and varies from 1 (inulobiose) to about 35 (inulin) [10]. Inulin is a reserve carbohydrate polymer present in some plants such as *Liliaceae*, *Iridaceae*, *Graminaceae* and *Compositae*. An interesting source of inulin is the Jerusalem artichoke tuber (J.A.T.) that contains a polyfructan with a degree of polymerization from 3 to 30 units [11]. The total potential sugar content of the J.A.T., expressed as inulin varies from 10 to 20 % of fresh weight. Fructose, expressed as percentage of the total potential sugar, varies from 75 to 98% according to growth and storage treatment of the tubers [10]. Such inulin source has recently received attention as a renewable raw material for fructose syrup production and ethanol fermentation [12].

In our previous paper [13], it was proved that the J.A.T. extract is suitable substrate for dietetic beverage obtaining by means of kombucha. The aim of this paper was monitoring quantity of some tea fungus metabolites (Llactic acid, Lascorbic acid, total and volatile acid contents) in dietetic kombucha beverage and comparing it with that obtained with the usual substrate, sucrose and black tea. L-lactic and L-ascorbic acids are very important cancer protective agents and organic acids are significant in a view of nutritional and sensorial characteristics and for beverage preservation, too.

Material and Methods

Local domestic kombucha determined by Markov et al. [14] was used for fermentation.

Jerusalem Artichoke (*Helianthus tuberosus* L.) was from fields of Scientific Institute of Agriculture (Bački Petrovac, Vojvodina, Yugoslavia).

Jerusalem Artichoke tubers (J.A.T.) extract was prepared in accordance with established procedure [13].

Kombucha beverages were obtained by fermentation on two different substrates:

1 liter J.A.T. extract and 1.5 g/L Indian black tea ("Vitamin", Horgoš, Yugoslavia),

70 g/l sucrose and 1.5 g/L Indian black tea

Inoculation, adjusting of fermentation parameters and sampling were described in our previous article [13].

pH values of the samples were determined with an electric pH meter.

Llactic acid was determined using the test of Boehringer Mannheim (Cat. No. 139084).

Lascorbic acid was determined using the test of Boehringer Mannheim (Cat. No. 409677).

Total acid content was determined using the volumetric method with sodium hydroxide and bromothymol blue as indicator [15].

Volatile acid content was determined using the volumetric method with sodium hydroxide and phenolphthalein as indicator [15].

Results and Discussion

The obtained results are presented in **Table 1** and **Figures 1** and **2**.

During the fermentation period on sucrose and J.A.T. substrates, pH value continuously decreased (Table 1). Significant pH value decreasing is noticed on the 3rd day of fermentation. It can be the result of intensive metabolic activity of acetic bacteria and yeasts after fermentation is started. The consequence of that activity is the beginning of organic acid biosynthesis, which affect on pH value. After 3 days of fermentation, there are no enormous changes of pH value (0.46 for J.A.T. substrate and 0.62 for usual), so it may be supposed that synthesized organic acids and mineral matters act as a buffer [16].

The dynamics of biosynthesis of total and volatile acids was the same on both substrates (**Table 1**). The organic acid content is higher on the J.A.T. substrate during the entire fermentation period. These results are not in accordance with pH value, which is significantly lower on the sucrose substrate. Mineral matters content is higher on the J.A.T. substrate [17], and it can be supposed that mineral matters and produced organic acids buffered on higher pH value [16].

Fermentation	J.A.T. substrate			Sucrose substrate		
time [days]	pH	Total acids [g/L]	Volatile acids [g/L]	pH	Total acids [g/L]	Volatile acids [g/L]
0	6.58	0.63	0.08	6.62	0.17	0.01
3	4.92	3.34	1.36	3.40	6.38	3.57
7	4.69	7.94	3.88	3.34	8.14	6.93
10	4.54	12.45	7.15	3.25	13.04	7.11
14	4.36	22.16	8.45	3.06	13.59	5.62
21	4.30	27.65	15.54	2.94	12.45	5.33

Table 1

It is known that insufficient amount of L-lactic acid in the human body causes poor cell respiration, and there are suitable conditions for producing of L and D-lactic acid racemic substance. That racemic substance has effects on cancer cell formation. It is also known that L-lactic acid affects on pH blood regulation [18]. In accordance with these facts L-lactic acid is one of the most important tea fungus metabolites. The results of L-lactic acid analysis in the kombucha samples are shown in **Figure 1**.

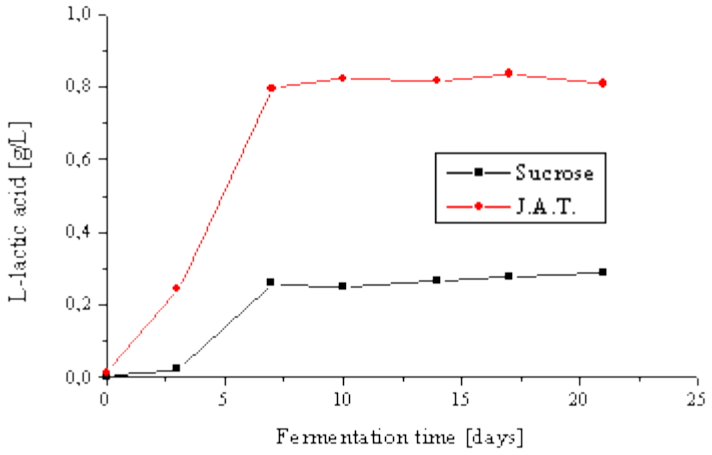

Figure 1. L-lactic acid production during tea fungus fermentation.

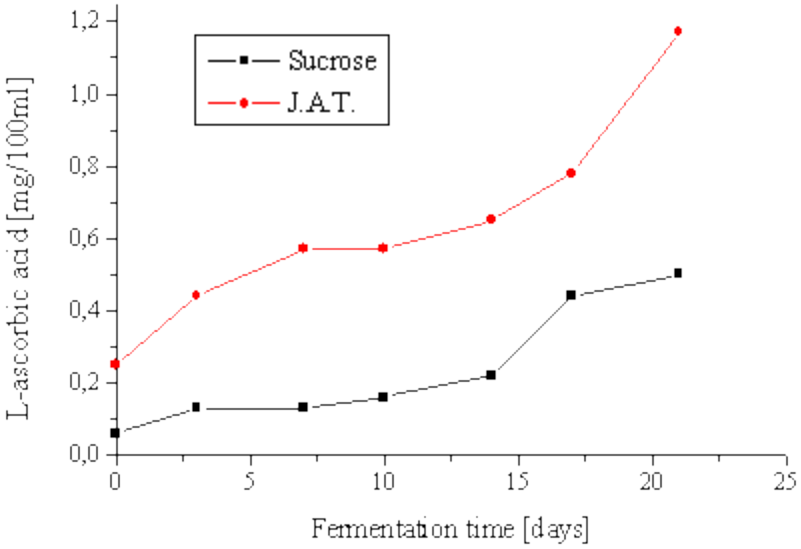

Figure 2. L-ascorbic acid production during tea fungus fermentation.

Dynamics of Llactic acid production is the same on both substrates (**Fig. 1**). After 7 days and till the end of fermentation Llactic acid content is about 4 times higher in J.A.T. samples. It probably means that the pH value of the J.A.T. substrate is more suitable for bacteria that synthesize Llactic acid.

The highest Llactic acid content on sucrose substrate is measured at the end of fermentation (0.29 g/l). This result is significantly different than in some literature data [8,

19] although applied analytical method was the same. The difference is surely the consequence of the diverse culture origin.

L-ascorbic acid possesses the strong antioxidative potential in humans. Trend of production of Lascorbic acid during fermentation is the same on sucrose and J.A.T. substrates (**Fig. 2**). The higher level of Lascorbic acid content is noticed in J.A.T. samples. It is obvious that tea fungus microorganisms are capable of producing this vitamin using inulin hydrolysis products. Results of Lascorbic acid analysis on sucrose substrate are different than the some in literature data [1-2, 5]. But, those authors have applied analytical methods, which are not too specific, especially for complex samples.

We discussed L-lactic and L-ascorbic acid as very important metabolites in cancer prevention. Glucuronic acid is also a necessary substance for human nutrition and it is a powerful detoxicator in the human body. The glucuronic acid in human liver binds the toxins making them water-soluble and, therefore, easier for elimination. On the sucrose substrate the kombucha culture produces glucuronic acid beside some other organic acids [20]. On the J.A.T. substrate kombucha does not produce glucuronic acid (unpublished observations) and it is one of the shortages of beverage obtained on this substrate.