

CULTIVATION OF TEA FUNGUS ON MALT EXTRACT MEDIUM

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The possibility of application of malt extract as a source of carbohydrate in a medium for tea fungus was investigated. The beverage obtained on such medium was compared with that prepared in a traditional way with sucrose medium. The presence of easily adoptable sugars, glucose and fructose, as dominant in malt medium results in a very effective fermentation, which gives much more sour beverage for the same time and makes it possible to reduce the fermentation period. The obtained beverage has satisfactory sensorial characteristics.

KEY WORDS: tea fungus, malt extract, fermentation, organic acids

INTRODUCTION

Tea fungus (kombucha) is a beverage of a sourish and sweetish taste and lightly carbonated, obtained by fermentation of sweetened black or green tea. It has been used for centuries in China, Russia and Germany (1), and its great popularity nowadays is a consequence of the interest in traditional and preventive medicine, as well as in ecological and biologically valuable nutrition. Between 1925 and 1950 several medical studies confirmed therapeutical value attributed to kombucha from ancient times: antibiotic activity, positive effects on the gastrointestinal tract, arthritis, gout, hemorrhoids, cholesterol value, arteriosclerosis, nervous system (2). There are data that daily consumption of kombucha significantly reduces the risk of cancer (2). In addition to tea components and sugar the beverage contains also acetic acid, gluconic acids, L-lactic acid (3-6), amino acids and biogenic amines (7), vitamins from B-complex and vitamin C (8). One of the most important metabolites from therapeutical point of view is glucuronic acid, a carrier of detoxification activity of kombucha (9,10).

Microbiological population of tea fungus consists of acetic acid bacteria (*Acetobacter xylinum*, *A. xylinoides*, *Bacterium gluconicum*) and yeasts (*Schizosaccharomyces pombe*,

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Saccharomyces ludwigi, *Saccharomyces cerevisiae* and others) (11,12). Yeasts and bacteria of kombucha are in a symbiotic association in which the yeasts by their invertases decompose sucrose (the most common source of carbon in cultivation media) and ferment its hexose units to ethanol and carbon dioxide. The bacteria of the *Acetobacter* genus oxidize ethanol through acetaldehyde to acetic acid (13). From glucose, the bacteria of acetic acid synthesize, first of all, gluconic acid and cellulose (13). Cellulose occurs as a membrane on the surface of fermented liquid maintained by the produced carbon dioxide. Fermentation under aerobic conditions usually lasts 4-6 days, resulting in the beverage with optimal acidity; longer incubation gives a beverage of higher acidity, as like as vinegar (14).

In the available literature, there are no data on using malt as carbohydrate base in media for kombucha, which induced us to investigate the possibility of its application and to follow the fermentation process during the incubation period of 5 days by measuring the pH value and cell count of kombucha microorganisms, especially concerning the dynamics of sugar adoption from the medium. The results were compared with those obtained for conventionally prepared kombucha on the medium with sucrose and black tea.

EXPERIMENTAL

The medium for tea fungus cultivation was prepared with 10 % malt extract as a commercial product "Maltex" and tap water. After boiling black tea was added to the solution in a quantity of 0.2 %. The extraction of tea components lasted 30 minutes, and then the rest of tea was removed by filtration. The medium was sterilized by tyndallization. Inoculation of cooled medium was performed by addition of starter from previous original fermentation in a quantity of 10 %. Cultivation of prepared tea fungus substrates lasted 5 days at temperature of 28°C.

The conventional kombucha beverage was prepared as follows:

After boiling of 7 % - sucrose/tap water solution, 0.2 % black tea was added. After 30 min the rest of the tea was removed by filtration and the prepared medium was inoculated with starter in a quantity of 10 %. Fermentation lasted 5 days at 28°C. One portion of the prepared kombucha beverage was used for inoculation of malt medium. pH values of kombucha beverage were determined using electronic pH-meter.

Total acidity was measured by conductometric titration with sodium hydroxide (15).

Easily volatile organic acids were distilled with water vapor, and their content was determined by titration with sodium hydroxide and phenolphthalein as indicator (15).

Total content of sugar in malt medium was estimated by antron method (16).

Qualitative analysis of sugar in medium was performed by thin-layer chromatography on silica gel G, using the solvent system chloroform-acetic acid-water (1:6:3, v/v/v). Spots were detected by spraying with a solution of 50 % sulfuric acid in ethanol, followed by heating for 10-15 min at 120 °C.

Total count of cells was determined by indirect method. For determination of total count of acetic fermentation bacteria YPM medium was used (3). Immediately after pouring, actidione as antimycotic (cycloheximid, naramic A) was added to the medium in a quantity of 300 mg/l (17,18). The medium was incubated for 7 days at temperatures up to 30°C. To determine total count of yeasts cells, malt agar "Novi sladni agar" was used (19). Incubation lasted 2-3 days at 28°C.

RESULT AND DISCUSSION

Black tea sweetened with sucrose is mostly used substrate for tea fungus microorganisms. All attempts to replace sucrose as source of C atoms with some other carbohydrate were unsuccessful. Because of residual sucrose and sugars originated from its hydrolysis, the beverage prepared in this manner is unacceptable for diabetics. In that sense, inulin and topinambur as its source appeared as an alternative (20).

The content of sucrose in liquid (medium) for fermentation usually amounts to 5-10 %, where by the direct relation between sucrose content and the content of metabolites present in the prepared beverage is ascertained (6). Using fructose as only source of C atoms the beverage containing solely acetic acid is obtained, while in solutions of maltose, lactose and dextrin the fungus grows well, producing very small quantities of acids (11).

Using honey, which contains many substances important to human organism, did not give desirable results since its active principles changed microbiological composition of tea fungus, and such effect is attributed to the antibiotic activity of honey components on kombucha bacteria (9).

From the viewpoint of active organisms, that is microbial population of tea fungus, black tea is a source of N atoms and mineral matter and it is added in a quantity of 0.2 % - 0.5 %. Recently, green tea is more and more used in media for tea fungus since its stimulating effect on fermentation and more expressed antioxidative activity has been confirmed (1).

The cellulose cover formed during fermentation contains kombucha microorganisms is used for inoculation and to start the subsequent fermentation. However, instead of cellulose membrane, a certain volume of fermented liquid (starter) containing the entire microbial population of kombucha can be used for inoculation (21). This method of inoculation was applied in our investigation since it enables the determination of the cell count of yeasts and bacteria of acetic fermentation at the very beginning of fermentation, and consequently the following of the dynamics of growth and multiplication of tea fungus microorganisms during fermentation.

As a rich source of sugar, malt forced itself as a potential source of carbon atoms in the medium for kombucha. Commercial product contains easily adoptable carbohydrates glucose and fructose in quantities up to 10 % and higher sugars, maltose and maltotriose (55 %), proteins (5-6 %). Besides due to the presence of proteins, sodium, potassium, phosphorus, calcium, magnesium, iron, vitamin B-complex, provitamin A, etc., malt has a great biological value. Using malt in media for kombucha cultivation can also be considered as economically reasonable, since its application during the off-season in breweries increases the level of exploitation of this part of the plant.

The values for pH and for total cell count of yeasts and acetic fermentation bacteria (BAF) of kombucha prepared on sucrose medium (5-day fermentation) used for inoculation are presented in Table 1. This kombucha inoculation is also the control sample, since it is prepared by usual method on the medium with sucrose and black tea, serving for comparison with the kombucha beverage obtained on malt medium.

Table 1. Parameters of kombucha inoculum

pH value	Total acidity (g/l)	Cell count of yeasts (ml ⁻¹)	Cell count of BAF (ml ⁻¹)
3.20	2.56	1.28· 10 ⁶	1.35· 10 ⁶

The acidity of the medium with 10 % malt extract and that inoculated with starter was low (Table 2) The acidity of the medium mostly originates from the fermented medium from previous charge added in a quantity of 10 % to the malt extract medium.

Table 2. Results of chemical and microbiological analyses of fermented malt medium

	Fermentation time (hours)				
	Beginning of fermentation		24	72	120
	non- inoculated medium	inoculated medium			
pH value	6.00	5.25	4.65	3.80	3.60
Volatile acidity (g/l)	-	-	0.277	2.76	5.16
Total acidity (g/l)	0.189	0.427	2.52	3.52	5.775
Total yeast count (ml ⁻¹)	-	1.28· 10 ⁵	1.20· 10 ⁷	-	1.77· 10 ⁷
Total count of BAF (ml ⁻¹)	-	1.35· 10 ⁵	1.31· 10 ⁷	-	1.85· 10 ⁷

The dynamics of fermentation on malt medium was followed by determining total contents on acids ("total acidity"), easily volatile organic acids ("volatile acidity"), pH value, total cell count of yeasts and acetic fermentation bacteria in the fermented medium. The results of these investigations (Table 2) indicate a very fast growth and multiplication of microorganisms accompanied by intensive metabolic activity. After 24 hours, a significant increase of beverage acidity is noticeable through the decrease of pH value and the increase of total acidity. Total acidity reaches the value of 2.52 g/l, which is by two units higher in comparison to the initial value. Similar changes were registered after 72 and 120 hours. The acidity in the first phase of fermentation originates mostly from the acetic acid obtained by oxidation of ethanol, confirming thus a strong activity of yeasts in the medium. The content of easily volatile acids after 24 hours is low (0.277 g/l), which is absolutely reasonable in view of the fact that these acids are secondary metabolites which need time to accumulate. The content of easily volatile acids is constantly increasing during the fermentation period to be dominant in total acidity after 120 hours (total acidity/volatile acidity = 5.775/5.16).

The changes in the beverage acidity during the fermentation period are in accordance with the changes in cell count of yeasts and acetic fermentation bacteria. Cell count shows a constant increase, reaching at the end of fermentation a value of the order of 10⁷

cell/ml, which is one hundred times higher compared to the initial value (Table 2). The growth and multiplication of kombucha microorganisms and their metabolic activity results in the corresponding acidity of beverage.

Comparing the values for the beverage acidity on the malt medium with those obtained by other authors who cultivated tea fungus on a medium with sucrose and black tea in comparative quantities (23), the total contents on acids and of easily volatile acids of the beverage on malt is several times higher. For illustration, the total acid content of 5.00 g/l in the above study is attained after 12 days of fermentation. This was also confirmed by comparing of the acidity of control kombucha from our experiment with the acidity of the kombucha beverage obtained with malt medium. Thus, in the same period of fermentation of 5 days malt kombucha was two times more acidic, i. e. in terms of total acidity content 5.775 g/l to 2.56 g/l.

Table 3. Total content of sugars in the medium with 10 % malt

	Fermentation time (hours)			
	Inoculated malt medium	24	72	120
Total sugar content (g/100ml)	8.9492	8.2109	7.8559	6.9203

Total content of sugars in the medium with 10 % malt was determined by antron method (Table 3). The initial value of 8.9492 g/100 ml (calculated on glucose) was expected and is in accordance with declared composition of commercial malt. It is obvious that sugar content in the applied medium is not a limiting factor in the process, so that after 5 days of fermentation a significant quantity of carbohydrates is left, enabling undisturbed further fermentation. However, further fermentation under the given conditions has no sense, because it would give a beverage of unacceptable acidity. With the aim to get a more detailed picture on adoption of malt sugars, especially maltose, recognized after hydrolysis by antron method as two molecules of glucose, qualitative analysis of sugars by TLC chromatography was performed (Figure 1).

Chromatographic analysis of inoculated medium at the beginning of fermentation confirmed the presence of declared sugars: glucose, maltose and maltotriose. Glucose and fructose as easily adoptable sugars are consumed first by kombucha microorganisms and their quantities in the medium after fermentation of 72 hours are below the sensitivity limit of the method. The presence of these sugars in malt medium and their effective utilization is the reason for higher acidity of the obtained beverage in comparison to that obtained on sucrose media. High acidity of the beverage, and especially high increase of acidity in the first fermentation phase, is a consequence of the presence of fructose in the malt medium, which is adopted by cells faster than glucose (3). While acetic fermentation bacteria convert glucose mostly to gluconic acid, fructose is metabolized almost completely to acetic acid, and never to gluconic acid (13).

The dominant carbohydrate in the malt medium after 2-3 days of fermentation is primarily maltose, which can be adopted by yeasts cells, enabling thus further fermentation and probably of trisaccharide maltotriose.

The beverage obtained with malt after 3-4 days of fermentation has a satisfactory taste and acidity

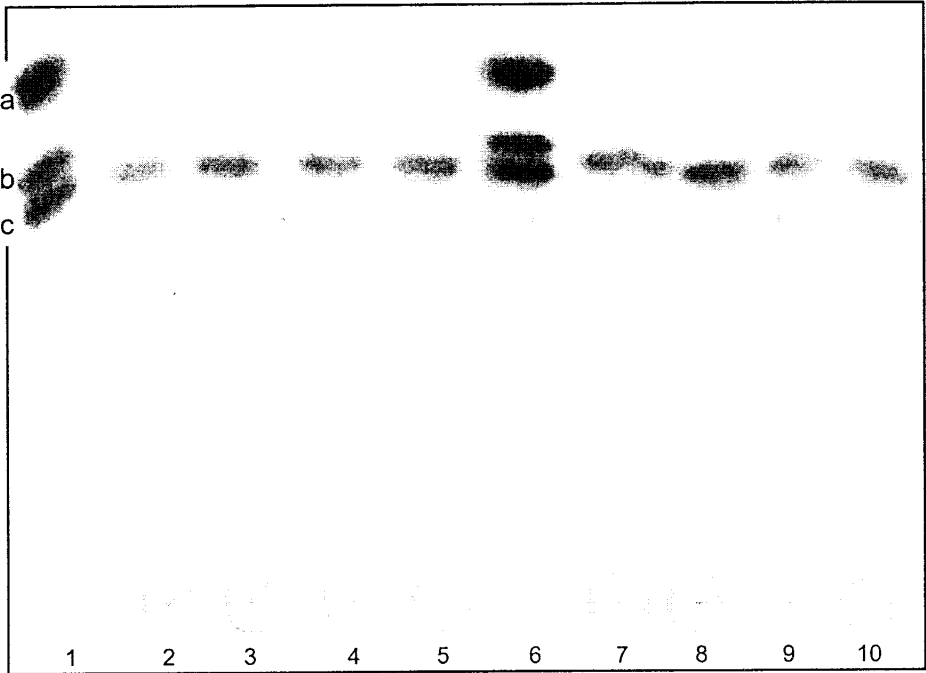


Fig. 1. TLC chromatogram of sugars in malt medium

1,6: standard (glucose, fructose, sucrose, maltose); 2,3: inoculated medium at the beginning of fermentation; 4,5: fermented liquid after 24 hours; 7,8: fermented liquid after 72 hours ; 9,10: fermented liquid after 120 hours. a-fructose and glucose, b-sucrose, c-maltose.

CONCLUSION

The results of the analysis of fermented malt media confirmed our assumption on the possibility of using malt as a source of carbohydrates in the medium for kombucha. The presence of easily adoptable sugars, glucose and fructose, in this medium contributes to faster fermentation and obtaining a beverage of acceptable flavor and taste in a shorter time compared with that prepared in traditional way. Kombucha on malt is biologically even more valuable beverage considering the fact that additional quantities of biologically valuable substances are taken in. Further investigations will be focused on investigation of the effects of reducing malt quantity in the medium for tea fungus cultivation on the development of fermentation and synthesis of metabolites.

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КУЛТИВАЦИЈА ЧАЈНЕ ГЉИВЕ НА ПОДЛОЗИ СА ЕКСТРАКТОМ СЛАДА

Драгољуб Д. Цветковић и Синиша Ј. Марков

Испитивана је могућност употребе екстракта слада (сладовине) као извора угљеникових атома у подлози за чајну гљиву. Напитак добијен на оваквој подлози упоређиван је са оним добијеним на традиционалан начин на подлози са сахарозом. Присуство лако усвојивих шећера, глукозе и фруктозе, као доминантних састојака подлоге са сладовином доводи до врло ефикасне ферментације којом се добија много киселији напиток за исто време, што пружа могућности за скраћење ферментационог периода. Добијени напиток је задовољавајућих сензорних карактеристика.

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