PERIODICO di MINERALOGIA Volume 88, No. 1, 2019

Sucrose and Inulin Balance During Tea Fungus Fermentation

R. V. MALBAŠA*, E. S. LONČAR, LJ. A. KOLAROV

Dept. of Applied Chemistry, Faculty of Technology, University of Novi Sad, Bul. Cara Lazara 1, 21000 Novi Sad, Yugoslavia, Fax 381 021 450 413

Abstract

Tea fungus or kombucha is a symbiosis of several yeast strains and acetic bacteria. This symbiosis is capable of converting a very simple substrate in a slightly carbonated, acidic, refreshing beverage. The usual substrate for tea fungus fermentation is black tea sweetened with sucrose. The significant amount of sucrose stays unfermented in the beverage after fermentation and this is the reason why it is not recommended for dietetic nutrition. We investigated tea fungus fermentation on two different substrates, one with sucrose and the other one with Jerusalem Artichoke tubers (J.A.T.) extract, which contains polyfructan inulin and its polyfructan fractions. The aim of this paper was the investigation of the percentage of conversion of basic source of carbon and its distribution.

Keywords: tea fungus, kombucha, fermentation, jerusalem artichoke, inulin, sucrose

Introduction

Tea fungus or kombucha is the most usual name for a symbiosis of several yeast strains and acetic acid bacteria [1, 2]. The basic bacterium is *Acetobacter xylinum*. It produces a cellulose floating net on the surface of the fermentative liquid. That net is the secondary metabolite of tea fungus fermentation, but also one of the main characteristics of that culture. Yeast strains belong to the genera *Zygosaccharomyces*, *Schizosaccharomyces*, *Saccharomyces*, *Brettanomyces*, *Saccharomycodes*, *Pichia*, *Torulaspora* and *Candida* [3, 4]. Tea fungus converts black tea sweetened with sucrose into a slightly carbonated, acidic, refreshing beverage. Chemical analysis proved the presence of sugars, gluconic, glucuronic, L-lactic, acetic, malic, tartaric, malonic, citric, oxalic acid, ethanol, 14 aminoacids, water soluble vitamins, antibiotically active matters and some hydrolytic enzymes [1, 5-9].

Fermentation is traditionally carried out by inoculating a previously grown culture into a freshly prepared decoction and incubated statically under aerobic conditions for 7 to 10 days. After tea fungus fermentation, the significant amount of sucrose stays unfermented. Because of this, the kombucha beverage is not recommended for dietetic nutrition.

Tubers of composite Jerusalem Artichoke (*Helianthus tuberosus* L.) accumulate fructan of inulin type that consists of a homologuous series of linear molecules with a degree of polymerization between 3 and approx. 50 [10]. Fructose, as the percentage of total potential sugar, varies from 75 to 98% according to the growth and storage treatment of tubers [11]. It has been noticed that inulo-oligosaccharides present in the J.A.T. tubers extract

are regarded as a type of soluble dietary fibers and expected to increase the population of resident bifidobacteria in the human intestinal flora [12].

The aim of this paper was the examination of tea fungus metabolism on two different substrates and its comparison. We followed the D-glucose and D-fructose contents, calculated as a percentage of fermented sucrose (on substrate with sucrose) and a percent of fermented inulin (on substrate with J.A.T. extract). The presence of fructo-oligosaccharides is also examined.

Materials and Methods

Tea fungus culture originates in Russia.

Jerusalem Artichoke tubers were from experimental fields of Institute of Agriculture (Bački Petrovac, Vojvodina, Yugoslavia).

Sliced J.A.T. and water in ratio 1:1 (w/w) have been mixed and heated for 15 minutes, at 80^{o}C. The mass obtained was filtered through cheesecloth and thus obtained filtrate was sterilized at 121^{o}C for 30 minutes. After sterilization, sediment was removed by centrifugation at 3000 rpm for 10 minutes. Thus the extract obtained and the tea were used for fermentation.

Kombucha was cultivated on two different substrates [13]:

-70 g/l sucrose and 1,5 g/l of Indian black tea ("Vitamin", Horgoš, Yugoslavia)

-1l J.A.T. extract and 1,5 g/l of Indian black tea ("Vitamin", Horgoš, Yugoslavia)

The substrates prepared in glass jars with wide orifice were inoculated with 10% (v/v) fermentative liquids from previous cultivation (after 21 days). Incubation temperature was 28^{o}C. Samples were taken periodically.

D-glucose and D-fructose are determined enzymatically using the test of Boehringer Mannheim (Cat. No. 139106).

Unfermented sucrose is hydrolyzed with invertase (NOVO, Danmark).

Unfermented inulin and inulin type oligosaccharides are hydrolyzed with inulase "Fructozim" (NOVO, Danmark).

Cellulose floating net is dried and hydrolyzed with cellulase (Merck, Germany).
Hydrolyzed samples are used for analysis of D-glucose and D-fructose.

J.A.T. samples are analyzed by TLC on silica gel G with mobile phase chloroform-acetic acid-water (1:6:3). Spots are staining with 50% H_2SO_4 and heating at 120^{o}C.

Results and Discussions

The results obtained confirmed that significant amount of sucrose stays unfermented during tea fungus fermentation on substrate with sucrose (**Table 1**).

Table 1. Sucrose utilizing during tea fungus fermentation on usual substrate.

Fermentation time (days)	Sucrose utilizing during fermentation (%)				
	D-glucose	D-fructose	Cellulose floating net	The other metabolites	Unfermented sucrose
0	0.04	0.04	-	-	99.92
3	9.20	5.15	0.06	29.94	55.65
5	9.85	8.47	0.12	32.32	49.24
7	11.74	9.99	0.12	44.09	34.06
10	18.81	10.25	0.22	43.34	27.38
14	19.60	8.10	0.58	51.97	19.75

21	17.95	7.69	1.10	53.98	19.28

In our survey, we focused on samples obtained after 7 days of fermentation, when kombucha beverage is recommended for consumption. It has 34.06% unfermented sucrose and also 11.74% and 9.99% D-glucose and D-fructose, respectively. It is significant value, because of the fact that usually daily consumption dose of kombucha beverage is 0.3 to 0.5 l. This is the reason why this beverage is not suitable for dietetic nutrition. The highest amount of the other metabolites consists of organic acids (unpublished observations). The rest of substances which tea fungus produces take less amount, but their importance is huge. We also noticed that after 21 day of fermentation 19.28% of sucrose stays unfermented. In comparison with results of the other authors [3, 4], this value is more or less different. The reason for this differences resides in the different culture applied to microorganisms.

D-glucose and D-fructose contents in J.A.T. samples are lower in comparison with the values obtained for samples on usual substrate (**Table 2**).

Table 2. Inulin utilizing during tea fungus fermentation on J.A.T. substrate

Fermentation time (days)	**Inulin* utilizing during fermentation (%)**				
	D-glucose	**D-fructose**	**Cellulose floating net**	**The other metabolites**	**Unfermented inulin***
0	1.40	3.36	-	-	95.24
3	0.04	6.56	8.97	21.67	62.76
5	0.59	10.41	15.52	33.63	39.85
7	0.02	5.91	17.45	37.55	39.07
10	0.42	2.47	19.00	46.70	31.41
14	0.04	2.71	21.90	50.50	24.85
21	0.21	4.15	25.47	52.22	17.95

*MW of inulin is approx. on 5000 [11], inulo-oligosaccharides are also approximated.

After 7 days of fermentation, the beverage obtained on the J.A.T. extract contained much less D-glucose and D-fructose when compared to the beverage obtained on the sucrose substrate. The D-glucose content of 0.02 g/l can be observed as the absence of this substance and the D-fructose content is about 2 times lower in comparison with the usual one. In generally, we can notice the huge difference between cellulose floating nets on the J.A.T. and the usual substrate. The cellulose floating net produced on the J.A.T. substrate was much thicker, it had a higher mass, and for the net production on this substrate kombucha microorganisms utilized higher percentage of the outset source of carbon. After 7 days of fermentation, this difference is over 100 times. The distribution of the other metabolites is similar to the same on sucrose substrate.

The TLC analysis represented in **Figure 1** was the confirmation that the kombucha beverage obtained on the J.A.T. extract contains fructo-oligosaccharides of inulin type.

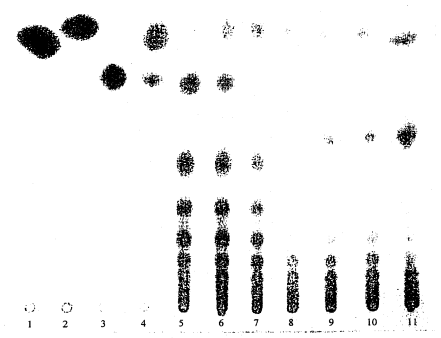

Figure 1. Chromatogram of tea fungus samples obtained on the J.A.T. substrate
1-3: Standard solutions of glucose, fructose and sucrose, respectively.
4: Mixture of standard solutions.
5-11: Tea fungus samples after 0, 3, 5, 7, 10, 14 and 21 day of fermentation, respectively.

It is obvious that the J.A.T. extract before the beginning of the fermentation contains inulo-oligosaccharides, but we can also see the presence of these substances during fermentation. It is very important because of dietary fiber characteristics of these substances, as we mentioned in the introduction.

Sensorial characteristics of the beverage obtained with kombucha fermentation on the J.A.T. extract can be corrected using microfiltration and adding of mynth and hibiscus tea concentrates and artificial sweetener. With these corrections, we have achieved better sensorial quality of the beverage in comparison with the usual tea fungus beverage with sucrose [14].

Conclusion

From the presented results, we can conclude that the tea fungus beverage obtained on the J.A.T. substrate is suitable for dietetic nutrition, because of the low D-glucose and D-fructose content, and also because of the presence of inulo-oligosaccharides which act as a dietetic fibers. The percent of basic source of carbon utilized for the formation of the cellulose floating net is much higher (17.45%) in comparison with the usual one (0.12%), after 7 days of fermentation. The distribution of the other metabolites on this substrate is very similar to the same one on the usual substrate.

References

1. L. N. KONOVALOV, M. .N. SEMENOVA, *Bot. Žurnal (Moskva)*, **40**(4), 567-570 (1955).
2. K. E. STEIGER, E. STEINEGGER, *Pharm. Acta Helv.*, **32,** 133-154 (1957).
3. M. SIEVERS, C. LANINI, A. WEBER, U. SCHULER-SCHMID, M. TEUBER, *System. Appl. Microbiol.*, **18**, 590-594 (1995).
4. C. CHEN, B.Y. LIU, *J. Appl. Microbiol.*, **89**, 834-839 (2000).
5. L.T. DANIELOVA, *Trudy Erevanskogo zooveterinarnogo Instituta*, **17**, 201-216 (1954).
6. P. H. LIST, W. HUFSCHMIDT, *Pharm. Zentralhalle*, **98**(11), 593-598 (1959).
7. S. PETROVIĆ, E. LONČAR, *Mikrobiologija*, **33**(2), 101-106 (1996).
8. J. REISS, *Dtsch. Lebensm.-Rundsch.*, **83**, 286-290 (1987).
9. E. S. LONČAR, S. E. PETROVIĆ, R. V. MALBAŠA, R. M. VERAC, Nahrung **44**, 138-139 (2000).
10. S. P. MARX, J. NÖSBERGER, M. FREHNER, *New Phytol.*, **135**, 267-277 (1997).
11. S. M. BYUN, B. H. NAHM, *J. Food Sci.*, **43**, 1871-1873 (1978).

12. T. NAKAMURA, A. SHITARA, S. MATSUDA, T. MATSUO, M. SUIKO, K. OHTA, *J. Ferm. Bioeng*., **84**(4), 313-318 (1997).
13. S. E. PETROVIĆ, E.S. LONČAR, L. J. A. KOLAROV, R. V. MALBAŠA, *Acta Periodica Technologica*, **28**, 67-72 (1997).
14. E. S. LONČAR, R. V. MALBAŠA, L. J.A. KOLAROV, M. M. AČANSKI, L. R. JEVRIĆ, *1st INTERNATIONAL SYMPOSIUM "FOOD IN THE 21st CENTURY", Book of Abstracts*, Subotica, Yugoslavia, 2001.