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TEA FUNGUS FERMENTATION ON A SUBSTRATE WITH IRON(II)-IONS

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

Iron is essential element for human metabolism and it is a constituent of both heme-containing and nonheme proteins. Its deficiency can cause serious diseases, i.e. iron-deficiency anemia, with some fatal consequences.

Tea fungus beverage has high nutritional value and some pharmaceutical effects. It is widely consumed all over the world and its benefits were proved a number of times.

The aim of this paper was to investigate tea fungus fermentation on a substrate containing iron(II)-ions and the possibility of obtaining a beverage enriched with iron. We monitored pH, iron content and also the production of L-ascorbic acid, which is very important for iron absorption in humans.

KEY WORDS: tea fungus, kombucha, fermentation, iron

INTRODUCTION

Tea fungus or kombucha beverage is a popular healthy drink that is used for refreshing and as an alternative therapeutic means. It is consumed worldwide and it is believed to have prophylactic and therapeutic benefits in a different variety of ailments like intestinal disorders, rheumatism, arthritis insomnia, stimulation of hair growth, cancer, ageing and stimulation of immune system (1). Although there have been a few clinical studies performed in America, Russians, Germans, Swedes and others compiled the data on the benefits of kombucha beverage for nearly 100 years. Frank (2) lists much evidence from Russian and German doctors who have claimed many benefits to their patients consuming it regularly. Despite so many testimonials and endorsements by herbalists regarding the clinical benefits of this beverage, there is no scientific evidence supporting any clinically relevant pharmacological activity of tea fungus beverage (3).

Radomir V. Malbaša, M.Sc., Assist., Dr Eva S. Lončar, Prof., Dr Ljiljana A. Kolarov, Assist. Prof., University of Novi Sad, Faculty of Technology, Dept. of Applied Chemistry, 21000 Novi Sad, Bulevar Cara Lazara 1, Yugoslavia

Kombucha beverage is usually prepared by fermentation of a substrate composed of black or green tea sweetened with sucrose. The inoculum is tea fungus consisting of symbiotic colony of yeasts and bacteria. The primary bacterium is *Acetobacter xylinum* and yeasts of the genera *Zygosaccharomyces, Saccharomyces, Saccaromycodes, Brettanomyces* and *Torulopsis* were identified in tea fungus culture (1). Usually kombucha beverage is prepared for a period of 7 to 10 days at room temperature. *A. xylinum* forms a cellulose floating net on the top of the liquid during fermentation.

Tea fungus beverage is composed of a variety of substances. Glucose liberated from sucrose is metabolized for the synthesis of cellulose and gluconic acid by *Acetobacter* and *Gluconabacter* strains, respectively. Fructose is metabolized into ethanol and carbon dioxide by yeasts. Ethanol is oxidized to acetic acid by *Acetobacter* strains. The organic acids formed shield the symbiotic colony from contamination with unwanted foreign microorganisms (4). Glucuronic, L-lactic acid, amino acids, water-soluble vitamins and some hydrolytic enzymes are also present in this beverage, and they are very important for its quality (5-7).

Iron is essential for human body. It binds to proteins either by incorporation into a protoporphyrin IX ring or by interaction with other protein ligands. Iron(II)- and iron(III)-protoporphyric IX complexes are designated heme and hematin, respectively. Heme-containing proteins include those that transport (hemoglobin) and store (myoglobin) oxygen, and certain enzymes that contain heme as a part of their prostetic groups (e.g., catalase, peroxidases, tryptophan pyrrolasse, prostaglandin synthase, guanylate cyclase, and the microsomal and mitochondrial cytochromes). Nonheme proteins include transferrin, ferritin, different redox enzymes that contain iron at the active site, and iron-sulfur proteins (8).

A normal 70-kg male has 3 to 4 g of iron, of which only of 0.1% is in the plasma. In Table 1 is listed the distribution of iron in humans.

Table 1. Iron distribution for a 70-kg man (8)

	g	%
Hemoglobin	2.5	68
Myoglobin	0.15	4
Transferrin	0.003	0.1
Ferritin, tissue	1.0	27
Ferritin, serum	0.0001	0.004
Enzymes	0.02	0.6
Total	3.7	100

Iron status in humans depends on nutrition. The known disorders in iron metabolism are iron-deficiency anemia and also iron overload that causes infection (8).

In this paper we report our findings on tea fungus fermentation on usual substrate, sucrose and black and green tea, with iron(II)-ions. We monitored pH, L-ascorbic acid and iron content during fermentation. The objective was to enrich the beverage with iron.

EXPERIMENTAL

Tea fungus cultivation

Tea fungus culture from household was defined by Markov et al. (9). 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) with 10 mg/l iron(II)-sulphate (BT substrate)
- 70 g/l pure sucrose and 1.5 g/l green tea ("Milford", Grüner tee, China) with 10 mg/l iron(II)-sulphate (GT substrate)

We chose to add iron(II)-sulphate to substrates because it may be required for iron therapy in some cases of iron deficiency and it is usually provided in the form of oral iron(II)-sulphate tablets.

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.

Methods of analysis

pH value was measured on a pH-meter (MA 5730, "Iskra", Kranj, Slovenia).

Iron content in fermentative liquid was measured according Ribéreau-Gayon and Peynaud (10).

Vitamin C content was determined by Boehringer-Mannheim (Cat. No. 409677).

All samples were analyzed in three replications.

RESULTS AND DISCUSSION

Tea fungus fermentation was usual in view of visual observation. The floating nets formed had normal color and thickness. Fermentative liquids were clear but clearer on a substrate with green tea. Taste of the beverages that we consumed was correct and the applied amount of iron(II)-ions did not affect standard taste quality (internal standard). The obtained results of analysis are presented in Tab. 2 and Figs. 1-2.

Changes of pH value were very similar to those observed previously on the usual and some alternative substrates (11, 12). The highest pH value decrease was observed after 3 days of fermentation (Fig. 1) and after that period there were no significant changes. After 7 days of fermentation, when the beverage is optimal for consuming because of its sensorial characteristics, pH is lower in the one obtained on GT substrate. It was also noticeable that its initial pH value was for 0.5 units lower than for the BT beverage and it also holds for the final values. In the neutral and alkaline pH ranges, the redox potential of iron in aqueous favors the iron(III) state, while in an acidic medium iron(II) state is favored. Iron(III) forms slowly large polynuclear complexes with hydroxide ion, water, and other anions that may be present. These complexes can become so large as to exceed their solubility products, leading to their aggregation and precipitation with pathological consequences (8). It is obvious that in kombucha beverage samples the favorite iron state is iron(II) because of the low pH value, especially for samples obtained on GT substrate, where pH is significantly lower.

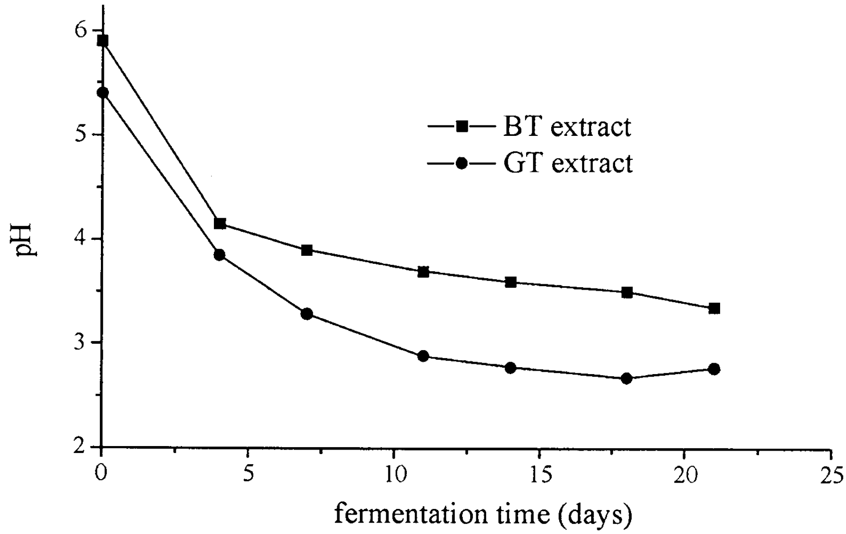

Fig. 1. Changes of pH value during tea fungus fermentation.

Table 2. Iron content during tea fungus fermentation.

Fermentation time (days)	Iron (mg/l)		Percent of iron used by tea fungus during the growth	
	BT substrate	GT substrate	BT substrate	GT substrate
0	9.87±0.06*	9.97±0.02	-	-
4	8.73±0.04	7.37±0.04	11.5	26.1
7	8.72±0.03	7.40±0.01	11.7	25.8
11	8.70±0.01	7.17±0.06	11.9	28.1
14	8.48±0.06	7.08±0.02	14.1	29.0
18	8.47±0.03	6.88±0.06	14.2	31.0
21	8.33±0.07	5.50±0.07	15.6	44.8

* mean value ± standard deviation

On the basis of the presented results (Tab. 2) we calculated that average percent of iron consumed for tea fungus growth from the 4^{th} to the 21^{st} day was 13.1% for BT substrate and 30.8% for GT substrate. This means that in the tea fungus beverage obtained on BT substrate remained a larger amount of the added iron in comparison with the other one. It is interesting in a view of pH, which is lower in case of the GT substrate and may be one of the reasons for such a behavior. In tea fungus fermentative liquid iron-ions can be bonded chemically with some of metabolites, i.e. amino acids, some vitamins, peptides, tea flavanols, etc., and also adsorb on the surface of yeast and bacteria cell or stay in its inorganic state. We cannot conclude what is the dominant mechanism but the important fact is that tea fungus consumes iron in the course of fermentation. Optimal kombucha fermentation time is about 7 days and iron daily dose for human adult is from 11 to 17 mg (13). This means that by consuming the usual daily dose of such kombucha beverage (0.3 to 0.5 l) human can satisfy part of iron daily needs. This is significant because inorganic iron metabolism in humans causes constipation. On the other hand, it is known that tea fungus beverage is a mild laxative. We can also suppose that iron absorption can be better from kombucha beverage because of the possibility of binding of some of metabolites mentioned above. Absorption of inorganic iron is poor in comparison with iron in the form of helates or some other organic complexes (8).

L-ascorbic acid is very important for iron status in biological systems. The results of L-ascorbic acid production during tea fungus fermentation are presented in Fig. 2.

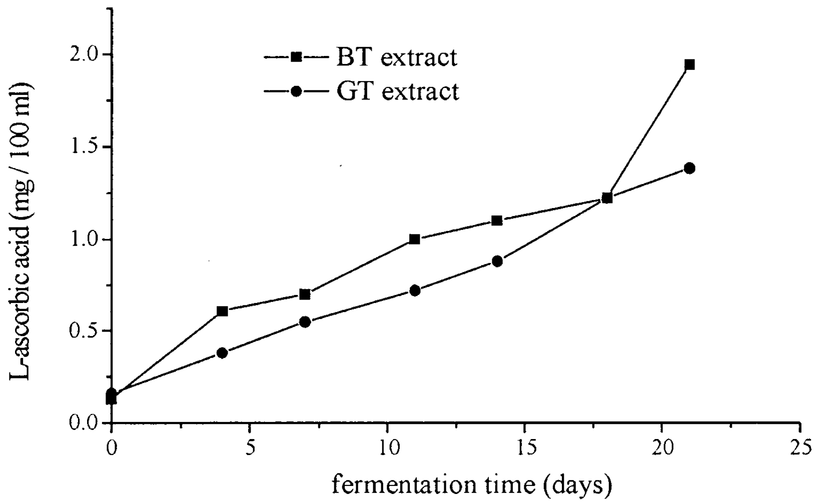

Fig. 2. L-ascorbic acid production during tea fungus fermentation.

The amount of L-ascorbic acid produced on these substrates is similar to those on the usual ones and some other substrates that we investigated (11,12). If we consider the whole fermentation period, production of L-ascorbic acid was greater on BT substrate but after 7 days its amounts were almost the same (about 0.6 mg/100 ml). Vitamin C is very important for absorption of iron in humans. Cooking of food facilitates the breakdown the ligands attached to iron, increasing the availability of the metal in the gut. The low pH of stomach contents permits the reduction of iron(III) to iron(II), facilitating dissociation from ligands. The latter requires the presence of an accompanying reductant, which is usually achieved by adding ascorbate to the diet. Stomach misfunction reduces substantially the amount of iron that is absorbed (8).

CONCLUSION

Tea fungus was fermented on two different substrates, on black and green tea sweetened with sucrose and addition of 10 mg of iron(II)-sulphate per liter, at 28°C, for 21 days.

Tea fungus beverage contained inorganic and probably bonded iron was obtained so that by consuming the usual daily dose of such kombucha beverage (0.3 to 0.5 l) human can satisfy part of iron daily needs.

L-ascorbic acid as a very important factor for iron absorption in humans was produced in the usual limits for tea fungus fermentation, and after 7 days of fermentation its content was about 0.6 mg per 100 ml.

In accordance with internal standard, the taste of kombucha beverages was correct.

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ФЕРМЕНТАЦИЈА ЧАЈНЕ ГЉИВЕ НА СУПСТРАТУ СА ГВОЖЂЕ (II)-ЈОНИМА

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

Гвожђе спада у есенцијалне елементе за метаболизам човека и конституент је протеина, како оних који садрже хем, тако и оних који га не садрже. Недостатак гвожђа у организму може изазвати озбиљне болести, као што је анемија, са неким чак и фаталним последицама.

Напитак од чајне гљиве 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.

Циљ овог рада је био да се испита ферментација чајне гљиве на супстрату који садржи гвожђе (II)-јоне, као и могућност добијања напитка обогаћеног гвожђем. Праћени су pH, садржај гвожђа и такође продукција Л-аскорбинске киселине, која је веома битна за ресорпцију гвожђа код људи.

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