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Characterization of Kombucha Beverages Fermented with Various Teas and Tea Fungus

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Abstract

Kombucha beverages were made from sweetened tea by Oriental, European and Tibetan tea fungus starters. The hot water extracts of green tea, black tea, Gugija and Omija were mixed with white and/or brown sugar, and were fermented under a static culture at 30°C. Titrable acidity, pH, color and cellulose production in kombucha beverages were evaluated. All tea fungus starters showed a higher acid production in green/black tea extracts rather than Gugija and Omija extracts. In green/black tea extracts, Oriental tea fungus produced a kombucha beverage with a higher titrable acidity and lower pH than those of European and Tibetan tea fungus starters. By the static fermentation of green/black tea extract for 18 days, Oriental, Tibetan and European tea fungus starters produced cellulose pellicles of 0.43 g, 0.16 g, and 0.19 g (dry weight) on the top in the culture, respectively. As a mother starter, the cellulose pellicle was more efficient in acid production compared with tea fungus broth. Oriental/Tibetan mixed tea fungus showed the best acid production in the green/black tea extract supplemented with brown sugar.

Key words: kombucha, tea fungus, acidity, cellulose

INTRODUCTION

Kombucha, an acetic acid flavored fermented tea beverage, is made from sweetened tea by tea fungus fermentation (1,2). The consumption of kombucha beverage was practiced in 220 B.C. in Manchuria and then spread to Russia (3). Now, it is widely consumed in various parts of the world and has more recently become a fad in the United States (4). Its consumption is popular mainly because of its refreshing power, curative effects (including detoxifying properties) and nutritive value (3,5). Additionally, it can be produced in the home, and can reduce blood pressure, relieve arthritis, increase the immune response and possibly cure cancer (4).

The symbiotic culture of *Acetobacter xylinum* and yeasts in tea fungus is grown traditionally on black tea with sucrose and gives a pleasantly sour and sparkling kombucha beverage under aerobic conditions (3). The transformation of sucrose into glucose, fructose, gluconic acid, ethanol, and acetic acid during tea fungus fermentation has been determined (2). In particular, *Acetobacter xylinum* synthesizes a floating cellulose pellicle in which the cells are embedded. The film produced is essentially a microbial cellulose similar to the "mother of vinegar" that forms on the surface of wines or ciders being converted to vinegar by the *Acetobacter aceti* subspecies *xylinum* oxdizing ethanol to acetic acid (4).

Bacterial cellulose pellicles have been used as food ingredients that are closely related to Philippine nata (6). This material is currently used by Sony Corporation in the production of high-end audio speaker systems because of its excellent acoustic properties (7). This biofilm also displays several advantages as a biological dressing, and hence, it is valuable as a temporary skin substitute in the treatment of skin wounds (8,9). Furthermore, different methods for the formation of hollow fibres during biosynthesis were investigated for various applications as a biocompatible material in medicine (10). Recently, cellulose biosynthesis and function in *Acetobacter* species were investigated at the genetic level (11,12).

Kombucha beverages should be regarded principally as a food unusually rich in nutritive properties, rather than just a healthy drink. Kombucha has a wide range of organic acids, vitamins and enzymes that give it its extraordinary value (1). The specific organic acids that are found in the kombucha beverage will vary according to the proportion of bacteria to yeast cells, and to the particular species of each (1). Therefore, the production of organic acids and cellulose pellicles will depend upon the type of tea fungus used. Generally, tea provides nitrogen, minerals, vitamins, and other substances essential for nutrition, and promotes the growth of micro-organisms and the cellular construction of the kombucha beverage. Sugar plays an essential part in kombucha's brewing process, providing a nutrient solution for the culture. It also initiates the fermentation process. The role of brown sugar in kombucha fermentation by various tea fungus starters has not been reported, although the effect of white sugar on kombucha fermentation was investigated using a tea fungus (13). It is necessary to understand the role of brown sugar and various tea fungus starters in various kombucha fermentation. In addition to black tea, various traditional Korean teas with particular

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properties can be exploited for kombucha beverages.

In this report the role of tea, sugar, tea fungus in the production of acid and cellulose pellicles in kombucha beverages was investigated.

MATERIALS AND METHODS

Materials

Green tea (Taepyungyang Co, Korea) and black tea (Lipton Tea Co, Singapore) were used as ingredients for kombucha fermentation. Omija and Gugija were obtained from a local market. Oriental, European and Tibetan tea fungus starters were purchased from Harmonic Harvest (P.O. Box 82, Harwood, TX, USA). White and brown sugars (Cheiljedang Co., Korea) were used as carbon sources. Mineral water (JinRo Food Co., Korea) was used for tea extraction.

Preparation of media for kombucha fermentation

A 20% (w/v) of sugar solution (white or brown) was prepared and autoclaved at 121°C for 15 min. One part of the brown sugar solution was mixed with nine parts of white sugar solution to prepare a 20% mixed sugar solution. Two green tea bags (3.08 g) and one black tea bag (2.10 g) were added to 500 ml of hot mineral water (90~95°C) and left to draw for 5 min. To prepare Gugija and Omija extract, 3 g of Gugija or Omija was extracted in 150 ml of hot water for 5 min. 100 ml of tea extract was placed into a sterile 500 ml beaker and then mixed with 100 ml of 20% white sugar solution or 20% white/brown sugar mixture (9:1, v/v). The tea extract (200 ml) containing 10% sugar was cooled down at 25°C before inoculating with a tea fungus starter.

Production of kombucha beverages

Kombucha beverages were prepared with various tea extracts and tea fungus starters. In order to activate the tea fungus, the green/black tea extract was inoculated with 5% of tea fungus broth and then was fermented in the static culture at 30°C. After fermenting for 7~10 days, each kombucha beverage with about 1.0% (w/v) titrable acidity was used as a mother starter for kombucha fermentation. In addition, a cellulose pellicle obtained from the kombucha culture was divided into four pieces and then each cellulose pellicle (3.5~5.0 g, wet weight) was added to the kombucha mixture. 200 ml of tea extract containing 10% sugar (white or brown) was inoculated with 5% tea fungus broth or cellulose pellicle. Kombucha fermentation was carried out under a static culture at 30°C for 30 days. The physicochemical properties of kombucha beverages were analyzed to determine the role of each tea fungus as well as ingredients such as sugar and tea.

Analysis of Kombucha beverages

The pH of kombucha beverages was determined with a pH meter (Digital pH meter 110, Wheaton, USA). The titrable acidity was measured by titrating 10 ml of kombucha beverage with 0.1 N NaOH (14). The viable cell count of yeast and bacteria in the tea fungus starters was determined with YM (Yeast extract malt) and SH (Schramm Hestrin) agar plate, respectively. The Hunter color value (L, a, b) of the kombucha beverage was measured with a Color Reader (Minolta CR-10, Japan). The cellulose pellicles were separated from the top of culture broths and then boiled in water for 5 min to remove the soluble solid. The yield of cellulose pellicles was determined by drying at 105°C (15).

RESULTS AND DISCUSSION

Microflora in tea fungus

Microflora in each tea fungus starter was evaluated using a selected medium for yeast and *Acetobacter* species. The microbial population of each tea fungus starter is shown in Table 1. The tea fungus starter contained yeasts with small or big colonies and bacteria assuming as *Acetobacter* species. Yeasts were confirmed by examining their morphology with a microscope. *Acetobacter* species were distinguished by their dry cell surface due to the cellulose production. Tibetan starter had a low number of yeast and *Acetobacter* species. European starter contained a higher population of *Acetobacter* species. It has been reported that the tea fungus (kombucha) is a symbiosis of *Acetobacter*, including *Acetobacter xylinum* as a characteristic species, and various yeasts (16,17). Different yeast species were also identified in different tea fungus cultures (18).

Kombucha beverages fermented by tea fungus starters

In order to compare the acid production in kombucha fermentation by three tea fungus starters, each tea fungus was grown in the green/black tea extract containing white sugar. Table 2 shows the pH, titrable acidity and yield of cellulose pellicles in the kombucha beverages. Among three tea fungus starters, Oriental tea fungus showed the best acid production indicating 1.3% (w/v) acidity after fermentation for 12 days. On the other hand, European tea fungus showed lower acid production compared with other tea fungus starters. All kombucha beverages showed very low pH values below 3.0. Oriental tea fungus was able to produce a kombucha beverage with 1.0% acidity within 10 days. For all kombucha beverages, the content of cellulose pellicles synthesized gradually increased by fermentation for 18 days. In particular, Oriental tea fungus showed a higher production of cellulose pellicle in the green/black tea mixture containing white sugar. The cellulose pellicle produced by Oriental tea fungus was 0.33 g (dry weight) from 200 ml of kombucha beverage after fermentation for

Table 1. Microbial counts in Oriental, European and Tibetan tea fungus starters used for kombucha fermentation (CFU/ml)

Tea fungus	Yeast[1)]	*Acetobacter*[2)]
Oriental	1.2×10^7	1.4×10^7
European	3.8×10^7	3.6×10^7
Tibetan	5.2×10^6	1.3×10^6

[1)]YM (yeast extract malt medium) for yeast
[2)]SH (Schramm Hestrin medium) for *Acetobacter*

Table 2. Production of acid and cellulose pellicle in kombucha beverages fermented by various tea fungus starters

Measurement	Time (days)	Tibetan	Oriental	European
pH	0	3.78	3.92	4.08
	6	3.01	2.77	3.07
	12	2.82	2.61	2.90
	18	2.74	2.54	2.84
Titrable acidity (%, w/v)	0	0.05	0.05	0.04
	6	0.54	0.37	0.42
	12	1.07	1.30	0.94
	18	1.37	2.12	1.27
Cellulose pellicle[1)] (g, dry wt)	0	-	-	-
	6	0.08	0.13	0.04
	12	0.12	0.33	0.14
	18	0.16	0.43	0.19

[1)]Cellulose obtained from 200 ml green/black tea mixture at 30°C (500 ml beaker, Ø8.6 cm)

12 days. Therefore, Oriental tea fungus was superior in its production of acids and cellulose pellicle in green/black tea extract. These results imply that the production of acids and microbial cellulose is dependent upon the type of *Acetobacter* sp. rather than number of viable cell counts in tea fungus. It has been reported that caffeine and related phenolic compounds (theophylline, theobromine) are identified as activators for bacterial cellulose production in *A. xylinum* (19). The microbial cellulose pellicle was considered a by-product for the production of acetic acid by *Acetobacter aceti*. Now, microbial cellulose as a biological membrane is being considered for various applications in the food and medical fields (3). Therefore, the selection of the tea fungus starter and ingredients will be crucial to enhance the production of kombucha beverages, as well as biological membranes.

Effect of tea extracts on the acidity of kombucha beverages

In order to determine the acid production in green/black tea, Gugija and Omija extract, each tea fungus broth (10 ml) was transferred to 200 ml of tea extract containing 10% white sugar. As shown in Fig. 1, the Oriental tea fungus starter showed a higher acid production, especially in the green/black tea mixture. Oriental tea fungus drastically increased the acid production after 9 days. Titrable acidity reached 1.4% (w/v) after 15 days. European and Tibetan tea fungus starters showed 0.9% (w/v) acidity after 15 days. The European starter showed very weak acid production in Omija and Gugija extracts. Therefore, three tea fungus starters resulted in higher acid production in the green/black tea mixture. In contrast to European and Tibetan tea fungus starters, Oriental tea fungus showed relatively higher acid production in Omija and Gugija extracts. This implies that Omija and Gugija extracts can be used as ingredients for kombucha fermentation by Oriental tea fungus.

Kombucha beverages fermented by cellulose pellicles

Cellulose pellicles as a tea fungus starter were used for kombucha fermentation to evaluate the acid production. Cellulose pellicles were separated from each kombucha beverage fermented with Oriental, Tibetan or European tea fungus and then added to either the green/black tea mixture, Omija or Gugija extracts. Fig. 2 shows the acid production in kombucha beverages. Three tea fungus starters also showed higher acid production in green/black tea mixture compared with Gugija and Omija extracts. In particular, Oriental tea fungus showed higher acid production from the green/black tea mixture showing 1.1% (w/v) acidity after fermentation for 9 days. Other tea fungus starters indicated 1.0% (w/v) acidity after fermentation for 12 days. It was confirmed that the cellulose pellicles as a mother starter enhanced the acid production in kombucha fermentation more than tea fungus broth. The symbiotic cul-

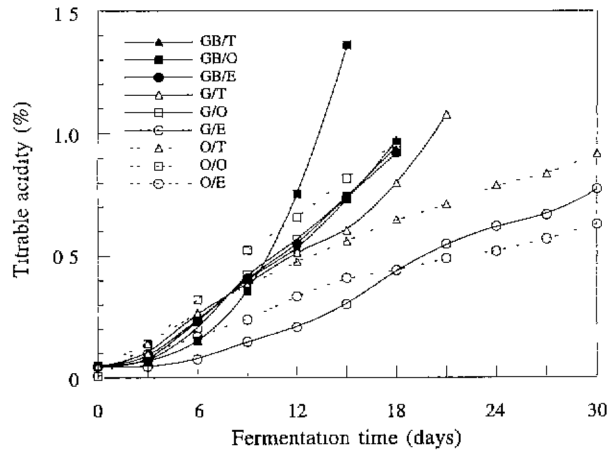

Fig. 1. Acidity in kombucha beverages made from green/black tea, Gugija and Omija extract by Oriental, European and Tibetan tea fungus starters. Tea fungus broth was used as a mother starter. GB, green/black tea; G, Gugija; O, Omija; T, Tibetan; O, Oriental; E, European tea fungus.

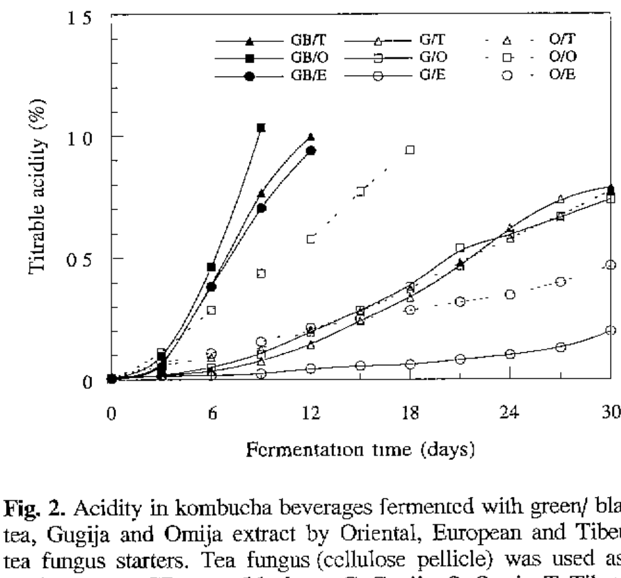

Fig. 2. Acidity in kombucha beverages fermented with green/ black tea, Gugija and Omija extract by Oriental, European and Tibetan tea fungus starters. Tea fungus (cellulose pellicle) was used as a mother starter. GB, green/black tea; G, Gugija; O, Omija; T, Tibetan; O, Oriental; E, European tea fungus.

ture of *Acetobacter xylinum* and yeasts produced a cellulose pellicle (tea fungus) that is similar in composition to mother of vinegar (3). Therefore, *Acetobacter* sp. embedded in cellulose pellicles may contribute the acid production in kombucha fermentation. Tibetan tea fungus indicated a weak acid production in Omija or Gugija extracts. European tea fungus showed a very weak acid production in Gugija and Omija extracts except the green/black tea mixture. But Oriental tea fungus indicated 0.95% (w/v) acidity from Gugija extract after fermentation for 18 days. It is confirmed that a green/black tea mixture is a more suitable medium for kombucha fermentation than Omija and Gugija extracts. Acid production in kombucha fermentation is dependent upon the type of tea extract. Thus, the acid production in kombucha beverages fermented with Gugija and Omija extracts could be enhanced by combining a green/black tea mixture.

Effect of mixed starters and brown sugar on kombucha fermentation

Generally, kombucha beverages are made from black tea and white sugar by a single tea fungus starter (2,4). The effect of Oriental and Tibetan mixed tea fungus and brown sugar on kombucha fermentation was evaluated. As shown in Fig. 3, Oriental or Tibetan tea fungus enhanced the acid production by the addition of brown sugar in green/black tea extract. A mixed starter including Oriental and Tibetan tea fungus starters accelerated the acid production compared with a single tea fungus. In green/black tea containing brown sugar, a mixed tea fungus starter showed 1.35% (w/v) acidity after fermentation for 7 days. Other kombucha beverages fermented by a single tea fungus only indicated acidity below 0.5% (w/v). Therefore, the acid production in kombucha beverages fermented with green/black tea was enhanced by the addition of a mixed starter and brown sugar. Considering the role of yeast and *Acetobacter* sp. in kombucha fermentation, the stimulative effect in acid production may be due to the mutual symbiotic relationship of a mixed starter.

Kombucha beverages made from sweetened black tea indicated a typical brown color. The color of the kombucha beverage was dependent upon the type of tea and sugar used. Table 3 shows the color value of kombucha beverages fermented

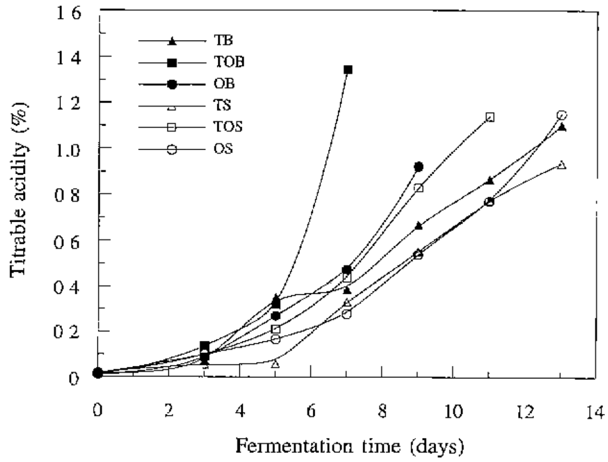

Fig. 3. Effect of sugar and mixed tea fungus on the acidity in kombucha beverages fermented with green/black tea. TB, Tibetan starter with brown sugar; TOB, Tibetan/Oriental starter with brown sugar; OB, Oriental starter with brown sugar; TS, Tibetan starter with white sugar; TOS, Tibetan/Oriental starter with white sugar; OS, Oriental starter with white sugar.

with green/black tea or Gugija extract by Oriental or Tibetan tea fungus starters. The color of each kombucha beverage was evaluated until the acidity of the kombucha beverage indicated 0.8 to 1.0% (w/v). Kombucha beverages made from green/black tea showed a lower Hunter color value (L) than that of Gugija tea. The addition of brown sugar produced a kombucha beverage with a dark brown color resulting in a lower L value. The Hunter color value (b) of Green/black tea was higher than that of Gugija extract. During fermentation the color value (L) of the kombucha beverages slightly increased in the case of both white and brown sugar. Therefore, the kombucha beverages had an indigenous color derived from the tea extract used and could be manipulated by the addition of brown sugar and other ingredients.

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Table 3. Change in color of kombucha beverages fermented with green/black tea and Gugija extract

Days			0			7			11		
Tea fungus	Sugar	Tea	L	a	b	L	a	b	L	a	b
O	B	GB	27.8	5.3	11.8	29.9	5.4	13.1	-	-	-
		G	28.7	3.0	7.2	31.0	4.9	10.4	31.9	5.0	10.3
	W	GB	28.1	5.9	11.7	30.6	5.3	11.8	29.3	5.2	11.7
		G	32.0	4.4	9.2	32.4	4.3	8.2	33.8	4.5	9.5
T	B	GB	28.3	6.6	13.5	29.8	6.2	14.2	-	-	-
		G	29.9	4.8	10.4	30.8	4.7	9.7	-	-	-
	W	GB	29.9	6.2	13.1	30.8	5.1	12.0	29.3	4.9	12.2
		G	30.7	4.2	6.3	31.5	5.1	7.9	32.8	4.4	8.2

O, Oriental starter; T, Tibetan starter; B, brown sugar; W, white sugar; GB, green/black tea; G, Gugija extract.

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