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RIBONUCLEIC ACIDS IN DIFFERENT TEA FUNGUS BEVERAGES

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In human nutrition, nucleic acids have to be balanced and limited up to 2 g/day because purines are degraded to urate, and excessive production of urate is a cause of gout which primarily affects adult males.

Tea fungus beverage is a well known drink with high nutritional value and certain curative effects. Its benefits have been proved in a number of studies but it is still necessary to examine some potential harmful effects of this beverage.

The aim of this paper was to investigate content of ribonucleic acids (RNA) produced during tea fungus fermentation on a usual substrate, sweetened black tea, and on Jerusalem artichoke tubers (J.A.T.) extract using method by Munro and Fleck (1966). pH, ribonucleic acids and also the production of proteins that affect purity of nucleic acids preparations were monitored.

A higher value of RNA has been noticed in J.A.T. beverage (0.57 mg/ml) and with observation of usual daily dose of the beverage it is completely safe and useful one.

KEY WORDS: tea fungus, kombucha, fermentation, ribonucleic acids

INTRODUCTION

The symbiotic culture of acetic bacteria and yeasts produces a zoogleal mat. The name tea fungus is a misnomer and arises from the bacteria's unique ability to synthesize a floating cellulose network that resembles a surface mold on non-agitated medium. This cellulose network is similar in composition to "mother of vinegar" (1). The other very popular name for tea fungus is kombucha.

This symbiotic culture is grown traditionally on black tea with sucrose for 7 to 10 days and gives a pleasantly sour and sparkling beverage. Longer incubations results in increasing production of acetic acid and in the formation of a mild vinegar (2). Besides traditional substrate, tea fungus grows on some different mediums. *Helianthus tuberosus* L.,

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or Jerusalem artichoke tubers (J.A.T.), extract is one of it. The beverage produced on that extract by kombucha is suitable for dietetic nutrition (3).

During tea fungus fermentation, it produces a number of substances like glucuronic, gluconic, L-lactic, acetic and many other organic acids, amino acids, water-soluble vitamins and some hydrolytic enzymes (4). Because of microbiological preparation of this beverage, it normally contains nucleic acids.

DNA is polynucleotide produced by polymerization of deoxyribonucleotides. RNA is an unbranched linear polymer of ribonucleoside monophosphates. The purines found in RNA are adenine and guanine; the pyrimidines are cytosine and uracil. Except for uracil, which replaces thymine, these are the same bases found in DNA. Purines are degraded to urate in humans (5). The pathway of degradation of adenosine monophosphate (AMP) is presented in Fig. 1.

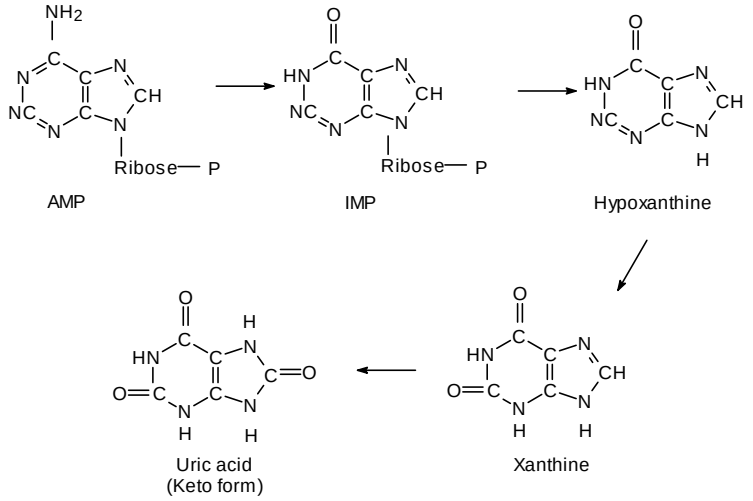

Fig. 1. Degradation of AMP to uric acid

The subsequent reactions leading to the free base hypoxanthine follow the general pattern. Xanthine oxidase, a molybdenum and iron-containing flavoprotein, oxidizes hypoxanthine to xanthine and then to urate. Molecular oxygen, the oxidant in both reactions, is reduced to hydrogen-peroxide, which is decomposed to water and oxygen by catalase. Xanthine is also an intermediate in the formation of urate from guanine. In humans, urate is a final product of purine degradation and is excreted in the urine. Excessive production of urate is a cause of gout. Gout is a disease that affects the joints and leads to arthritis. The major biochemical feature of gout is an elevated level of urate in the serum. Inflammation of the joints is triggered by the precipitation of sodium urate crystals. Kidney disease may also occur because of the deposition of urate crystals in that organ. Gout primarily affects adult males (5).

The objective of this article was to investigate the amount of RNA in different tea fungus beverages. This paper contains the findings on tea fungus fermentation on usual substrate, sucrose and black tea, and on J.A.T extract, while the values of pH, RNA and protein content were monitored during 21-day long fermentation.

EXPERIMENTAL

Tea fungus cultivation

Tea fungus culture from household was defined by Markov et al. (6). In addition to acetic and gluconic bacteria, the presence of yeasts *Saccharomycodes ludwigii, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis* sp. and *Zygosaccharomyces* sp. species was established. The culture was cultivated on two different substrates:

- 70 g/l pure sucrose and 1.5 g/l Indian black tea ("Vitamin", Horgoš, Yugoslavia)
- J.A.T. extract prepared according usual procedure (3). J.A.T. originated from the fields of the Institute of Field and Vegetable Crops, Novi Sad, in Bački Petrovac (Serbia and Montenegro).

Substrates were inoculated with 10% (v/v) fermentative liquids from previous fermentation (21-days long). Fermentation time was 21 days, at 28°C, and samples were taken periodically without stirring.

Methods of analysis

pH value was measured on a pH-meter (MA5730, "Iskra", Kranj, Slovenia).

RNA content in fermentative liquid was measured according to Munro and Fleck (7).

Protein content was determined by Lowry et al. (8).

All samples were analyzed in three replications.

RESULTS AND DISCUSSION

Tea fungus fermentation was usual in view of visual observation and in accordance with internal standard the taste of kombucha beverage was correct. The floating net formed on J.A.T substrate was much thicker in comparison with the one on black tea substrate. Fermentative liquids were clear but clearer on a substrate with black tea. The obtained analytical results are presented in Figs. 2-4 and Tab. 1.

During fermentation period on both substrates pH value continuously decreased (Fig. 2). Significant pH value decrease was noticed after 3 days of fermentation. It is a consequence of intensive metabolic activity of acetic bacteria and yeasts after the beginning of fermentation. As a result of this activity the biosynthesis of organic acids takes place, which affects the pH value. After first three days of fermentation, there were no large changes of pH value, so it may be supposed that the synthesized organic acids and mineral matter act as a buffer (9). After 7 days of fermentation, when the beverage was optimal for consuming in view of its sensorial characteristics, pH was lower in the beverage obtained on the substrate with black tea. Thus lower pH was noticed on the medium with sweetened black tea, the beverage taste was more acidic on the substrate with J.A.T. extract, and even total acid content was higher on that substrate (10). The method for RNA determination was slightly modified because it was developed for solid samples. The characteristic UV spectrum is presented in Fig. 3 and all the results are collected in Table 1.

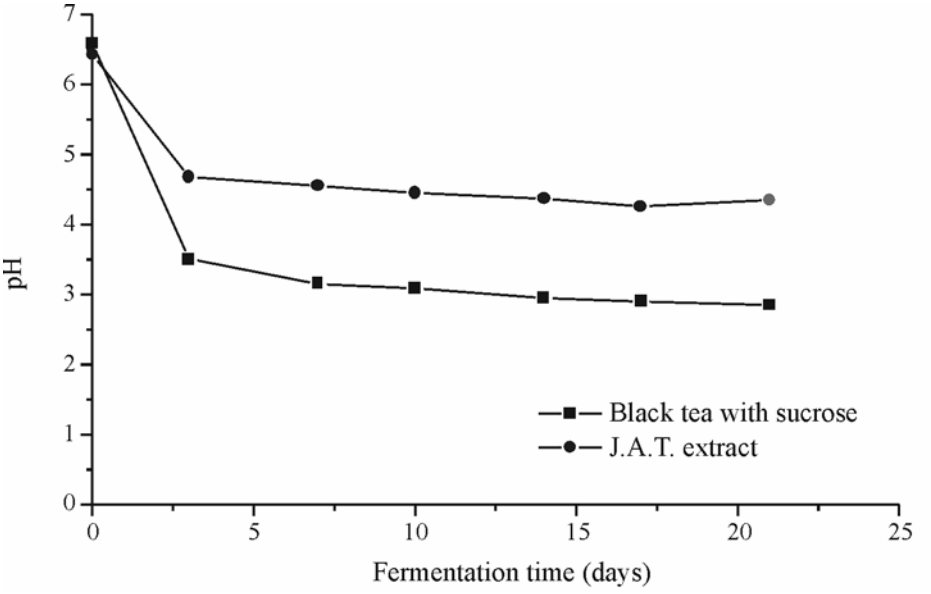

Fig. 2. Changes of pH value during tea fungus fermentation.

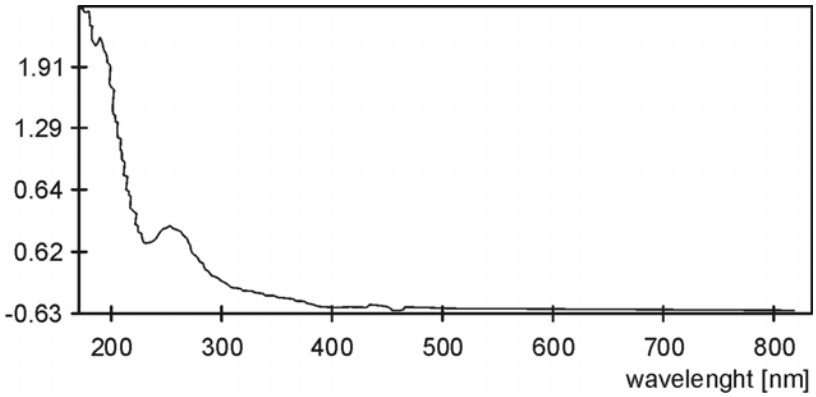

Fig. 3. Characteristic UV spectrum of RNA in the beverage obtained after 7 days of fermentation on sweetened black tea.

Before the fermentation started, it was found that RNA content in J.A.T. extract is about 4.3 times higher than in sweetened black tea. This is logical because J.A.T substrate is a much richer substrate than the other one. On the black tea substrate RNA content increased significantly after 3 days of fermentation and till the end it was almost constant. On the basis of the presented results (Tab. 1) it is calculated that average value of RNA for tea fungus liquid obtained on sweetened black tea, from the 3rd to the 21st day, was about

0.265 mg/ml. Microorganisms from the tea fungus inoculum contributed significantly to the RNA content on the black tea substrate.

Table 1. RNA content and A^{260}/A^{280} ratio during tea fungus fermentation.

Fermentation time (days)	Black tea substrate		J.A.T. substrate	
	RNA (mg/ml)	A^{260}/A^{280} (sample purity)	RNA (mg/ml)	A^{260}/A^{280} (Sample purity)
0	0.17±0.01	1.2	0.71±0.06	1.1
3	0.26±0.03	1.2	0.61±0.06	1.0
7	0.28±0.03	1.2	0.57±0.05	1.0
10	0.25±0.02	1.2	0.41±0.03	1.1
14	0.27±0.03	1.2	0.45±0.04	1.1
17	0.24±0.02	1.2	0.47±0.04	1.1
21	0.29±0.03	1.2	0.48±0.04	1.1

On the J.A.T. substrate RNA content decreased constantly until the 10^{th} day of fermentation and after that there were not significant changes till the end of fermentation. The reason for such behavior was probably sedimentation during the incubation clarifying the fermentation liquid. It was estimated that a number of yeast cells remained in the sediment and it affected the RNA decrease, because the samples were taken without stirring.

It is known that urate is further degraded in some organisms. Mammals other than primates excrete allantoin, which is formed by oxidation of urate. Teleost fish excrete allantoate, which is formed by hydration of allantoin. The degradation proceeds a step further in amphibians and most fish. Allantoate is hydrolyzed to two molecules of urea and one of glyoxylate. Finally, some marine invertebrates hydrolyze urea to ammonium ion and carbon(IV)-oxide. It seems likely that the enzymes catalyzing these reactions were progressively lost in the evolution of primates (5). Deficiency of an enzyme which would derive or degrade urate in humans limited nutrition daily dose of nucleic acids to 2 g/day (11).

After 7 days of fermentation when the tea fungus beverage was optimal for consumption the RNA content was about two times higher in the beverage obtained on J.A.T. extract, amounting to 0.57 mg/ml (Table 1). Because of tea fungus beverage recommended daily dose of 0.3 to 0.5 L, it is obvious that tea fungus beverage is not harmful to humans in a view of nucleic acids.

Purity of samples was defined by the A^{260}/A^{280} ratio (7). If that value is in a range from 2 to 2.2, the nucleic acid preparation is pure. The presented results (Table 1) show that for a pure nucleic acid preparation it is necessary to carry out a more detailed protein separation but it was not the aim of this paper.

The changes in protein content during tea fungus fermentation are represented in Fig. 4.

Proteins in tea fungus fermentative liquids originated from plant material and also from microorganisms. After the beginning of fermentation, there were no significant changes in protein content. This means that the kombucha microorganisms do not affect much the mentioned value. Average protein content was about 3.2 times higher in samples produced on J.A.T. extract as a substrate. It is a consequence of the more complex and richer substrate that also affected nucleic acid content. These values were expected because the

visually observed fermentation process on J.A.T. extract was more intensive than on the conventional substrate.

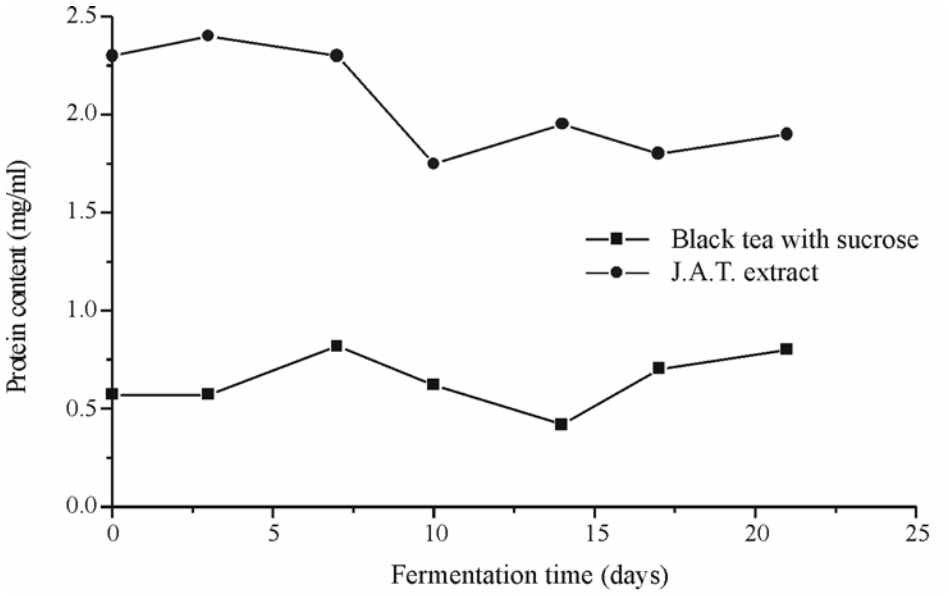

Fig. 4. Protein content changes during tea fungus fermentation

CONCLUSION

Tea fungus was fermented on two different substrates, on sweetened black tea and J.A.T. extract, at 28°C, for 21 days.

Tea fungus beverages produced on both substrates contained RNA and that value was about two times higher in the beverage with J.A.T. extract, but the consumption of a usual daily dose of such kombucha beverages (0.3 to 0.5 l) is not harmful to humans in view of the permitted RNA nutrition value (2 g/day).

pH value of the produced beverages was lower on the substrate with black tea.

Protein content was higher in samples fermented on the J.A.T. extract.

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РИБОНУКЛЕИНСКЕ КИСЕЛИНЕ У РАЗЛИЧИТИМ НАПИЦИМА ОД ЧАЈНЕ ГЉИВЕ

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

Нуклеинске киселине су есенцијални конституент сваке ћелије. У људској исхрани морају бити избалансиране и ограничене су на 2 г/дан, због тога што се у човечијем организму пурини деградирају у урате, а повишени садржај урата може проузроковати гихт, који примарно погађа одрасле мушкарце.

Напитак од чајне гљиве је добро познат и спада у производе високе нутритивне вредности, који уз то поседују и нека лековита својства. Овај напитак се конзумира широм света, а његови позитивни ефекти су доказани више пута, међутим неопходно је испитати и потенцијалне токсичне ефекте овог напитка.

Циљ овог рада је био да се испита продукција рибонуклеинских киселина током ферментације чајне гљиве на уобичајеном супстрату, заслађеном црном чају, и на екстракту топинамбура, користећи метод који су развили Мунро и Флек (1966). Осим тога, праћена је и вредност pH, као и продукција протеина, која утиче на чистоћу препарата нуклеинских киселина.

Вредности pH, садржај рибонуклеинских киселина и протеина били су већи на супстрату са ектрактом топинамбура. Ипак, узевши у обзир садржај нуклеинских киселина у напитку са екстрактом топинамбура (0,57 мг/мл) и уобичајену дневну дозу овог напитка (300 до 500 мл), може се закључити да је он са тог становишта потпуно безбедан и не представља ризик за потенцијалне конзументе.

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