J. Food Sci. Nutr.
Vol. 7, No. 1, p.37~42 (2002)

Production of Microbial Cellulose and Acids in Kombucha

Han-Sup Soh and Sam-Pin Lee†

Department of Food Science and Technology, Keimyung University, Taegu 704-701, Korea

Abstract

Factors affecting the production of bacterial cellulose and organic acids in Kombucha fermentation were investigated. Kombucha was obtained by the fermentation (for 12 days at 30°C) of the green/black tea extract, supplemented with 10% white sugar, using an Oriental tea fungus as starter. Higher initial pH increased acid production with decreased cellulose production. With a cellulose pellicle or tea fungus broth as a starter, a 1~3 mm thick cellulose layer developed as a top layer every four days, and was removed subsequently while continuing fermentation. Addition of 30 mL tea fungus broth (13%, v/v) in Kombucha fermentation resulted in maximum production of a cellulose pellicle, indicating weak acid production. Yield of cellulose production at an early stage of fermentation was also higher when Kombucha was inoculated with a cellulose pellicle. In fact, addition of 1% (v/v) alcoholic beverage in the Kombucha fermentation activated the cellulose production, coupled with four times higher acid production.

Key words: Kombucha, cellulose pellicle, tea fungus

INTRODUCTION

Kombucha is a refreshing and health-promoting beverage, which is usually made by fermenting black tea and white sugar using a symbiotic culture (1). This beverage tasting like sparkling apple cider is often produced in the home by fermentation using a tea fungus. This beverage has been consumed in Asia, Europe and Russia from time immemorial. Now, it is widely consumed in various parts of the world and has more recently become a fad in the United States because of its beneficial effects on human health (2). The U.S. Food and Drug Administration has evaluated the practices of several commercial producers of the starter, which is often termed 'Kombucha mushroom' or 'tea fungus', and found no pathogenic organisms or other hygiene violations (3).

Black tea and white sugar have been used as the best substrates for the preparation of Kombucha, although green tea can be also used (1). The desired quality or composition of Kombucha can be obtained through the proper control of fermentation time (4,5). Bacteria and yeasts present in Kombucha produce acetic acid, gluconic acid and ethanol that play an important role for antimicrobial activity against pathogenic bacteria thereby providing protection against contamination of the tea fungus (6). The basic biochemistry and microbiology of the Kombucha fermentation is known. Kombucha fermentation depends upon the geographical location, kinds of tea fungus, ingredients, incubation time and temperature (7). In Kombucha the effect such as stimulation of the immune system, and anti-cancer and antibacterial effects may be the result of both attributes associated with tea and changes brought about by fermentation (8-12).

In the production of Kombucha by a tea fungus, a floating cellulose membrane is produced simultaneously by *Acetobacter* sp. The highly crystalline cellulose membrane embedded with cells is a thick and smooth cellulose pellicle (2). Production of a microbial cellulose pellicle (bio-film) in Kombucha greatly depended upon the type of tea fungus, composition of medium, temperature and antibiotics added (13-15). Caffeine and related xanthines derived from tea were identified as potent stimulators for the bacterial cellulose production in *Acetobacter xylinum* (16). Recently, structural modification of bacterial cellulose was carried out by the addition of antibiotics and incorporation of N-acetylglucosamine (17, 18). Bacterial cellulose pellicles have been used as food ingredients that are closely related to Philippine nata (19). Recently, this bacterial cellulose has been considered as a potent biological material to be used as a temporary skin substitute in the treatment of skin wounds, such as burns and ulcers (16,20). Furthermore, different methods for the formation of hollow fibres during cellulose biosynthesis were investigated for various applications as a biocompatible material in medicine (21,22).

†Corresponding author. E-mail: splee@kmu.ac.kr
Phone: 82-53-580-5554. Fax: 82-53-580-5554

Today, the aging of the population and limitations of modern medicine against chronic diseases have caused many people to look for new ways to improve their health (1). Currently, Kombucha is alternately considered as the ultimate health drink and therapeutic agent in countless diseases such as rheumatism, intestinal disorders and cancer (7,23). In addition, Kombucha also produced the microbial cellulose as a potent biological material for various applications in many fields. Therefore, it is very valuable to understand the factors affecting the fermentation of Kombucha. In this report factors affecting the production of cellulose pellicle and acids in Kombucha fermented by an Oriental tea fungus were investigated.

MATERIALS AND METHODS

Materials

Green tea (Taepyungyang Co., Korea) and black tea (Lipton Tea Co., Singapore) were used for preparing tea extract for Kombucha fermentation. An Oriental tea fungus was purchased from Harmonic Harvest (P.O. Box 82, Harwood, TX, USA) and used as a starter. White sugar (Cheiljedang Co., Korea) was used as carbon source. Mineral water (JinRo Food Co., Korea) was used for tea extraction. Commercial alcoholic beverage (Cham soju, Korea) was used for the fortification of tea extract. Other chemicals used were all analytical grade.

Tea extract for Kombucha fermentation

To prepare green/black tea extract, two green tea bags (3.0 g/ a tea bag) and one black tea bag (2.1 g/ a tea bag) were added to 500 mL of hot mineral water (90°C) and left for 5 min. A white sugar solution (20%, w/v) was autoclaved at 121°C for 15 min. One part of sugar solution was mixed with one part of green/black tea extract. Tea extract containing 10% (v/v) sugar was cooled down to 25°C before inoculating with a tea fungus. The initial pH 2.8 of tea extract containing 10% white sugar was increased to 4.0, 5.0 and 6.0 by using 0.5 M Na_2HPO_4, in combination with a little amount of 5 M NaOH. Green/black tea extract with 10% white sugar (200 mL) was fortified with 10 mL of commercial alcoholic beverage (23% alcohol).

Fermentation of Kombucha

Kombucha was prepared by fermentation of green/black tea extract using an Oriental tea fungus. A tea fungus was cultivated in green/black tea extract containing 10% white sugar at 30°C for 12 days. Kombucha with 1.0% (w/v) titratable acidity was used as a mother starter for Kombucha fermentation. A 200 mL of green/black tea containing 10% white sugar was placed in a sterile 500 mL beaker (8.6 cm diameter), and then inoculated with tea fungus broth (10 to 50 mL) or cellulose pellicle grown in 500 mL beaker. Kombucha was fermented in the static culture at 30°C for 12 days and was used for the analysis of acidity and cellulose production at every four days.

Analysis of Kombucha

The pH of Kombucha was determined with a pH meter (Digital pH meter, 110, Wheaton, USA). The titratable acidity was measured by titrating 10 mL of Kombucha with 0.1 N NaOH as a standard of acetic acid (24). The wet cellulose pellicles were harvested from the top of culture medium at four days intervals and then their thickness were measured by Vernier calipers (Mitutoyo, Japan). Cellulose pellicles were then boiled in water for 5 min to remove the soluble solid. After washing cellulose pellicles twice with hot distilled water, yield of cellulose pellicles was determined after drying at 105°C (25).

Formation of tubular cellulose pellicle

To prepare the tubular form of microbial cellulose, a cotton fiber (2 mm diameter) inoculated with tea fungus was connected to the nostril of the Burette (200 mL) that was filled with green/black tea extract containing 10% white sugar in aseptic condition. This assembly was placed at 30°C for 10 days.

RESULTS AND DISCUSSION

In Kombucha fermented with green/black tea mixture containing 10% white sugar and an Oriental tea fungus, the productions of cellulose pellicle and acids were affected by initial pH of tea extract, amount of tea fungus broth, type of starter and addition of alcohol. It has been reported that an Oriental tea fungus was superior to the production of acids and cellulose pellicle compared to European and Tibetan tea fungus starters (26).

Recently, microbial cellulose membrane has been known as valuable biomaterial as the substituent skin for burning (16,20). It also turned out that cellulose membrane with 1~3 mm thickness could be produced by fermentation using an Oriental tea fungus for 4 days. A cellulose membrane with 1~3 mm thickness can be utilized as biological membrane because of its durable physical properties. Therefore, the cellulose membrane was recovered from Kombucha in every four days during fermentation for 12 days at 30°C. At the same time, relationship between the acidity and cellulose production in Kombucha was evaluated.

Effect of pH

The initial pH value of tea extract affected the acidity and cellulose production during fermentation. As shown in Fig. 1, the lower initial pH in tea extract indicated the lower pH in Kombucha fermented for 4 days. Titratable acidity showed slight difference (0.36~0.47%) in all conditions. The pH of Kombucha gradually decreased according to the order of initial pH value. Regardless of initial higher pH, Kombucha showed very acidic pH values below 2.3 after fermentation for 12 days. It has been reported that the pH value dropped from an initial value of 3.75 to 2.42 as a result of acid formation during Kombucha fermentation for 60 days (27). The tea extract without sodium phosphate showed the gradual increase of acidity with 2.0% (w/v) acidity after fermentation for 12 days. On the other hand, the tea extract fermented at pH 6.0 by adding sodium phosphate indicated the 3.4% (w/v) acidity. In Kombucha fermentation by tea fungus, sucrose was transformed into glucose, fructose resulting in the accumulation of gluconic acid, acetic acid and cellulose pellicle (27). An initial pH 2.8 in tea extract resulted in the lower acid production compared with those of higher initial pH. It indicated that initial higher pH in tea extract with sodium phosphate might be related to higher acid production. But Kombucha with sodium phosphate showed slightly higher pH, despite higher acidity during fermentation. It implies the buffering capacity due to sodium phosphate in Kombucha.

During the initial stage of fermentation (4 days) with pH 4 or pH 5, Kombucha produced 0.25 g (dry wt.) of cellulose pellicle but with an initial pH 2.8 showed weak production of cellulose, while longer fermentation (12 days) with pH 2.8, showed higher production of cellulose, 0.7 g (dry wt.) at 12 days (Fig. 2). In spite of subsequent recovery of the cellulose pellicle from Kombucha, the removal of the cellulose pellicle did not affect the cellulose production in Kombucha during longer fermentation. The thickness of wet cellulose pellicle harvested from Kombucha was about 1~3 mm. It was concluded that cellulose production in the mixture of green/black tea and an Oriental tea fungus was dependent on the initial pH of Kombucha as well as fermentation time. Addition of sodium phosphate in tea extract enhanced acid production with reduction of cellulose yield in Kombucha. But it is not clear whether the higher pH or sodium phosphate affects the production of acids and cellulose in Kombucha. It has been reported that the initial medium pH 5.5 was optimum for cellulose synthesis by *Acetobacter xylinum* (28). The difference in optimum pH may be due to the microbial complexity of tea fungus for Kombucha fermentation.

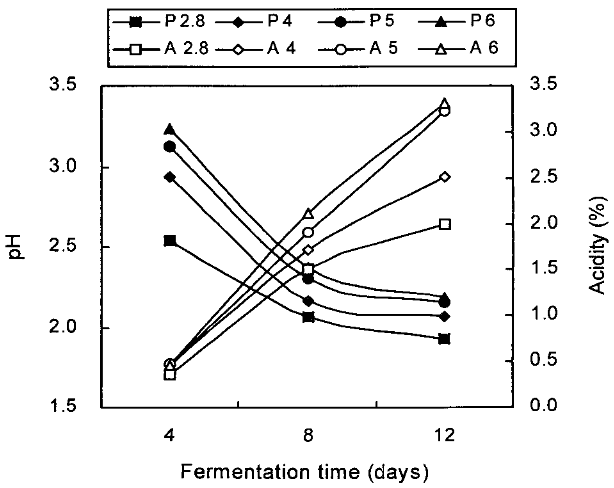

Fig. 1. Effects of initial pH of tea extract on the acid production and pH in Kombucha fermented by a tea fungus. Numbers indicate initial pH. P: pH, A: acidity.

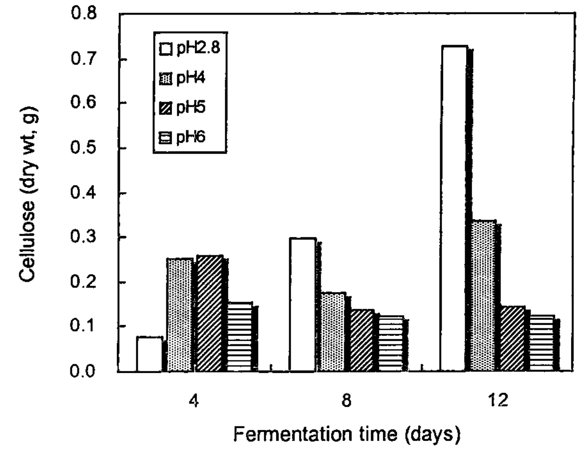

Fig. 2. Effect of initial pH on the cellulose production in Kombucha, fermented by a tea fungus. Cellulose was obtained from Kombucha grown in 200 mL tea extract using 500 mL beaker (diameter 8.9 cm).

Effects of tea fungus broth or cellulose pellicle

To evaluate the effect of the amount of tea fungus broth or cellulose pellicle on the production of cellulose and acids, different amounts of tea fungus broth were added as starter to the green/black tea extract. In addition, the cellulose pellicle for Kombucha fermentation was inoculated to the tea extract. As shown in Fig. 3, Kombucha fermented for initial 4 days showed pH between 2.5 and 2.8. By inoculating 10 mL tea fungus broth, Kombucha showed the higher acid production indicating lower pH during fermentation for 12 days (Fig. 3). The cellulose pellicle as a starter resulted in the higher production of acids. Cellulose pellicle and 10 mL tea fungus broth showed about 2.3% (w/v) acidity after fermentation for 12 days. In the case of mixing 30 mL tea fungus broth with 200 mL of tea extract, Kombucha showed the lowest production of acids with 1.3% (w/v) acidity. It has been reported that acid production from the fer-

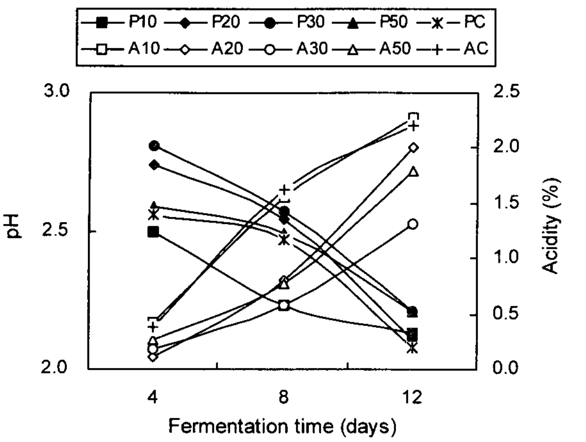

Fig. 3. Changes of pH and acidity in Kombucha fermented by a tea fungus broth or cellulose pellicle. Numbers indicate the amounts of tea fungus broth (mL) added to 200 mL tea extract. P: pH, A: acidity, C: cellulose pellicle as a starter.

mented black tea was reached to 1.5% in 12 days (15). Therefore, the amount of tea fungus may reduce the fermentation time of Kombucha. In Kombucha inoculated with cellulose pellicle as a starter, the cellulose production was higher than tea fungus broth for initial four days (Fig. 4). However, Kombucha fermented with 30 mL tea fungus broth was superior to the cellulose production for longer incubation (Fig. 4). It could be concluded that the cellulose production in Kombucha fermentation was inversely proportional to the acid production, and the amounts of tea fungus broth affected the production of cellulose and acids in Kombucha.

Effect of ethanol

The symbiotic culture of *Acetobacter* sp. and yeasts in a tea fungus efficiently produced acids and cellulose pellicle in Kombucha. During Kombucha fermentation, alcohol produced by indigenous yeast should be consumed by *Acetobacter* sp. to produce acetic acid similar to vinegar production. Yeast in a tea fungus may perform the weak alcohol fermentation, compared to the commercial alcohol fermenting yeast. For instance, Kombucha fermented by a tea fungus produced less than 0.5% (w/v) ethanol in 12 days of fermentation (27). Therefore, the addition of ethanol in Kombucha may allow *Acetobacter* sp. to accelerate the production of acids and cellulose pellicle. The addition of 1% alcohol in Kombucha resulted in the high acid production at an early stage of fermentation (Fig. 5). Acidity of Kombucha attained about 1.0% (w/v) after fermentation for 4 days. By increasing fermentation time, acidity was increased compared to Kombucha without alcohol. Also, addition of 1% ethanol in Kombucha fermentation increased four-times cellulose production in four days (Fig. 6). The thickness of the wet cellulose pellicle was about 2 mm. It has been reported that the production of cellulose was improved in optimized medium containing 1.36% ethanol. Acid production was also enhanced by the addition of ethanol (23). It implies that the addition of ethanol in Kombucha fermentation allowed *Acetobacter* to stimulate the productions of cellulose and acids. However, higher concentration of ethanol inhibited cellulose synthesis (unpublished data). Microbial cellulose membrane produced in Kombucha is suitable for application in the biological and medical fields, owing to their biocompatibility. In conclusion, the productions of acids and cellulose in Kombucha, fermented with green/black tea is greatly dependent upon the ethanol. In addition, initial pH and the amount of starter also influence the production of acids and microbial cellulose.

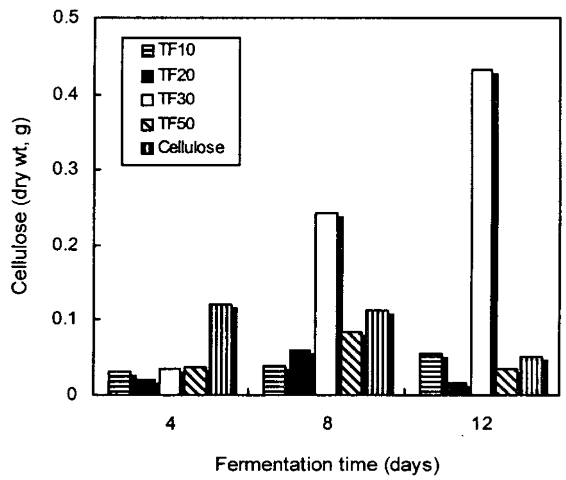

Fig. 4. Production of cellulose from Kombucha fermented by tea fungus broth or cellulose pellicle. Numbers indicate the amounts of tea fungus broth (mL). TF: tea fungus.

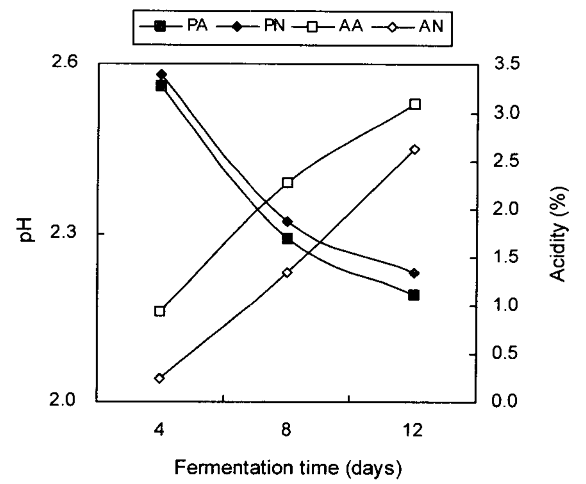

Fig. 5. Effects of ethanol on the acidity and pH in Kombucha fermented by a tea fungus. PA: pH of Kombucha with alcohol, PN: pH of Kombucha without alcohol, AA: acidity of Kombucha with alcohol, AN: acidity of Kombucha without alcohol.

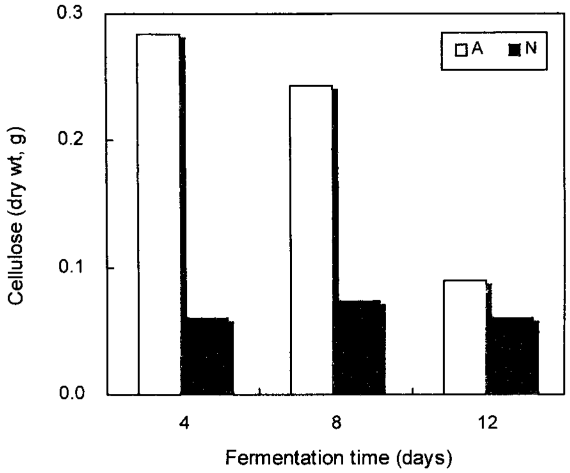

Fig. 6. Effect of ethanol on the cellulose production in Kombucha fermented by a tea fungus. A: ethanol added, N: without ethanol.

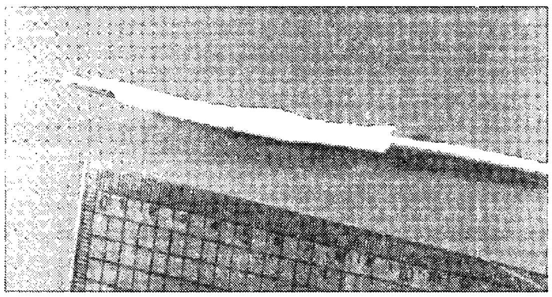

Fig. 7. Tubular form of cellulose pellicle.

Production of tubular cellulose pellicle

To produce the tubular form of cellulose, a cotton thread (2 mm diameter) was used as a support in Kombucha fermentation. A tubular cellulose membrane (90× 6 mm) was synthesized successfully from Kombucha fermented in 12 days (Fig. 7). To be used as a bio-film, it is necessary to evaluate and modify the physical properties of the microbial cellulose pellicle obtained in this manner. It is anticipated that the thickness and diameter of tubular cellulose pellicles can be manipulated using various supports and culture broth.

REFERENCES

1. Dufresne C, Farnworth E. 2000. Tea, kombucha and health: a review. *Food Research International* 33: 409-421.
2. Steinkraus KH, Shapiro KB, Hotchkiss JH, Mortlock RP. 1996. Investigations into the antibiotic activity of tea fungus/ kombucha beverage. *Acta Biotechnologic* 16: 199-206.
3. Kurtzman CP, Robnett CJ, Basehoar-Powers E. 2001. Zygosaccharomyces kombuchaensis, a new ascosporogenous yeast from kombucha tea. *FEMS* 1: 133-138.
4. Blanc PJ. 1996. Characterization of the tea fungus metabolites. *Biotechnology Letters* 18: 139-142.
5. Liu CH, Hsu WH, Lee FL, Liao CC. 1996. The isolation and identification of microbes from a fermented tea beverage. *Food Microbiology* 13: 407.
6. Chen C, Liu BY. 2000. Changes in major components of tea fungus metabolites during prolonged fermentation. *J Applied Microbiology* 89: 834-839.
7. Hartmann AM, Burleson LE, Holmes AK, Geist CR. 2000. Effects of chronic kombucha ingestion on open field behaviors, longevity, appetitive behaviors, and organs in C57-BL/6 mice: A pilot study. *Nutrition* 16: 755-761.
8. Bushman JL. 1998. Green tea and cancer: a review of the literature. *Nutrition and Cancer* 31: 151-159.
9. Hara Y, Luo SJ, Wickremashinghe RL, Yamanishi T. 1995. Uses and benefits of tea. *Food Reviews International* 11: 527-542.
10. Wang H, Provan GJ, Hellivell K. 2000. Tea flavonoids: their functions, utilization and analysis. *Trends in Food Sci Technol* 11: 152.
11. Chu DC, Kobayashi K, Juneja LR, Yamamoto T. 1997. Theanine-its synthesis, isolation, and physiological activity. In *Chemistry and applications of green tea.* Juneja T, Juneja LR, Chu DC, Kim M, eds. CRC Press LLC, Salem. p 129-135.
12. Sreeramulu G, Zhu Y, Knol W. 2001. Characterization of antimicrobial activity in kombucha fermentation. *Acta Biotechnologica* 21: 49-56.
13. Lee SP, Ha YD. 2001. Production and application of functional bacterial cellulose. *Food Industry and Nutrition* 6: 1-9.
14. Ross P, Mayer R, Benziman M. 1991. Cellulose biosynthesis and function in bacteria. *Microbiol Rev* 55: 35-58.
15. Choi MA, Kim JO, Choi KH. 1996. Effects of saccharides and incubation temperature on pH and total acidity of fermented black tea with tea fungus. *Korean J Food Sci Technol* 28: 405-410.
16. Fontana JD, Franco VC, de Souza SJ. Lyra IN. de Souza AM. 1991. Nature of plant stimulators in the production of *Acetobacter xylimum* ("tea fungus") biofilm used in skin therapy. *Appl Biochem Biotechnol* 28: 341-351.
17. Shirai A, Takahashi M, Kaneko H, Nishimura S, Ogawa M, Nishi N, Tokura S. 1994. Biosynthesis of a novel polysaccharide by *Acetobacter xylinum. Int J Biol Macromol* 16: 297-300.
18. Yamanaka S, Ishihara M, Sugiyama J. 2000. Structural modification of bacterial cellulose. *Cellulose* 7: 213-225.
19. Atacador-Ramos M, Azmey MSM, Basuki T, Dahiya DS, Ekmon TD, Ekundayo JA. 1996. Indigenous fermented foods in which ethanol is a major product. In *Handbook of indigenous fermented foods.* Steinkraus KH, ed. Marcel Dekker, Inc. New York. p 486.
20. Fontana JD, de Souza AM, Fontana CK, Torriani IL, Moreschi JC, Gallotti JJ, de Souza SJ, Narcisco GP, Bichara JA, Farah LF. 1990. *Acetobacter* cellulose pellicle as a temporary skin substitute. *Appl Biochem Biotechnol* 24: 253-264.
21. Geyer U, Heinze T, Stein A, Klemm D, Marsch S, Schumann D, Schmauder HP. 1994. Formation, derivatization and applications of bacterial cellulose. *Int J Biol Macromol* 16: 343-347.
22. Klemm D, Schumann D, Udhardt U, Marsch S. 2001. Bacterial synthesized cellulose-artificial blood vessels for microsurgery. *Progress in Polymer Science*, in press.
23. Galas E, Krystynowicz A, Tarabasz-Szymanska L, Pankiewicz T, Rzyska M. 1999. Optimization of the production of bacterial cellulose using multivariable linear regression analysis. *Acta Biotechnologica* 19: 251-260.
24. Amerine MA, Ough CS. 1980. *Methods for analysis of*

musts and wines. A Wiley-Interscience Publication, John Wiley & Sons, New York, p 46.
25. AOAC. 2000. *Official methods of analysis.* Association of official analytical chemists, Washington DC. Chap 32, p 31.
26. Lee SP, Kim CS. 2000. Characterization of Kombucha beverages fermented with various teas and tea fungus. *J Food Sci Nutr* 5: 165-169.
27. Sievers M, Lanini C, Wever A, Schuler-Schmid U, Teuber M. 1995. Balance in a kombucha Microbiology and fermentation cha beverage obtained from a tea fungus fermentation. *System Appl Microbiol* 18: 590-594.
28. Vandamme EJ, Baets SD, Vanbaelen A, Joris K, Wulf PD. 1998. Improved production of bacterial cellulose and its application potential. *Polymer Degradation and Stability* 59: 93-99.

(Received December 13, 2001; Accepted February 5, 2002)