

Production and characterization of a novel beverage from *Artemisia selengensis* Turcz juice through fermentation with kombucha consortium

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Abstract

The fermentation beverage was made by adding kombucha SCOBY into *Artemisia selengensis* Turcz juice, black tea and sugar water as raw materials. The single factor test was carried out by selecting inoculation amount, sugar amount, fermentation time and fermentation temperature. The optimal fermentation conditions were determined by orthogonal experiment design method, with total sugar, total acidity and sensory evaluation as indexes. The results showed that the optimal fermentation conditions were 6.0% kombucha SCOBY inoculation amount, 10.0% sugar content, 30 °C fermentation temperature and 168h fermentation period. The total sugar and acid of kombucha was 2.98 g/100mL and 3.66 g/100mL, respectively. The new fermentation beverage with bright color, sweet and sour taste and special aroma of artemisia was prepared.

Keywords

Kombucha; *Artemisia selengensis* Turcz; Fermented beverage.

1. Introduction

Kombucha is fermented from sugar and tea by a symbiotic culture of bacteria and yeast (SCOBY). There are some tea extracts, microbe and their metabolites in the kombucha, which mainly include gluconic acid, D-gluconedioate-1, 4-lactone, glucose, protein, amino acids, vitamins, trace elements, tea polyphenols, caffeine and so on. Therefore, kombucha has the function of reducing blood fat, cholesterol and protecting liver[1-3].

Artemisia selengensis Turcz is a perennial herb with a special fragrance, which is widely distributed in lakes and wetlands in the Yangtze river basin in China. *Artemisia* is not only delicious, but also has high medicinal value [4,5]. *Artemisia* contains crude protein 3.91%, crude fat 0.91%, soluble carbohydrates 60.11%, crude fiber 3.72%, and mineral 2.01%. *Artemisia* is not only nutritious, but also a rare green wild vegetable that is far from the industrial pollution area.

With the progress of society and the improvement of the quality of life, people have a stronger pursuit of healthy diet, and consumers' behavior on food choices has changed. Nowadays, products containing probiotics are getting attention. Fermentation is a biotechnology that helps preserve food, improve its nutritional value and enhance its sensory properties. Combined with the production method of folk kombucha, it was applied to *Artemisia selengensis* Turcz juice. Through the introduction of SCOBY for fermentation, the biological active substances in *Artemisia Selengensis* Turcz were integrated into the beverage. Through the optimization of fermentation process conditions,

the fermented beverage was sour, sweet and refreshing. It not only has the special aroma of tea and *Artemisia selengensis* Turcz, but also contains rich nutrients, which provides technical reference for developing and utilizing *Artemisia selengensis* Turcz resources and processing them into functional drinks with high added value.

2. Materials and methods

2.1 Preparation of artemisia juice

Wash artemisia and separate its stems and leaves. Cut the stem section of artemisia, add water to beat pulp in the ratio of 1:1, beat pulp with plant masher, filter with gauze, and take its juice. Artemisia leaves were added with water in the ratio of 1:2, then the pulp was beat with a plant blender and filtered with gauze. Artemisia juice was prepared by mixing the stem juice and the leaf juice of *artemisia annua* with the ratio of 3:1, and the sugar was 4.5°Brix. The juice was stored in the refrigerator at 4°C for later use.

2.2 Single factor test of fermentation

2.2.1 SCOBY inoculum size

The SCOBY inoculum size was tested as 2.0%, 4.0%, 6.0%, 8.0% and 10.0%, and 200mL *Artemisia selengensis* Turcz juice was taken and fermented for 168h at 25°C with 10.0% sugar.

2.2.2 The initial amount of sucrose

The initial sugar dosage was set as 8.0%, 10.0%, 12.0%, 14.0% and 16.0% for fermentation. 200mL *Artemisia selengensis* Turcz juice was taken and fermented for 168h at the SCOBY inoculum size of 6.0% and fermentation temperature of 25°C.

2.2.3 Fermentation temperature

Under the conditions of 6.0% SCOBY inoculum size and 10.0% sugar addition, 200mL *Artemisia selengensis* Turcz juice was cultured at 5 levels of 15,20,25,30 and 35°C, and fermented for 168h.

2.2.4 Fermentation period

The fermentation period was 96, 120, 144, 168, and 192 h, 200 mL of *Artemisia selengensis* Turcz juice was taken, and the inoculation amount was 6.0%, the amount of sugar was 10.0% and the fermentation temperature was 25°C.

2.3 Orthogonal experiment of fermentation parameters

The optimal fermentation conditions of *Artemisia selengensis* Turcz juice kombucha were determined by orthogonal test at three levels, including the amount of kombucha bacteria, the amount of sugar, the fermentation temperature and the fermentation time.

2.4 Determination of total acid in beverages

Refer to GB/T 15038-2006 for the specific determination method of total acid in *Artemisia selengensis* Turcz juice fermented beverage. The test was repeated for 3 times for each sample and its average value was taken for subsequent calculation. The acid concentration in the sample is calculated according to Equation (1).

$$X=cVK/m \times V_0/V_1 \times 100 \quad (1)$$

In the Equation (1), X is the total acidity of the sample, g/100 mL; m is sample mass, g; V is the volume of NaOH standard solution consumed by titration, mL; c is the concentration of NaOH standard solution, mol/L; V₀ is the total diluent volume of the sample, mL; V₁ is the volume of sample liquid absorbed during titration, mL; K is the coefficient of conversion to the major acid.

2.5 Determination of total sugar in beverages

Refer to the direct titration method in GB/ T5009.7-2008 for the determination of sugar content in *artemisia scoparia* kombucha fermented beverage. The test was repeated for 3 times for each sample

and its average value was taken for subsequent calculation. The sugar content in the sample is calculated according to Equation (2).

$$X=(V_0-V)\times c\times n\times 1000/5 \quad (2)$$

In the Equation (2), X is the sugar content in the sample, g/L; V₀ is the product of Portuguese standard liquid consumed in blank test, mL; V is the volume of standard Portuguese liquid consumed during sample determination, mL; c is the concentration of Portuguese standard liquid, g/mL; n is the dilution concentration of the sample.

3. Results

3.1 Effects of SCOBY inoculum size on sugar and acid contents of *Artemisia selengensis* Turcz juice fermented beverage

Under the conditions of 10.0% sugar content, 25°C fermentation temperature and 168 h fermentation, the sugar content and total acid content of the fermented beverage were taken as the judging indexes and supplemented by sensory evaluation to determine the optimal conditions of inoculation amount. The results are shown in Fig. 1. With the increase of inoculation amount, the total acid content in fermented beverage increased with the decrease of sugar content. When the inoculation amount was 4.0%~8.0%, the change trend of sugar content and total acid content tended to be gentle. It may be that the amount of kombucha liquid inoculation is too large in this range, reaching a stable period.

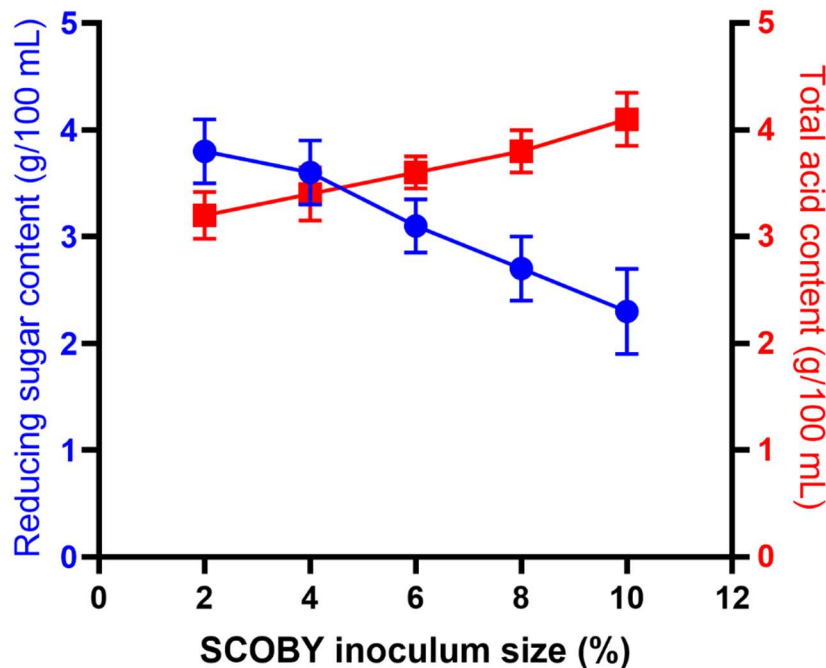


Fig. 1 Effects of SCOBY inoculum size on sugar and acid contents of *Artemisia selengensis* Turcz juice fermented beverage

According to sensory evaluation, the fermented beverage at 4.0%,6.0% and 8.0% had moderate sour and sweet taste, light mellow flavor and suitable taste. The fermented beverage at 2% had poor smell, and the fermented beverage at 10% was sour. Therefore, the optimal dose of SCOBY inoculation was 4.0%, 6.0% and 8.0%, the corresponding reducing sugar content was 3.62, 3.15 and 2.79 g/100mL, and the total acid content was 3.41, 3.65 and 3.89 g/100mL, respectively and the taste of the beverage was acceptable.

3.2 Effects of fermentation period on sugar and acid contents of *Artemisia selengensis* Turcz juice fermented beverage

Under the condition of 6.0% inoculation amount, 10.0% sugar addition and 25°C fermentation temperature. The optimal time conditions can be determined by taking the sugar content and total acid content in fermented beverage as the judging indexes and supplemented by sensory evaluation. The results are shown in Fig. 2. With the increase of fermentation time, SCOBY would consume oligosaccharides in *Artemisia selengensis* Turcz juice in the fermentation process, so the total acid content in the fermented beverage increased with the decrease of sugar content. When the fermentation time was 120-168h, the change trend of sugar content and total acid content tended to be gentle, which may be the lactobacillus reached a stable period in the long range of fermentation time.

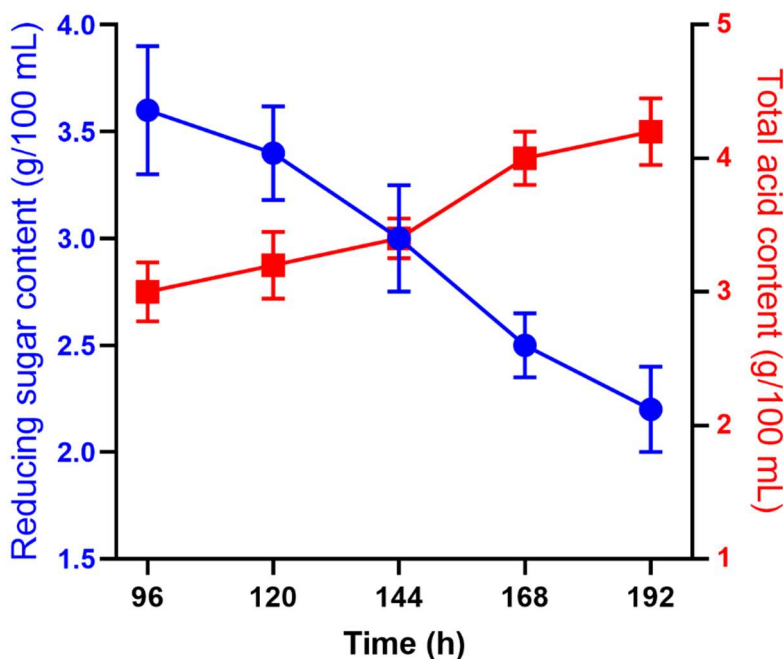


Fig. 2 Effects of fermentation period on sugar and acid contents of *Artemisia selengensis* Turcz juice fermented beverage

3.3 Effects of the initial amount of sucrose on sugar and acid contents of *Artemisia selengensis* Turcz juice fermented beverage

Under the conditions of 6.0% inoculation amount, 25 °C fermentation temperature and 168 h fermentation, the sugar content and total acid content of the fermented beverage were taken as the judging indexes, and supplemented by sensory evaluation, the optimal conditions of sugar content could be determined. The results are shown in Figure 2. With the increase of sugar content, the total acid content in fermented beverage increased with the decrease of sugar content. When the sugar content was 10.0%~14.0%, the change trend of sugar content and total acid content tended to be gentle.

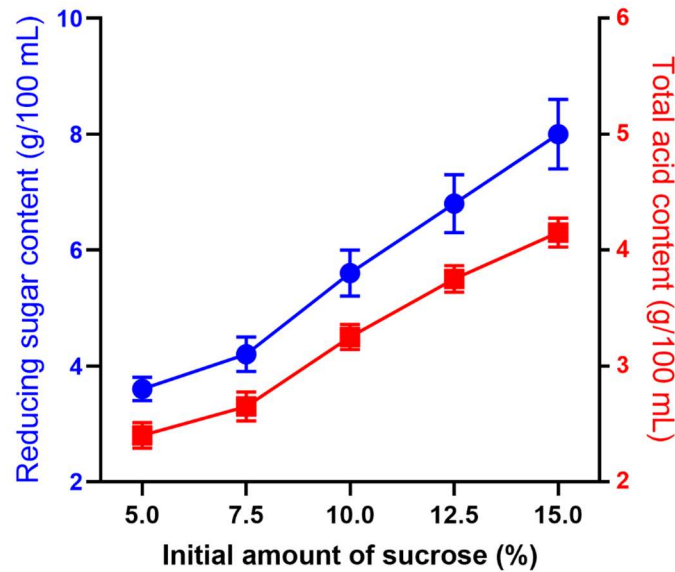


Fig. 3 Effects of the initial amount of sucrose on sugar and acid contents of Artemisia selengensis Turcz juice fermented beverage

3.4 Effects of fermentation temperature on sugar and acid contents of Artemisia selengensis Turcz juice fermented beverage

Under the conditions of 6.0% inoculation amount and 10.0% sugar addition and 168 h fermentation, the sugar content and total acid content of the fermented beverage were taken as the judging indexes and supplemented by sensory evaluation to determine the optimal fermentation temperature. The results are shown in Figure 3. With the increase of temperature, the total acid content in fermented beverage increased with the decrease of sugar content, and when the fermentation temperature was 20-30 °C, the change trend of sugar content and total acid content tended to be gentle. This is mainly because the optimal temperature of kombucha bacteria is in this range, which consumes the sugar in the fermented beverage and produces lactic acid..

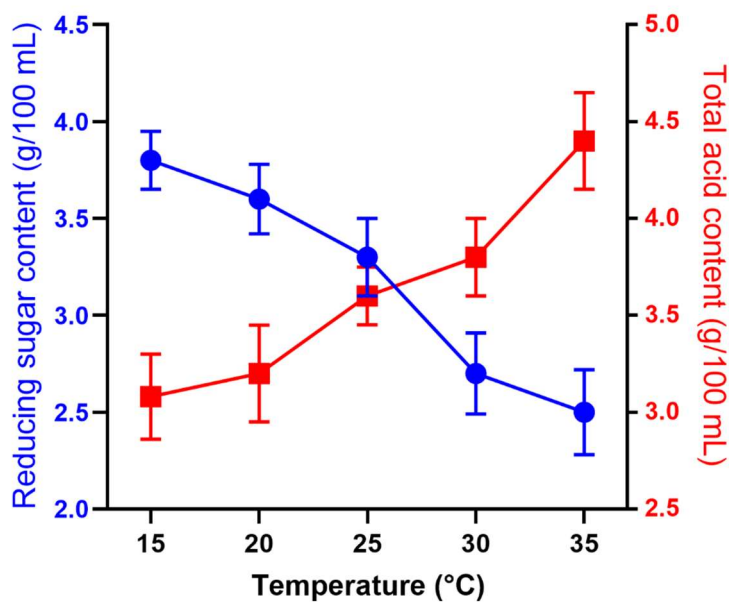


Fig. 4 Effects of fermentation temperature on sugar and acid contents of Artemisia selengensis Turcz juice fermented beverage

3.5 Orthogonal experiment

There are four main factors affecting the fermentation of *Artemisia selengensis* Turcz juice beverage, namely, SCOBY inoculation amount, sugar content, fermentation temperature and fermentation time. Sensory evaluation, sugar content and total acid content were used as the criteria, and the results were analyzed according to L9(34) orthogonal table, so as to determine the best fermentation conditions of *Artemisia selengensis* Turcz juice fermented beverage. The results showed that the amount of SCOBY inoculation, the amount of sugar added, the fermentation temperature and the fermentation time all affected the taste of *Artemisia selengensis* Turcz juice fermented beverage. According to the range analysis, the four factors all have different degrees of influence on the taste of *Artemisia selengensis* Turcz juice fermented beverage, among which the most important factor is the amount of SCOBY inoculation. Taking the sensory evaluation of *Artemisia selengensis* Turcz juice fermented beverage as the index, among the factors affecting sensory evaluation of *Artemisia selengensis* Turcz juice fermented beverage, the amount of SCOBY inoculation had the greatest influence, followed by the amount of sugar, fermentation temperature and fermentation time. In the process of orthogonal analysis, because the range of fermentation time was the smallest, the other three factors were selected for variance analysis. According to variance analysis, the amount of SCOBY inoculation has a very significant impact on the fermentative beverage of *Artemisia selengensis* Turcz juice, while the amount of sugar and fermentation temperature have a significant impact on the fermentative beverage of *Artemisia selengensis* Turcz juice. The results showed that the optimal fermentation parameters of *Artemisia selengensis* Turcz juice fermented beverage were as follows: SCOBY inoculation amount 6.0%, sugar content 10.0%, fermentation temperature 30°C, fermentation time 168h, total sugar 3.66g/100mL, total acid 2.98g/100mL.

4. Conclusion

The juice of *Artemisia annua* was used as raw material, and the beverage was fermented by SCOBY fermentation. Four single factors including SCOBY inoculation amount, sugar content, fermentation temperature and fermentation time were tested, and the optimal fermentation conditions of *Artemisia annua* juice fermentation beverage were obtained through orthogonal test. The results showed that under the optimum fermentation conditions, the bad smell and taste of *Artemisia scoparia* juice could be covered, and the fermented beverage with good taste and suitable sugar and acid could be obtained. The optimum fermentation conditions could be determined by orthogonal test. The main factors of the taste of *Artemisia selengensis* fermented beverage were determined in the order of SCOBY inoculation > fermentation temperature > sugar addition > fermentation time, and the SCOBY inoculation had the greatest influence. The optimal fermentation conditions were determined, namely, 6.0% inoculation amount of kombucha bacteria liquid, 10.0% sugar content, 30°C fermentation temperature, 168h fermentation time, under which the total sugar was 3.66g/100mL, the total acid was 2.98g/100mL, the sweet and sour beverage with bright color was prepared.

Acknowledgments

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