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Kombucha Production by Combinations of Black Tea and Apple Juice

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Abstract

This work aims to study the use of apple juice combined with black tea to produce Kombucha beverage, a tea-fermented beverage (AT-Kombucha) and to access its chemical and microbiological quality for food safety. After fermentation, AT-Kombucha was pasteurized and then filter sterilized. Predominant chemical compounds and microbial detection in AT Kombucha were analyzed. The results demonstrated that using apple juice as the culture substrate together with black tea to ferment AT Kombucha improved total phenols content. For the food safety view point, there was no foodborne pathogens detected followed the Notification of Thai Ministry of Public Health No. 356 (2013) for beverages in sealed container. Thus, the methods of fermentation and sterilization used in this study were proved to safe from biological hazard. In addition, there is a potential for commercial production of AT-Kombucha using the method and equipments in this study. In Thailand beverage market, there is no Kombucha product which fermented using fruit juice combined with black tea for increasing flavor and health-promoting.

Keywords: Kombucha, Fermentation, Healthy beverage, Apple juice, Total phenols, Tea

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1. Introduction

Kombucha is a famous tea-fermented beverage with a history of several thousand years in the East Asia and becoming popular in Europe since World War II [1, 2, 3, 4, 5]. The taste of this healthy beverage is slightly sweet and carbonated acidic. Some authors reported that it tastes like mild vinegar [1, 6]. In Thailand, Kombucha is still not well known while ready-to-drink tea products had market values around 16,053 million Baht in 2013 and the growth values of this product approximates 30.8% over the last 5 years [7]. There is only one commercial band of Kombucha production in domestic Market which is "madi kombucha". So, it has a market chance for developing this product in terms of improving flavor, enhancing health benefit compounds, optimizing the process of fermentation, and studying about consumer behavior, and etc.

Conventionally, Kombucha is produced by using black tea and sugar as a substrates. A tea fungus which is a symbiotic association of bacteria and yeasts forming is used as a culture for fermentation under aerobic condition at room temperature for about 14-18 days [4, 5, 8]. This fermentation process results in the formation of cellulose pellicles floating at the surface of the growth medium [9, 10, 11,12].

It has been reported that the main compounds in Kombucha beverage are acetic, lactic, gluconic and glucuronic acids, ethanol, glycerol, and B-complex vitamins [8, 13, 14]. This beverage provides lots of benefits on human health. Many researches exposed that this beverage assists digestion, reduces arthritis and gout symptom, inhibits microbial infection, and also resists stress and cancer. [1, 2, 8, 15, 16]. In addition, several authors revealed that the antioxidant properties in Kombucha mainly polyphenols come from tea, especially catechins [17, 18]. Green tea is a better source of antioxidant substances, especially catechins compared to black tea [19]. However, black tea affords to the unique taste and flavor of Kombucha [19]. Then, to enhance the antioxidant properties of Kombucha, other substrates such as fruits, vegetables or herbs might be added into Kombucha fermentation process. Recent works demonstrated that phenolics content in traditional Kombucha was increased when supplemented with wheatgrass juice [19] and application of molasses from sugar beet in Kombucha fermentation produce more lactic acid than the traditional one.

Apple juice is a broadly consumed. It is a rich source of phytochemicals including quercetin, catechin, phloridzin, chlorogenic acid [19], phenolic compounds mainly in terms of flavonoids [20]. All of them provide strong antioxidant properties [17, 19]. In addition, it contains a variety of vitamins and mineral which has a lot of advantageous effects on health in terms of suppressing cancer cell proliferation, reducing lipid oxidation, and cholesterol [19, 20].

Thus, this work aims to study the use of apple juice together with black tea as substrates to produce Kombucha beverage in order to improve

its quality and assess foodborne pathogens for commercial production development.

2. Materials and Experiment

2.1 Kombucha Fermentation

The composition of raw materials for Kombucha fermentation was presented in Figure 1. A black tea extracts was prepared from 5-6 g tea leaf/1 L of water. The initial total soluble solid (TSS) of tea extracts was measured approximately 0.2 Brix (°B). Apple juice (TSS=12°B) was added into tea extract about 15% (v/v) of tea extract. 50% (w/v) sucrose syrup was added into the mixed solution. In addition, 20 mL/L vinegar (5% acetic, 1.25 g acid) was put in to the mixed solution. Then, 3% (w/v) of starter culture, local tea fungus was inoculated in to the apple juice-tea extracts (AT) broth. The Kombucha fermentation was performed at room temperature for 10 days.

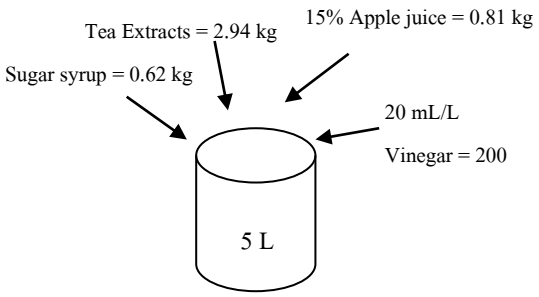

Figure 1 Schematic of raw material compositions for AT Kombucha fermentation

2.2 AT Kombucha Sterilization

After the end of the fermentation, AT Kombucha was pasteurized at 67-70 °C, for 10 minutes. After cooling down, it was sterilized using filter press (Figure 2). The rough filter is K150. The second filter is 7 EKS plate filters (20 × 20 cm) for removing yeast and bacteria cells.

Figure 2 Kombucha sterilization using filter press

2.3 Chemical Analysis

2.2.1 Determination of Titratable acidity

Total acids content was analyzed by the volumetric method with the standard solution of sodium hydroxide (0.1 N). Acetic acid was calculated from the equation (1)

Titratable acidity (% Acetic acid) = (L of NaOH used) x (0.1 N NaOH) x MW (60.05) / (grams of sample) x 100 (1)

2.2.2 Determination of Total polyphenol content

Total polyphenol was determined using Folin-Ciocalteu colorimetric method as described in Singleton and Rossi, 1965 [22]. The total polyphenol was expressed as gallic acid equivalents (GAE, mg/mL) from the calibration curve. (as gallic acid)

2.2.3 Determination of Alcohol content

Alcohol content was analyzed based on method of Gunduz et al., 2013 [23].

2.3 Biological Analysis

After fermentation and pasteurization, AT Kombucha was tested the presence of foodborne pathogens, yeast, and mold. Coliforms and *Escherichia coli* were examined using standard methods for examination of water and waste water [24]. *Bacillus cereus* [25], *Clostridium perfringens* [26], *Staphylococcus aureus* [27], yeast and mold [28] were inspected based on Bacteriological Analytical Manual, BAM. *Listeria monocytogenes* and *Salmonella* spp. were analyzed following the ISO 11290-1:1996, Amd 1: 2004 and ISO 6579: 2002, respectively [29, 30].

3. Results and Discussion

3.1 Chemical Composition of AT Kombucha

After 10 days fermentation, AT Kombucha was sterilized. Chemical compounds in AT Kombucha were then analyzed as shown in Table 1. The major antioxidant component, total polyphenols, was illustrsted as gallic acid equivalent (GAE) approximately 0.75 mg GAE/mL. Previous study proved that the total phenolics content in Kombucha statistically increased compared to the unfermented tea [31]. Furthermore, the longer fermentation time, the more production of phenolics compound. The explanation of enhancing in total phenols compounds during Kombucha fermentation process was elucidated that the microorganisms in tea fungus have capability to release polyphenol compounds by healing phytase enzyme [32]. However, the more extended time of fermentation than a month can lead to a reduction in polyphenols concentration [31, 33].

This total polyphenols content in AT Kombucha was higher than traditional Kombucha which made from fermented tea [34]. In addition the traditional Kombucha took longer fermentation time to reach that phenolics content compared to AT Kombucha. The improvement of total phenolics content in AT Kombucha possibly comes from apple juice which generally contains gallic acid and other antioxidant components [19, 20].

Acetic acid and alcohol in Kombucha come from metabolic activities of microorganisms in tea fungus, yeast and bacteria [1]. Yeast hydrolyzes sucrose into glucose and fructose, then produce ethanol alcohol. Acetic acid bacteria utilize alcohol to generate acetic acid. The acetic acid content in AT Kombucha was 4.03% which much higher than tradition Kombucha within the range of 0.5-1% [1]. Likewise, the analysis of alcohol content in AT Kombucha was estimated 1.68% (v/v) which was higher concentration than 1% recommended by Center for Disease Control (CDC) [35]. Further improvement might be performed by optimizing fermentation time in order to decrease acetic acid and alcohol contents in Kombucha.

3.2 Microbial Detection in AT Kombucha

From the food safety point of view, pathogenic bacteria have a highest risk to

contaminate Kombucha after the tea was cooled until fermented tea reached the pH lower than 4.2 [35]. In this work, foodborne pathogens including yeast and mold were determined in AT Kombucha. The examined results were presented in Table 2. No pathogens were found or not detected in AT Kombucha using standard methods as described in 2.3. For Coliforms, it was detected < 1.1 MPN/100 mL. This corresponded to the Notification of Thai Ministry of Public Health No. 356 (2013) for beverages in sealed container which allowed the presence of Coliforms < 2.2 MPN/100 mL [36].

Table 1 Some of compounds present in apple juice-tea extracts (AT) Kombucha.

Compounds	Content
Total phenol	0.75 mg GAE/mL
Titratable Acidity (Acetic acid)	4.02%
Alcohol	1.68 % (v/v)

Table 2 Biological testing of AT Kombucha.

Microorganisms	Testing results
Coliforms, MPN/100 mL	< 1.1
Escherichia coli/100 mL	Not detect
Bacillus cereus, CFU/mL	< 1 (None)
Clostridium perfringens, CFU/mL	< 1 (None)
Listeria monocytogenes / 25 mL	Not detect
Salmonella spp. / 25 mL	Not detect
Staphylococcus aureus / 0.1 mL	Not detect
Yeast and Mold, CFU/mL	< 1 (None)

4. Conclusions

This study demonstrated that Kombucha production using apple juice combined with black tea as culture substrates improved the antioxidant property of the beverage. However, alcohol and acetic acid contents were too high. Further improvement should focus on fermentation process optimization. For the food safety view point, it was not detected any foodborne pathogens in AT Kombucha So, the methods of fermentation and sterilization using in this work were verified.

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