Production and characterization of a novel beverage from maize silk through fermentation with kombucha consortium.

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Abstract. Maize silk was taken as raw material, boiled, cooled and fermented by kombucha fungus to prepare maize silk kombucha beverage. The inoculation amount, initial sugar content and fermentation temperature of kombucha were selected as the influencing factors, and the total acid content of kombucha beverage was taken as the response value. Based on the single factor test, the Box-Behnken response surface methodology was used to optimize the fermentation process. The results showed that the optimal fermentation conditions were as follows: kombucha fungus inoculation amount of 17%, initial sugar content of 18%, fermentation temperature of 30 ℃, the total acid content of Kombucha beverage was 4.85 g/dL, which was basically consistent with the predicted value of the model.

1. Introduction  
Consumption of soft drinks is a major contributor to obesity and related health problems. Alternatives to soft drinks are being developed to replace the consumption of soft drinks. Due to the high public health concern, consumer demand for healthy foods and beverages is considered to be the driving force for growth in the functional food industry.

Kombucha, a fermented beverage that has rapidly gained popularity in recent years, is generally considered to be a functional drink. Kombucha is prepared by using a combination of bacteria and yeast, known as SCOBY (a symbiotic culture of the epidermis of bacteria and yeast), using the fermentation of a sugary tea broth[1]. After the 20th century, the influence of kombucha tea consumption is bigger, because of the healthy properties of kombucha, it is a big increase in the worldwide popularity. Numerous studies have shown that kombucha is associated with anti-hypertensive, anti-microbial, anti-tumor and cardiovascular disease preventive effects, as well as properties that can prevent diabetes and neurodegenerative diseases[2]. To explore modern flavors and different beneficial effects, many studies have reported the use of alternative substrates. Recently, soybean milk, soy whey, and coconut water have been used as alternative substrates for the preparation of fermented beverages using kombucha starter[3-5]. In this study, the chemical and microbial properties of maize silk during fermentation were studied and compared with traditional black tea substrate to explore the possibility of maize silk as an alternative substrate for the production of kombucha. In addition, bioactive components and fermentation products such as organic acids were compared. Therefore, this study aimed to develop a
functional kombucha beverage with strong physical and chemical properties, antioxidant effect and nutritional value based on corn stalk as substrate.

2. Materials and methods

2.1. Materials and Reagents
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The mixed strains of kombucha were purchased from Anhui Shouyuan Biological Technology Co., Ltd. Black tea and sugar are bought in supermarkets. Standard products of rutin are purchased from Shanghai yuanye Bio-Technology Co., Ltd. Sodium nitrite, aluminum nitrate, sodium hydroxide, 3, 5-dinitrosalicylic acid, sodium tartrate and phenol are all from Sinopharm Chemical Reagents Co., Ltd.

2.2. Preparation of maize silk extract
Grind the dried maize silk using a shredder, then put 5 g of corn whisker powder into a tea bag and extract it with 100 mL boiling water (100 ℃) for 15 minutes. After cooling, filter it with a filter paper and take the supernate. Black tea soup is prepared in the same way.

2.3. Activation of kombucha fungus
Take 500ml filtered red tea soup, add 100g white granulated sugar, and mix well to get tea sugar water. 30% seed liquor of kombucha fungus was added to the tea sugar water, transferred to a sterilized glass container, sealed with double-layer gauze, and then placed in a constant temperature incubator at 28℃ for static fermentation for 7d. Activated kombucha fungus liquid could be obtained when the liquid level produced film.

2.4. Single factor experiment
The above treated maize silk soup was fermented for 5 days under the conditions of kombucha fungus inoculation amount of 12%, 16%, 20%, 24%, 28%; initial sugar content of 14%, 16%, 18%, 20%, 22%; fermentation temperature of 22 ℃,26 ℃,30 ℃,34 ℃,38 ℃ for single factor test. The influence of these three factors on the acid yield of kombucha fermentation was studied.

2.5. Response surface methodology
On the basis of single factor test, the inoculation amount of kombucha bacteria, initial sugar content and fermentation temperature were selected as the investigation factors, and the total acid content was selected as the response value. The Box-Benhnken experimental design was used to optimize the fermentation process of maize silk kombucha. The levels of each factor were shown in Table 1.

2.6. Titratable acidity and pH
The titratable acidity of kombucha samples during fermentation was determined by titration method, which was measured as acetic acid (g/dL). Use a pH meter to measure pH values.

2.7. Reducing sugar and soluble solids content
Dinitrosalicylic acid method (DNS) was used to determine the content of reducing sugar in kombucha samples. The content of soluble solids in kombucha fungus was measured by Abbe refractometer.

| Table 1. The factors and levels of response surface test |
|--------------------------------------------------------|
| **Levels** | **Symbols** | **Kombucha fungus inoculation amount (%)** | **Initial sugar content (%)** | **Fermentation temperature (℃)** |
|-----------|-------------|------------------------------------------|-------------------------------|---------------------------------|
| -1        | A           | 12                                       | 16                            | 26                              |
| 0         | B           | 16                                       | 18                            | 30                              |
| 1         | C           | 20                                       | 20                            | 34                              |
3. Results

3.1. Effect of kombucha fungus inoculation amount on total acid content
Under the conditions of initial sugar content of 18% and fermentation temperature of 30 ℃, the influence of different kombucha fungus inoculation amount on fermentation acid production was studied, and the results were shown in Figure 1. With the increase of the inoculation amount of kombucha, the total acid content showed a trend of increasing first and then decreasing slowly. This is because with the continuous increase of inoculation amount, the bacteria will proliferate massively. On the one hand, a large number of metabolites will be generated, leading to premature aging and autolysis of cells, etc. On the other hand, the content of nutrients needed by the bacteria for mass reproduction will decrease, leading to the decrease of total acid content. Therefore, selecting the appropriate inoculation amount of kombucha bacteria is beneficial to control the density of bacteria and total acid content, and the optimal inoculation amount is 16%.

![Fig. 1. Effect of Kombucha inoculation amount on the total acid content.](image)

3.2. Effect of initial sugar content on total acid content
Under the conditions of 16% kombucha fungus inoculation and 30 ℃ fermentation temperature, the effects of different initial sugar content on the acid production of corn stalk kombucha fermentation were studied, and the results were shown in Figure 2. With the increase of initial sugar content, the total acid content first increased and then decreased. This is because enough carbon sources can make the bacteria grow and metabolize to produce abundant organic acids and other beneficial components, but too much initial sugar content, that is, too much osmotic pressure of the mixture, will lead to the dehydration of the bacteria cells and lead to the separation of the plasma-wall, which is not conducive to their growth and fermentation. Therefore, an appropriate initial sugar content should be selected. Too high or too low is not conducive to fermentation, and the best initial sugar content is 18%.

![Fig. 2. Effect of initial sugar content on the total acid content.](image)
3.3. Effect of fermentation temperature on total acid content

Under the conditions of Kombucha fungus inoculation amount of 16% and initial sugar content of 18%, the effects of different fermentation temperatures on the acid production of maize silk kombucha fermentation were studied, and the results were shown in Figure 3. With the increase of temperature, the total acid content of fruit vinegar first increased and then decreased. Temperature is an important factor affecting the growth and fermentation of microorganisms. When the temperature increases, the enzyme activity in the microorganism cells increases, the reaction speeds up, and the acid production is higher. However, when the temperature is too high, the enzyme in the cell is inactivated, which is not conducive to the growth of microorganisms, thus producing less acid. Therefore, the optimal fermentation temperature of kombucha is 30 °C.

3.4. Response surface test results and factor interaction

According to the single factor test results and the level coding Design of test factors in Table 1, the Box-Behnken test Design was carried out, and the software Design-Expert 11 was used to perform multiple quadratic regression fitting on the test results. The regression equation of total acid content (Y) and the inoculation amount of kombucha (A), initial sugar content (B) and fermentation temperature (C) was obtained: $Y = 4.35 + 0.2628A + 0.093B + 0.11C - 0.0925AB - 0.066AC + 0.105BC - 0.3463A^2 - 0.2235B^2 - 0.00341C^2$. The correlation coefficient of regression equation $R^2 = 0.9686$, the determination coefficient (adjusted correlation coefficient) $R_{adj}^2 = 0.9584$, can explain the change of response value within the range of 95.84%, and the loss of fit term is not significant ($P = 0.455$), indicating that this model has good fitting degree and little error. It is suitable for optimizing the fermentation process of maize silk kombucha.

4. conclusion

Maize silk kombucha was prepared by fermentation of maize silk extract with yeast, acetic acid bacteria and lactic acid bacteria in kombucha. After controlling the initial sugar content to 18% and the amount of kombucha fungus inoculation to 17%, the finished product was fermented at 30 °C for 5 days, and the total acid content was 4.65g/dL. The process is simple and easy to control. The sensory and physicochemical indexes of the prepared kombucha are in line with the food standards, and it has good flavor.

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