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TLC ANALYSIS OF SOME PHENOLIC COMPOUNDS IN KOMBUCHA BEVERAGE

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Black and green tea contains a wide range of natural phenolic compounds. Flavanoids and their glycosides, catechins and the products of their condensation, and phenolic acids are the most important.

Kombucha beverage is obtained by fermentation of tea fungus on black or green tea sweetened with sucrose.

The aim of this paper was to investigate the composition of some phenolic compounds, catechin, epicatechin, quercetin, myricetin, gallic and tanic acid, and monitoring of their status during tea fungus fermentation.

The method used for this study was thin layer chromatography with two different systems. The main phenolic compounds in the samples with green tea were catechin and epicatechin, and in the samples with black tea it was quercetin.

KEYWORDS: Tea fungus; kombucha¨,, fermentation, phenolic compounds, TLC

INTRODUCTION

Kombucha is a popular beverage among many traditional fermented foods across the world. In the literature, kombucha is also frequently called tea fungus, although there is actually no fungus involved in the fermentation. This beverage reportedly exerts a number of medicinal effects against metabolic disease, arthritis, psoriasis, constipation, indigestion, and hypertension. By virtue of the numerous health-promoting aspects reported and the easy and safe preparation of this beverage at home, it has gained popularity as other traditional beverages. Tea fungus is a symbiotic growth of acetic bacteria and yeast strains cultured in sugared black or green tea (1). The fermentation is traditionally carried out by ino-

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culating a previously grown culture into a freshly prepared tea decoction and incubated statically under aerobic conditions for 7-10 days (2).

In the past few years, there has been a growing interest in the use of natural additives in preference to synthetic substances for the stabilization of foods. Because some synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, have been implicated in acute toxicity and chronic diseases in experimental animals, various plant extracts have gained attention as sources of safe antioxidants. The natural antioxidants in herbs, spices, and teas have been extensively studied (3).

Dietary factors play a major role in chronic diseases development. A number of these chronic diseases, cardiovascular diseases and cancer, have been linked to excess production of reactive oxygen species and oxidative damage to biomolecules. Derived from plant material and containing numerous biologically active compounds, tea beverage may be also proposed as a component of a healthy diet. Tea fungus beverage is also very useful in terms of antioxidant characteristics (unpublished results). The majority of tea beverage is prepared from two types of manufactured tea: black and green (4). The principal components of black and green tea beverage are shown in Table 1.

Table 1. The principal components of black and green tea beverage (5)

Component	Black tea	Green tea
Catechins	3-10	30-42
Flavonols	6-8	5-10
Other flavanoids	15-24	2-4
Theogallin	-	2-3
Other depsides	10-12	1
Ascorbic acid	-	1-2
Gallic acid	-	0.5
Quinic acid	-	2
Other organis acids	-	4-5
Theanine	-	4-6
Other amino acids	13-15	4-6
Metylxanthines	8-11	7-9
Carbohydrates	15	10-15
Minerals	10	6-8
Volatiles	<0.1	0.02
Protein	1	-

Note: Components measured in wt % of extract solids.

The objective of this article was to investigate the composition of some phenolic compounds during tea fungus fermentation. The separation and identification of catechin, epicatechin, gallic acid, tanic acid, quercetin and myricetin, in fermentative liquid samples of tea fungus was performed using thin layer chromatography (TLC).

EXPERIMENTAL

Tea fungus cultivation

Tea fungus culture from household was defined by Markov et al. (6). The culture was cultivated on two different substrates:

- 70 g/L pure sucrose and 1.5 g/L Indian black tea ("Vitamin", Horgoš, Serbia& Montenegro)
- 70 g/L pure sucrose and 1.5 g/L Chinese green tea ("Milford Grüner Tee", China Green)

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

Preparation of samples for TLC

Samples for the TLC on cellulose were prepared with vaporization of 15 mL of sample up to 1 mL.

Samples for the TLC on silica gel were prepared using extraction with ether. 15 mL of sample and 15 mL of ether were mixed in separation funnel for the 5 min. After separation, 15 mL of ether layer was vaporized up to 1 mL.

Solutions of standard substances, catechin, epicatechin, quercetin, myricetin, gallic acid and tanic acid, were prepared by dissolving of 10 mg in 1 ml of distilled water.

Methods of analysis

TLC was performed with two different chromatographic systems. Thin layers were prepared from microcrystalline cellulose and silica gel G (7). Standard solutions and samples were spotted on precoated plates of cellulose microcrystalline with fluorescent indicator and developed with mobile system of ethyl-acetate:formic acid:acetic acid:water (100:11:11:26, v/v/v/v). The other chromatographic system was silica gel GF_{254} as stationary and chloroform:ethyl-acetate:formic acid (5:4:1, v/v/v) as mobile phase. Substances were identified using UV detection at 254 nm. For visualization, plates were sprayed with $FeCl_3$ (2% in ethanol) (8).

RESULTS AND DISCUSSION

Preliminary TLC separation and identification of some phenolic compounds in kombucha beverages were performed using the chromatographic system with cellulose as stationary phase and the corresponding mobile phase mentioned above. The result of that analysis is presented in Fig. 1.

TLC on cellulose (Fig. 1) was accompanied by some problems in separation and identification of phenolic compounds. Even spots of standard solution were easy to detect, it is obvious that after detection of spots, the separation of standards was not successful, i.e. the R_f values of all investigated standards, except catechin, were very close. After spraying with $FeCl_3$, the spots color was dominantly blue. The analysis of sweetened black and green tea (Fig 1, spots 8 and 9) was not possible because of high amount of sucrose,

70 g/L, which prevented the development of the samples by forming a thick film over the start spots. The other samples were easier to analyze as a consequence of sucrose content decrease during fermentation (9). In fermentative liquids after 7 and 10 days of cultivation of tea fungus, it was noticed the presence of different phenolic compounds. Catechin and quercetin were identified in the samples after 7 days of fermentation on both substrates. The other spots were not identified because of the limited number of investigated standards in the mixture. It was also possible to notice the different phenolic composition between 7^{th} and 10^{th} day of fermentation (spots 10-11, 12-13). There were a smaller number of compounds in the samples after 10 days in comparison with 7^{th} day of fermentation. It indicates that the activity of tea fungus microbial system affected the phenolic composition in the substrate.

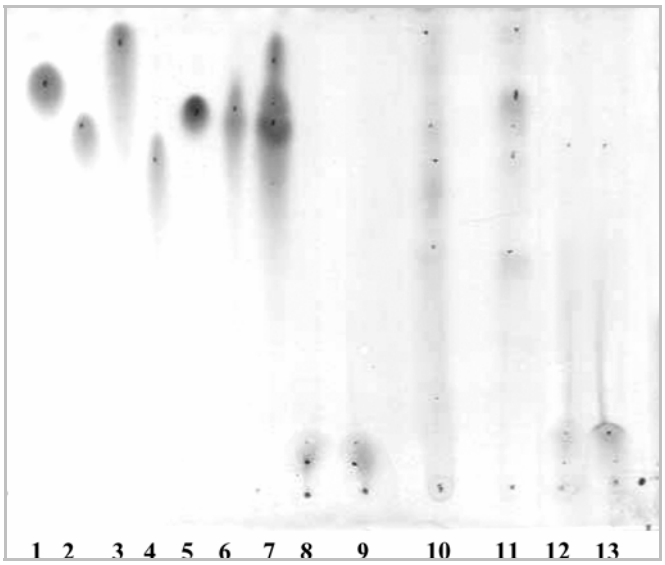

Fig. 1. TLC of catechin, epicatechin, quercetin, myricetin, gallic acid and tanic acid, and concentrated tea fungus samples on cellulose as stationary phase

1-6	Standards: catechin, epicatechin, quercetin, myricetin, gallic and tanic acid, respectively.
8 and 9	Sweetened black and green tea, respectively.
7	Mixture of standards.
10 and 11	7^{th} day of fermentation on black and green tea, respectively.
12 and 13	10th day of fermentation on black and green tea, respectively.

TLC on silica gel with chloroform:ethyl-acetate:formic acid (5:4:1, v/v/v) as mobile phase was performed as an attempt to improve the separation and identification of the investigated compounds especially in the samples of fermentative liquids. Hence, phenolic compounds were extracted from the samples with ether and then concentrated to increase the sensitivity of the analysis. The results of TLC analysis of some pnenolic compounds on silica gel layer with the above mobile phase are presented in Figs. 2 and 3.

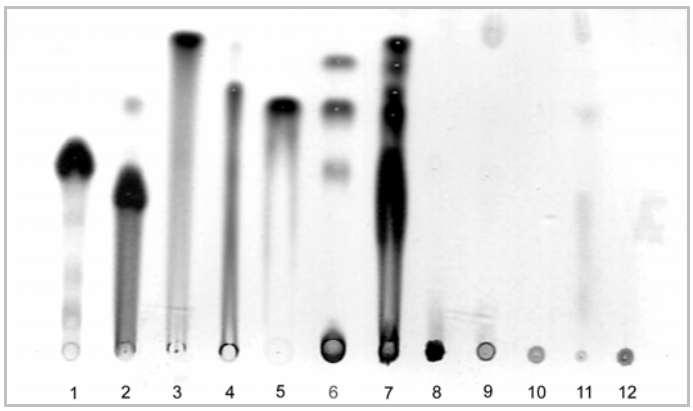

Fig. 2. TLC of catechin, epicatechin, quercetin, myricetin, gallic acid and tanic acid, and ether extracts of tea fungus samples (black tea) on silica gel as stationary phase

1-6 Standards: catechin, epicatechin, quercetin, myricetin, gallic and tanic acid, respectively.
7 Mixture of standards.
8 and 9 Water and ether extracts of black tea, respectively.
10, 11 and 12 Ether extracts of samples after 3, 7 and 10 days of fermentation on black tea, respectively.

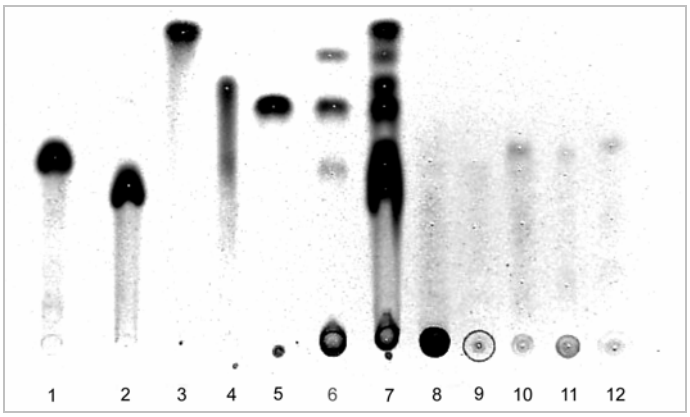

Fig. 3. TLC of catechin, epicatechin, quercetin, myricetin, gallic acid and tanic acid, and ether extracts of tea fungus samples (green tea) on silica gel as stationary phase

1-6 Standards: catechin, epicatechin, quercetin, myricetin, gallic and tanic acid, respectively.
7 Mixture of standards.
8 and 9 Water and ether extracts of green tea, respectively.
10, 11 and 12 Ether extracts of samples after 3, 7 and 10 days of fermentation on green tea, respectively.

The presented results of TLC analysis on silica gel with proper mobile phase (Figs. 2-3) have showed a higher sensitivity in terms of standards. Spots were blue after spraying with $FeCl_3$. In comparison with the TLC analysis on cellulose with proper mobile phase it was obvious that the different R_f values and their relative ratios were obtained. The main improvement of this analysis was a better separation of the standard solution mixture. The precise measurement of R_f value of each standard substance was possible.

The application of ether extracts of samples instead of water extract resulted in a better development of samples. Comparison between fermentative samples with black and green tea shows that more spots were identified in samples with green tea (Figs. 2 and 3). The R_f values of the spots of samples with green tea were dominantly in accordance to the R_f values of catechin and epicatechin and there were also noticed some substances with lower R_f values. The most obvious spots in the samples with black tea belong to quercetin. The difference in phenolic composition of fermentative liquids of tea fungus cultivated on sweetened black and green tea could be logical because of different composition of black and green tea (Table 1), especially in a view of the major differences of content of catechins and the other flavanoids (5).

In both chromatographic systems, the difference between the R_f values measured after UV identification at 254 nm and after visualization with $FeCl_3$ was not significant.

CONCLUSION

Tea fungus was fermented on two different substrates, on sweetened black and green tea, at 28°C, for 10 days, and its composition was investigated for the presence of some phenolic compounds.

TLC analysis of catechin, epicatechin, quercetin, myricetin, gallic acid and tanic acid, and concentrated ether extracts of tea fungus fermentative liquids samples, on silica gel with chloroform : ethyl-acetate : formic acid (5:4:1, v/v/v) as mobile phase, showed good separation and sensitivity. The procedure is suitable for the analysis of phenolic compounds in kombucha beverages.

The dominant phenolic compounds in the samples cultivated on green tea were catechin and epicatechin, and in the samples with black tea it was quercetin.

ACKNOWLEDGEMENTS

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АНАЛИЗА НЕКИХ ФЕНОЛНИХ КОМПОНЕНАТА У НАПИТКУ ОД ЧАЈНЕ ГЉИВЕ ТАНКОСЛОЈНОМ ХРОМАТОГРАФИЈОМ

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

Црни и зелени чај поседују широк спектар природних ароматичних једињења. Најважнији су флавоноиди и њихови гликозиди, катехини и њихови кондензациони производи, као и фенолне киселине. Напитак од комбухе се добија ферментацијом црног или зеленог чаја, који је заслађен сахарозом, при чему је производна култура микроорганизама симбиоза више врста квасаца и сирћетних бактерија, позната под називом комбуха или чајна гљива.

Циљ овог рада било је испитивање квалитативног састава неких фенолних једињења (катехин, епикатехин, кверцетин, мирицетин, гална и танинска киселина), који су веома важни антиоксиданти, као и праћење њиховог присуства током ферментације комбухе.

За анализу наведених супстанци коришћена је хроматографија на танком слоју. Показало се да су доминантне фенолне компоненте у ферментативној течности са зеленим чајем катехин и епикатехин, а кверцетин у узорцима са црним чајем.

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