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PRELIMINARY RESEARCH ON THE PRODUCTION PROCESS OF KOMBUCHA TEA FROM THE FRUIT BODY AND THE SOLID MEDIUM OF *CORDYCEPS MILITARIS* IN VIETNAM

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Abstract

The study was conducted with the aim of determining the factors affecting the fermentation of Kombucha tea (F1) in order to diversify Kombucha tea fermentation products from Cordyceps militaris, including the fruit body and the solid medium, has not been widely applied. The surveys included fruit body and solid medium content, sugar content, amount of SCOBY starter culture, and fermentation duration. The results of sensory evaluation of the product showed that the most suitable conditions in this study were 2g fruiting body or solid medium, sugar content 12.5 % (w/v) supplemented with 10% supplemented with 10% (v/v) of starter cultures solution Scoby with pH of 3.237 and 3.02, respectively, % Brix of 12.07% B and 10.6 % B respectively, and fermentation for 7 days and hours at room temperature achieved the highest sensory results. This is the first Kombucha tea product that has been successfully researched and produced on a laboratory scale from the fruiting body and the substrate of Cordycepin militaris in Vietnam.

Key words: Kombucha, Cordyceps militaris, fruit body, solid medium, SCOBY starter, fermentation duration, fermented tea product

1. Introduction

Kombucha tea is a beverage fermented by a symbiotic microflora between yeast and bacteria on tea water supplemented with sugar that has many health benefits [1]. Vietnam is a country with a tropical climate, so the demand for soft drinks is huge. Low-alcohol fermented water product is a new products with high nutritional value and health benefits. The alcohol content is not high in the product, which stimulates digestion, making the meal delicious without causing intoxication like some other alcoholic beverages. The combination of tea fermentation has created a kombucha drink with all the advantages of ingredients and properties. Some of the uses of Kombucha have been scientifically proven such as: lowering cholesterol, avoiding atherosclerosis, lowering blood pressure as well as purifying toxins in the body [2]. Besides, the need to use herbs of natural origin to prevent and treat diseases is becoming more and more popular, including cordyceps, an herb with miraculous medicinal values. Cordyceps, scientifically known as Cordyceps militaris, has many medicinal values and is widely used in functional foods and pharmaceuticals because it contains many biologically active compounds, including cordycepin. 3'-deoxyadenosine) - has been shown to have anti-tumor, antioxidant, antibacterial, antifungal, tumor cell lines, anti-inflammatory, and anti-angiogenic activities [3]. In addition to fruit body as the main product, the solid medium is also considered valuable due to the high content of the bioactive compounds Cordycepin. In our country Kombucha has not been industrially produced but is only a product made by people, so the product is unstable and often contaminated with mold, bacteria or wild yeast, which affects the product quality. Therefore, it is essential to create commercial Kombucha products of consistent quality. A kombucha drink that can be developed and commercialized requires a lot of laboratory research. This paper studies the factors affecting the main fermentation of F1 Kombucha from fruit body and solid medium of Cordyceps militaris.

2. Research materials and methods

2.1 Materials

- Fruit body and solid medium of Cordyceps militaris.

- Scoby Organic stay Tam Dao starter cultures solution with yeast concentration (1,1 $\times$ 105 CFU/mL), bacteria (3 $\times$ 102 CFU/mL).

- Bien Hoa natural golden sugar.

2.2 Experimental method

2.2.1 Fermentation process Experiment with fruit body fermented beverage following these steps (same process as solid medium): Ingredients Preliminary preparation of ingredients (separation of fruit body and solid medium) → Boil water → Tea extraction → Filter → Stir in sugar → Cool → Add starter cultures solution and Scoby mushrooms → Fermentation → Filter → Pasteurization → Product .

2.2.2 Experimental layout

Conduct experiments with cordyceps fruit body and solid media and repeat 3 times.

Experiment 1: Investigate the material content of Kombucha tea fermented extracts.

- Fixed factor: extracted by ultrasonic wave method in 60 minutes – 65^0C.

- Starter cultures solution Scoby 10% by volume (Scoby 5% w/v). Fermentation for 7 days at room temperature. - Variable factor: Content of extracted ingredients 1g, 2g, 3g in 200ml of water.

- Monitoring parameters: sensory.

Experiment 2: Investigate factors affecting Kombucha tea fermentation

• Effect of sugar content

- Fixed factor: the optimal parameters were investigated in the above experiment.

- Variable factor: sugar content in proportion: 10%, 12.5%, 15% (w/v) respectively.

- Monitoring parameters: pH, % Brix, sensory.

• Effect of starter cultures solution Scoby

- Fixed factor: the optimal parameters were investigated in the above experiments.

- Variable factor: starter cultures solution Scoby content 5% (w/v): 5%, 10%, 15%(v)

- Monitoring parameters: pH, %Brix, sensory.

• Effect of fermentation time

- Fixed factor: The optimal parameters were investigated in the above experiments.

- Fixed factor: The optimal parameters were investigated in the above experiments.

- Variable factor: Fermentation time: 5, 7, 9 (days).

- Monitoring parameters: pH, % Brix, sensory.

3. Analytical method

3.1 Determination of pH

Use a Hanna pH meter.

3.2 Determination of % Brix

Using Alpha Atago refractometer.

3.3 Sensory assessment

In scoring methods, the senses rely on assessment to give a score on a certain scale. Based on TCVN 3215 - 79. This standard uses a 20-point system built on a unified scale with 6 steps (0 to 5) and gives 5 as the highest score of the standard. When evaluating samples, testers base on the recorded results and compare them with the table describing these criteria.

3.4 Data processing

Processing statistics, using MS.Excel 2007 software.

4 Results and discussion

4.1. Effect of the percentage of extracted materials during fermentation

The amount of ingredients affects the state, color, and taste of the product. Almost every Kombucha product on the market uses black and green tea, so using the fruit body and growing media of Cordyceps to create a novel product. The effect of the number of ingredients also results in different sensory evaluations. Sensory results are one of the important criteria to evaluate product quality and decide whether the product can be commercialized or not. With fermentation conditions is room temperature: The temperature fluctuates in the range of 25^0C – 27^0C. The sensory results of the experimental samples according to fermentation time are shown in Figure 1.

Sensory evaluation according to the method of scoring consumer tastes with 10 testers and results. The color, smell, and taste of the surveyed products varied with the weight of the ingredients (Figure 1). After fermentation, the product in the samples (1g of raw material) has a light yellow color, clear, very light aroma, difficult to perceive but no strange smell, and slightly sour taste makes the taste less harmonious, less stored. The taste of the product sample with the material and water ratio of 2:200 (g/mL) was rated the highest

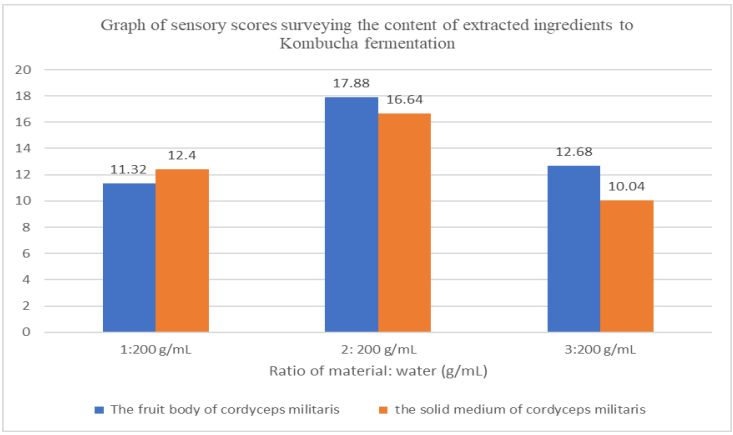

Figure 1: Effect of the percentage of extracted materials during fermentation

score and was significantly different from the other experiment. The sweetness of sugar and the aroma of Cordyceps are harmonized with the mild sourness of acids, the slightly acrid taste of tannins, the transparent water without turbidity, and the small foreign bodies. The color is completely specific to the product. Therefore, the amount of tea (2 g) that was evaluated for sensory evaluation with a high score, a taste suitable for consumers, and a statistically significant difference in the 95% confidence interval was selected as a parameter for further experiments follow.

4.2. Factors affecting Kombucha tea fermentation

4.2.1 Sugar content

The nutritional environment of microorganisms includes many different essential nutrients, this nutrient composition depends on the essential needs of each microbial species. The main element that is indispensable in the growth of organisms, in general, is a source of carbon and in beverage products, the main source of carbon used is sucrose. Carbohydrates provide the nutritional requirements for yeast growth and metabolism [4]. Sugar is an essential substrate for fermentation, so it greatly affects the efficiency of fermentation. During growth and development, microorganisms are affected by sugar content. The fermentation process was investigated for sugar content at the values of 10%, and 15% (w/v) Fermentation time for 7 days, at room temperature, the material content of 2g extracted in 200ml boiling water with assisted extraction by ultrasonic bath for 60 min at 65^0C. The results of the fermentation are shown in Figures 2 and 3.

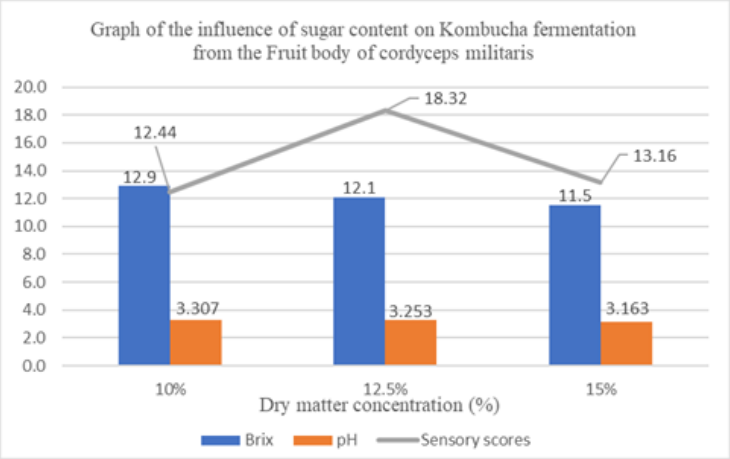

Figure 2: Effect of sugar content on sensory quality of kombucha tea products from fruit bodies

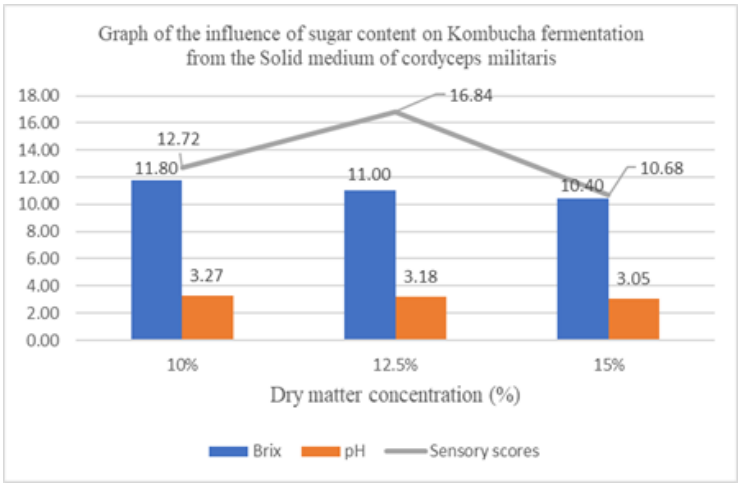

Figure 3: Effect of sugar content on sensory quality of kombucha tea products from solid medium

In the process of symbiotic fermentation of bacteria, yeast will use the substrate in the environment and metabolize it to create mostly acidic products, so the pH of the medium will decrease after fermentation. Based on Figures 2 and 3, it is shown that the initial sugar change has a great influence on pH and % Brix. This proves that there is an activity using the substrate of yeast. According to Larpent (1991), about 10% of sugar is used for biomass growth, the rest is used to convert to ethyl alcohol and other by-products such as glycerol, and pyruvate. In addition, a sensory evaluation showed that, under the same fermentation conditions, the sugar content was different. At 12.5% (w/v) sugar content, all were rated better, and taste, color, and clarity preferences also differed significantly from products at 10% and 15% (w/v). Thus, to promote the fermentation process with the highest efficiency and highest sensitivity, we chose to add sugar content at the rate of 12.5% (w/v) to carry out the next experiments.

4.2.2 Starter cultures solution Scoby

Based on the results obtained in Figure 4 and Figure 5, we found that the change in the percentage of starter cultures added affected the quality of Kombucha tea. Increasing the percentage of starter cultures leads to a decrease in pH and Brix of the post-fermentation solution. In the percentages of starter cultures additions surveyed, the experiment added 15% for the product with the lowest pH (2.597) and OBrix (9.433).

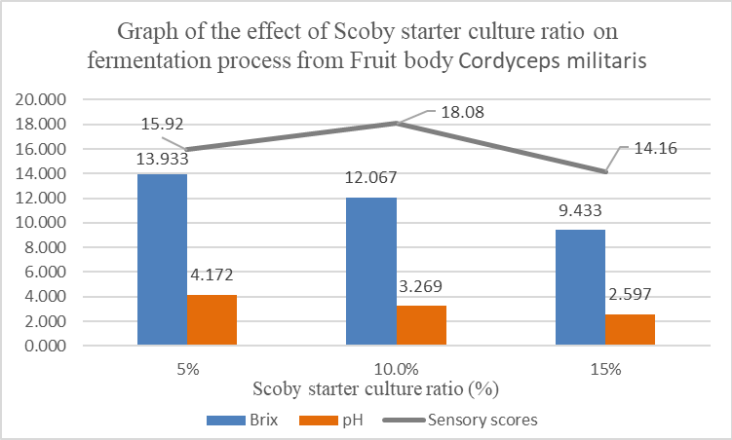

Figure 4: Effect of starter cultures of Scoby content on sensory quality of kombucha tea products from fruit bodies

Looking at the results of sensory evaluation of the product (Figure 4 and Figure 5), it was found that the samples that received the preference in terms of taste, color, and clarity were samples with 12.5% of the starter cultures. In

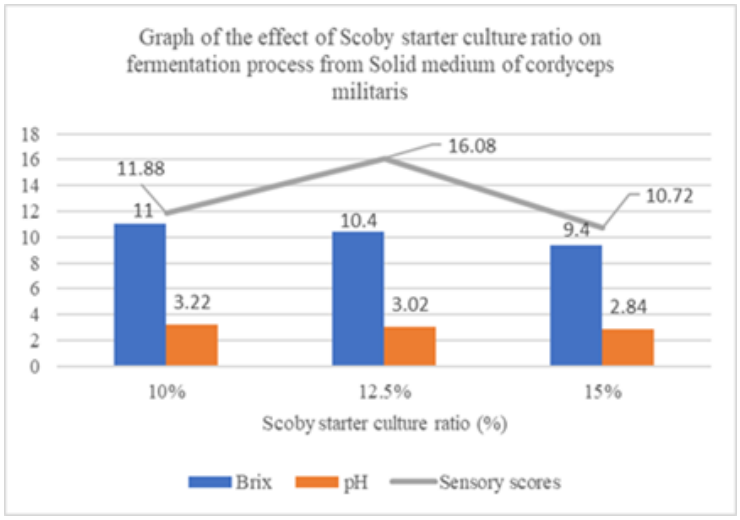

Figure 5: Effect of starter cultures of Scoby content on sensory quality of kombucha tea products from solid medium

the sample with 10% and 15% of the starter cultures solution, the product is less preferred than the other sample. The rate of 5% of the starter cultures solution gives a mild and moderate sour taste, color, and clarity at satisfactory levels. However, when compared with the sample with a ratio of 10% of the starter cultures solution, the taste buds of this sample were judged to have the most suitable sour taste, with a sweet and sour aftertaste, the transparency was still opaque and the sediment was characteristic of fermented tea water and the characteristic yellow color of Cordyceps tea makes a difference from other products on the market. While at the rate of 15%, the post-fermentation product has a strong taste due to the high alcohol concentration, the strong sour taste, and the pungent odor causing discomfort to the senses. Experimental results investigated the effect of a low percentage of the starter cultures solution on slowing down the fermentation process and reducing alcohol production [5]. These batches of fermentation often give poor efficiency and poor quality of the finished product. However, if too much bait tea is added, the fermentation speed is fast at first, but later it can hinder the next fermentation process [6]. Moreover, using the starter cultures solution with a dense density, the propagation time is longer and more expensive. Based on the results of physical, chemical, and sensory indicators, we chose the ratio of the starter cultures solution to be 12.5% (v/v), for the next survey.

4.2.3 Fermentation time According to the results of Figure 6 and Figure 7,

Brix and pH decreased sharply with increasing fermentation time.

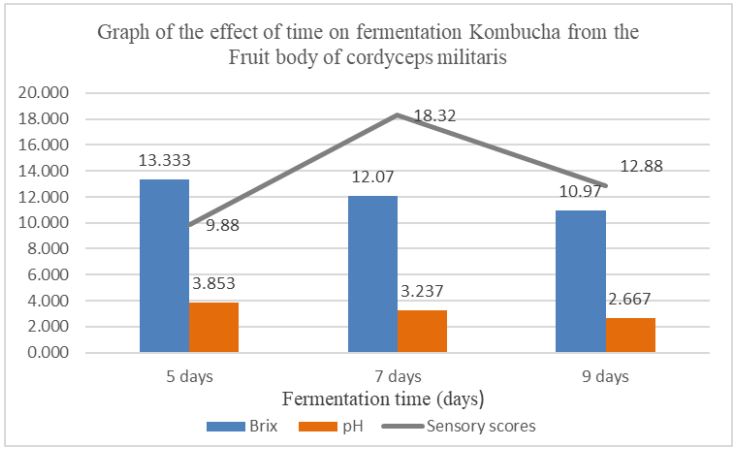

Figure 6: Effect of fermentation time on sensory quality of kombucha tea products from fruit body

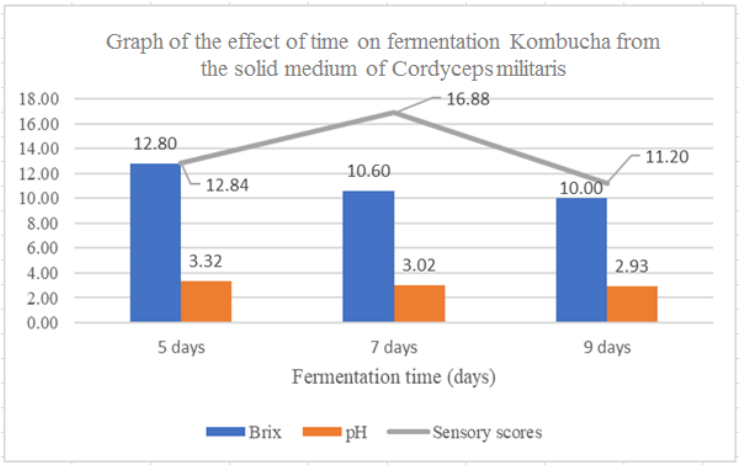

Figure 7: Effect of fermentation time on sensory quality of kombucha tea products from solid medium.

However, after the 7th day of fermentation in the product from the fruit body, Brix and pH decreased faster than the product from the solid medium. From the 7th to 9th day, the Brix of the product from the solid medium changed insignificantly. In contrast, the product from the fruit body decreased sharply.

This shows that fermentation time significantly affects Kombucha tea fermentation. If it continues for a long time, it corresponds to the growth and development stage of microorganisms due to entering the stage of decline. The fermentation medium at this stage has high acidity, low pH is not suitable for yeast growth, and poor metabolism of substances. According to sensory evaluation results, for Kombucha tea fermented from fruit body, the sample fermentation time of 5 days is

not enough sour taste, the product sample is preferred at the fermentation time of 7 days and 9 days. In particular, the product is fermented for 7 days, harmonious taste, suitable for many types of consumers. For the product fermented from solid medium, the 5 days, 7 days sample has a higher preference than the 9 days sample. However, the difference between 5 days and 9 days is not great. The change is because the longer the fermentation time, the less substrate in the fermentation process, the microorganisms start at the end of the equilibrium period, competing for nutrients, along with the fermentation by-products. yeasts, specifically organic acids that inhibit microbial growth, reduce the pH and also the soluble solids concentration of the finished product. Besides, the short fermentation time will make the product not achieve the characteristic properties such as color, taste, metabolites and by-products. Through sensory evaluation by the method of scoring consumer tastes with 10 testers, the results show that the fermented sample at 7 days is rated the highest score and shows a significant difference with the remaining samples.

5. Conclusion

The experimental results of fermented beverage from the fruit body and solid medium of Cordycep militaris initially showed that the suitable fermentation conditions were: 2g of fruit body or solid medium of Cordyceps, 12.5% sugar content, supplemented with 10% of starter cultures solution Scoby and fermentation for 7 days at room temperature gives the product the best organoleptic quality. This is the first Kombucha tea product that has been successfully researched and produced on a laboratory scale from the fruiting body and the substrate of Cordycepin militaris in Vietnam

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