Chapter 9

Kombucha Production and Its Bioactive Compounds Analysis

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Abstract

Kombucha is a fermented beverage obtained from the fermentation of sweetened tea by a consortium of yeasts and bacteria. The beverage contains many bioactive compounds from tea extraction and microbial synthesis. In this chapter, we describe two preparation methods of Kombucha using a symbiotic culture of bacteria and yeast or a synthetic microbial community as a starter. Moreover, the determination of bioactive compounds, including organic acids, sugars, and catechins, has been introduced.

Key words Kombucha, Tea, Yeast, Bacteria, Bioactive compounds

1 Introduction

Kombucha is a functional beverage produced by the fermentation of sugared tea broth with a symbiotic culture of bacteria and yeast [1, 2]. There are many active compounds found in Kombucha [3, 4], such as tea polyphenols, vitamins (B1, B2, B6, B12, and C), organic acids (acetic acid, gluconic acid, glucuronic acid, among others), and D-saccharic-1,4-lactone acid. These active compounds provide Kombucha with potential benefits for human health [4, 5], including antioxidant, antimicrobial, and hepatoprotective effects.

Kombucha is composed mainly of various acetic acid bacteria and yeasts, sometimes containing a little lactic acid [6]. The traditional culture method of Kombucha uses a symbiotic culture of bacteria and yeast (SCOBY) as a starter [7, 8], in which a cellulose film is formed. Still, it is difficult to control the microorganisms. Therefore, more and more researchers use a synthetic microbial community (SMC) as a starter for fine control [9, 10]. In this chapter, the preparation method of Kombucha using SCOBY or

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SMC has been introduced in detail. In addition, the determination of bioactive compounds, including organic acids, sugars, and catechins, has also been described.

2 Materials

2.1 Sugared Tea Broth

The composition of the sugared tea broth included the following materials (g/L) [11]: sucrose, 50–100; tea, 1.5–10.

2.2 Yeast Extract Peptone Dextrose (YPD) Broth

The composition of the YPD broth included the following materials (g/L) [12]: yeast extract, 10; peptone, 20; D-glucose, 20.

2.3 Hestrin-Schramm (HS) Medium

The composition of the HS medium included the following materials (g/L) [13]: glucose, 20; peptone, 5.0; yeast extract, 5.0; disodium phosphate (anhydrous), 2.7; and citric acid (monohydrate), 1.15; and final pH 5.0 ± 0.1.

2.4 De Man Rogosa Sharpe (MRS) Medium

The composition of the MRS medium included the following materials (g/L) [14]: glucose, 20; tryptone peptone, 10; beef extract, 8; yeast extract, 4; sodium acetate, 5; diammonium hydrogen citrate, 2; dipotassium hydrogen phosphate, 2; magnesium sulfate, 0.2; manganous sulfate, 0.05; Tween 80, 1; and final pH 6.5 ± 0.1.

3 Methods

3.1 Preparation Method of Kombucha

1. Preparing the sugared tea broth [15]: Mix 6 g of tea leaves with 100 g of sucrose in 1 L of boiling water and steep for 15 min (*see* **Note 1**). Then, filter the tea leaves and transfer the sugared tea broth into a sterilized glass jar. Cool the sugared tea broth to room temperature (*see* **Note 2**).
2. Inoculating starter cultures (SCOBY (a) or SMC (b)):
   (a) Use a SCOBY (*see* **Notes 3 and 4**) as a starter (Fig. 1). Inoculate 10 g of SCOBY into the glass jar bottle with the sugared tea broth, and seal with sterile gauze.
   (b) Use an SMC as a starter. Inoculate single colonies of yeast, acetic acid bacteria, and lactic acid bacteria into YPD broth, HS medium, and MRS medium (*see* **Note 2**), respectively, and culture at 30 °C with 160 rpm agitation for 24 h, at 30 °C with 160 rpm agitation for 48 h, and at 37 °C with no agitation for 24 h, respectively. Inoculate combinations of the three strains into the glass jar bottle with the sugared tea broth, and seal with sterile gauze.

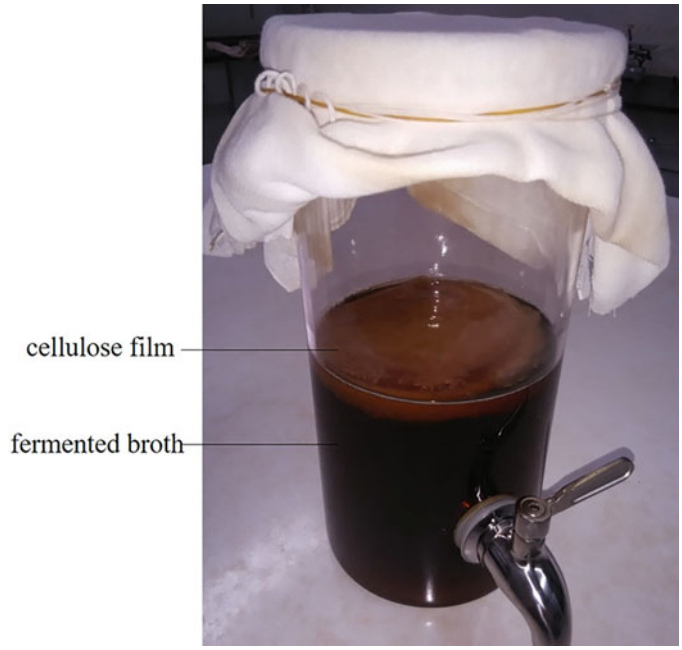

Fig. 1 Kombucha production using a SCOBY as starter

3. Culturing Kombucha: place the Kombucha cultures in an incubator (*see* **Note 5**) at 28 ± 2 °C, and let them stand for 1–2 weeks.
4. Post-fermentation treatment: after the fermentation process, remove the cellulose film (*see* **Note 4**), and collect the fermented broths (*see* **Note 6**).

3.2 Determination of Organic Acids

1. Mobile phase preparation.
   (a) Solvent A: Mix 970 mL of 0.1% phosphate solution and 30 mL of methanol in a 1 L glass bottle, then filter the mixture using a 0.45-μm microporous membrane.
   (b) Solvent B: 500 mL of 100% methanol (HPLC-grade).
2. Sample preparation.
   Centrifuge the fermented broths at 8000 rpm for 10 min, and collect the supernatants (*see* **Note 7**). Then, filter the supernatants using a 0.45-μm microporous membrane.
3. Instrument selection.
   HPLC equipped with a UV-DAD detector and an Agilent ZORBAX® SB-C18 column (4.6 × 150 mm, 5 μm).
4. LC settings [15].
   (a) Detection wavelength: 210 nm.
   (b) Column temperature: 40 °C.

(c) Injection volume: 10 μL.

(d) Flow rate: 1 mL/min.

(e) Gradient program: Maintain solvent A at 100% from 0 to 10 min, linearly ramp to 100% solvent B over 1 min, maintain solvent B at 100% for 5 min, linearly ramp to 100% solvent A over 1 min, and then maintain solvent A at 100% for 5 min.

3.3 Determination of Sugars

1. Mobile phase preparation.

   Solvent A: Mix 700 mL of acetonitrile and 300 mL of water in a 1 L glass bottle, and then filter the mixture using a 0.45-μm microporous membrane.

2. Sample preparation.

   Centrifuge the fermented broths at 8000 rpm for 10 min, and collect the supernatants. Then, filter the supernatants using a 0.45-μm microporous membrane.

3. Instrument selection.

   HPLC equipped with an evaporative light-scattering detector and a Waters XBridge™ Amdie column (4.6 × 150 mm, 5 μm).

4. LC settings [15].

   (a) Column temperature: 40 °C.

   (b) Injection volume: 10 μL.

   (c) Flow rate: 1 mL/min.

   (d) Elution mode: Isocratic mode.

   (e) Detector setting: Set drift tube temperature at 80—90 °C and nitrogen pressure at 350 kPa.

3.4 Determination of Catechins

1. Mobile phase preparation.

   (a) Solvent A: Add 90 mL acetonitrile, 20 mL acetic acid, and 2 mL EDTA-2Na solution (10 mg/mL) into a 1 L volumetric flask, add water to make a final volume of 1 L, and then filter the mixture using a 0.45-μm microporous membrane.

   (b) Solvent B: Add 800 mL acetonitrile, 20 mL acetic acid, and 2 mL EDTA-2Na solution (10 mg/mL) into a 1 L volumetric flask, add water to make a final volume of 1 L, and then filter the mixture using a 0.45-μm microporous membrane.

2. Sample preparation.

   Centrifuge the fermented broths at 8000 rpm for 10 min, and collect the supernatants. Then, filter the supernatants using a 0.45-μm microporous membrane.

3. Instrument selection.

   HPLC equipped with a UV-DAD detector and a Waters Symmetry C18 column (4.6 × 250 mm, 5 μm).

4. LC Settings [16].

   (a) Detection wavelength: 278 nm.

   (b) Column temperature: 35 °C.

   (c) Injection volume: 10 μL.

   (d) Flow rate: 1 mL/min.

   (e) Gradient program: Maintain solvent A at 100% from 0 to 10 min, linearly ramp to 68% solvent A and 32% solvent B over 15 min, maintain solvent A at 68% and solvent B at 32% for 10 min, linearly ramp to 100% solvent A over 1 min, and then maintain solvent A at 100% for 5 min.

4 Notes

1. The tea types used in Kombucha production are commonly black tea or green tea. Therefore, the time for extracting tea juice with boiling water should not be too long, which can avoid the flavor deterioration of tea juice.
2. All the media mentioned in "2 Materials" should be sterilized and cooled to room temperature before inoculation.
3. Starter cultures, SCOBY in particular, should not be contaminated by undesired microbes. If any mildew is found, the cultures should be discarded.
4. The SCOBY could be stored in a tea base liquid mixture. This can be kept at room temperature for up to 3 weeks, but for more extended storage, the SCOBY should be placed in the refrigerator at 4 °C.
5. Because vitamins in Kombucha are easily degraded under light, Kombucha should be cultivated in clean and dark conditions.
6. When the fermentation time is too long, the acidity of Kombucha might be too high to drink directly. Therefore, it can be appropriately diluted and mixed with sugar or honey before drinking.
7. When the organic acids of Kombucha are determined, the fermented broths should be diluted at a suitable multiple. It is due to the high concentration of organic acids in Kombucha.

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