METABOLIC ACTIVITY OF TEA FUNGUS ON MOLASSES AS A SOURCE OF CARBON

Eva S. Lončar; Radomir V. Malbaša, Ljiljana A. Kolarov

Tea fungus or kombucha, an acetic flavoured fermented tea beverage, is widely consumed in various parts of the world. This is partly due to the fact that it can be prepared at home, and it is reported to be medicinal against arthritis, psoriasis, chronic fatigue, constipation, indigestion and metabolic diseases. Among 264 references from 1852 to 1961, there are reports of antibiotic activity of kombucha.

The aim of this paper was to examine the possibility of tea fungus fermentation on molasses as a source of carbon, instead of pure sucrose. Molasses is a byproduct of sugar manufacturing, which is very rich energetically and contains maximum of 50% of sucrose. Fermentation course is monitored by measuring pH, vitamin C content, reducing sugars content, organic acids (total and volatile) content and alcohol. It was concluded that metabolic activities of tea fungus on molasses and on pure sucrose are very similar.

KEY WORDS: tea fungus, kombucha, molasses, sucrose, metabolites

INTRODUCTION

Tea fungus or kombucha is a symbiosis of several yeast strains and Acetobacter species. It is capable to convert sugared black tea into refreshing beverage with high nutritive and pharmacological value. Tea fungus first appeared in Asia and its use spread over Russia to Central Europe in about 1950 (1). Different yeast species belonging to the genera Zygosaccharomyces, Pichia, Brettanomyces, Schizosaccharomyces, Saccharomyces, Saccharomyces, Torulaspora, Candida were identified in different tea fungus cultures (2). On the surface of a cultivation liquid, tea fungus produces gelatinous membrane that proved to be pure cellulose by its chemical composition. Acetobacter xylinum acts as a producer of this membrane (3). Caffeine and related compounds (theophylline, theobromine) are identified as activators for cellulose production in A. xylinum (4). Chemical analysis of tea fungus beverage 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 (5-10).

Dr. Eva S. Lončar, Assoc. Prof., Radomir V. Malbaša, M. Sc., Assist., Dr. Ljiljana Kolarov, Assist. Prof., University of Novi Sad, Faculty of Technology, Dept. of Applied Chemistry, 21000 Novi Sad, Bul. Cara Lazara 1, Yugoslavia.
Molasses is a byproduct of sugar manufacturing and it is very rich in energy. It contains about 50% of sucrose, about 30% of non-sugar substances (organic and inorganic) and about 20% of water. Molasses contains between 0.1 to 0.5% invert sugar (hydrolyzed sucrose) and 0.5% of raffinose. Some kinds of molasses contain some three-saccharides and some pentoses (L-arabinose and D-xylose) (11).

The aim of this paper was to investigate tea fungus metabolic activity on molasses as a source of carbon, and its comparison to tea fungus metabolism on pure sucrose. The objective for undertaking such an investigation was the fact that molasses, being a byproduct of sugar manufacturing, has not been sufficiently utilized. Fermentation course was followed by measuring pH, alcohol, reducing sugars, organic acids (total and volatile) and vitamin C content.

EXPERIMENTAL

**Tea fungus cultivation**

Tea fungus, originated from Russia, was cultivated simultaneously on two different substrates:
- 70 g/l pure sucrose (7%) and 1.5 g/l Indian black tea ("Vitamin", Horgoš, Yugoslavia)
- 291.7 g/l previosly sterilized molasses (7% sucrose) and 1.5 g/l black tea.

Substrates were inoculated with 10% (v/v) fermentative liquid from previous tea fungus fermentation (14 days long). Fermentation time was 14 days, at 28°C, and samples were taken periodically.

**Methods of analysis**

pH value is measured on a pH-meter (MA5730, "Iskra", Kranj, Slovenia).
Alcohol content and organic acids content (total and volatile) were determined according to Jazić and Ružić (12).
Reducing sugars content was determined by DNS method (13).
Vitamin C content was determined by the method reported by Plummer (14).

RESULTS AND DISCUSSION

Tea fungus fermentation was similar on both substrates, as observed from the thickness of membrane formed, and changes in fermentative liquid colour. Chemical analysis showed some similarities and some differences, the obtained results being presented in Figs. 1-5.

![Fig. 1. Changes of pH during tea fungus fermentation](image-url)
The highest pH value decrease was observed after 3 days of fermentation, for 3.41 pH units on pure sucrose and for 0.96 units on molasses (Figure 1). After this period, there was no significant changes of pH value. It is supposed that the produced organic acids together with mineral matter in tea fungus beverage act as a buffer. Substrate with molasses possesses a higher amount of mineral matter in comparison to the substrate with pure sucrose (11) and it can be an explanation for the smaller pH decrease on the substrate with molasses.

![Fig. 2. Organic acids production during tea fungus fermentation on the substrate with pure sucrose](image)

Total and volatile acids were measured while non-volatile acids content is calculated. Organic acids production was mainly a consequence of the metabolic activity of tea fungus bacteria, primarily *A. xylinum*. The obtained results for organic acids production on pure sucrose (Fig. 2) show that volatile acids make approximately 70% of total acids content.

![Fig. 3. Organic acids production during tea fungus fermentation on the substrate with molasses](image)

Total acids content on the substrate with molasses is 2.8 times lower in comparison to the usual substrate (Fig. 3). On the substrate with molasses, volatile acids production is the most intensive to the third day of fermentation, while non-volatile acids production is effective after 7 days of fermentation. This is a difference in comparison to the situation observed for the usual substrate.
The results presented in Fig. 4 show that sample with pure sucrose reasonably does not contain reducing sugars before the start of fermentation. Logically, after fermentation started and during the whole tea fungus fermentation period, reducing sugars content is increasing. It is the consequence of invertase production by kombucha microorganisms. Invertase hydrolyses sucrose into D-glucose and D-fructose, and mentioned monosaccharides are then suitable for kombucha growth and metabolism. The highest reducing sugars content is at the end of fermentation, as could be expected. These results are in accordance with some literature data (4).

The substrate with molasses contained 3.8 g/l of reducing sugars before starting the fermentation. This is expected because molasses contains invert sugar (11). Reducing sugars content during tea fungus fermentation on that substrate is about 3 times lower in comparison with the same on usual substrate. This means that invertase activity is lower. The presence of reducing sugars before the start of fermentation prolonged invertase initiation.

Sweetened black tea, usual substrate for tea fungus fermentation, does not contain vitamin C. After 14 days of fermentation vitamin C content was 0.75 mg/100 ml, and its content increased continuously. We found similar literature data (3, 6).

Substrate with molasses contains 0.06 mg/100 ml of vitamin C. Because of that all the obtained results were corrected for that value so that only the vitamin C content produced during fermentation was observed. At the end of fermentation, vitamin C content was 0.69 mg/100 ml. It is also obvious that the bysynthesis dynamics is not the same on the two substrates.
For alcohol content determination we measured the density of samples, which is a method commonly used for alcohol determination in wines and hard drinks (detection limit for this method is 0.05%). We did not determine the presence of alcohol in any of our samples, as it was possibly under the detection limit. Some authors (9) detected from 0.05 to 0.1% ethanol in kombucha beverage on usual substrate.

Generally speaking, the quantity of investigated metabolites is similar in both beverages, except for reducing sugars content, which is about 4 times lower after 7 days of fermentation, when tea fungus beverage is the most suitable for consuming.

Sensorial characteristics of usual tea fungus beverage was typical light brown coloured, slightly carbonated, acidic and refreshing. Tea fungus beverage obtained on the substrate with molasses was dark, slightly carbonated, sweet, with typical molasses smell.

CONCLUSION

Tea fungus is fermented on two different substrates, on pure sucrose and on molasses, at 28°C, for 14 days.

The quantity of investigated metabolites on different substrates is more or less different but, in general, kombucha metabolism on them is very similar.

Sensorial characteristics are better on usual substrate, if we observe them without corrections.

ACKNOWLEDGMENT

We want to thank Mrs. Irena Došenović for molasses preparation.

REFERENCES


МЕТАБОЛИЧКА АКТИВНОСТ ЧАЈНЕ ГЉИВЕ НА МЕЛАСИ КАО ИЗВОРУ УГЉЕНИКА

Ева С. Лончар, Радомир В. Малбаш и А. Коларов

Конзумирање напитка од чаяне гљиве или комбухе, ферментисаног чаяног напитка благог мириса на сирпне, широко је распрострањено по свету. Ово је последица чињенице да се може припремати код куће, а такође да има лековито дејство на различите тегобе као што су артритис, нервници, хронични замор, констипација, лоше варење и метаболички поремећаји. У 264 литературна навода, од 1852. до 1961. постоје подаци о антибиотској активности комбухе.

Циљ рада је било испитивање могућности ферментације чаяне гљиве на меласу као извору угљеника, уместо чисте сахарозе. Меласа је нупроизvod производње шећера. Садржи масимално 50% сахарозе и енергетски је веома богата. Динамика ферментације комбухе је праћена мерењем pH, садржаја витамина Ц, редукције угљеника, органских киселина (укучних и непарнолиних) и алкохола. Може се закључити да је метаболичка активност чаяне гљиве на меласи и чистој сахарози веома слична.

Received 2 April 2001
Accepted 7 June 2001