

Research Article

Research on Fermentation Technology for a *Dendrobium officinale* Leaf–Sweet Potato Koji Compound Beverage

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Dendrobium officinale leaf contains various physiologically active substances. However, no previous studies have reported on the use of *D. officinale* leaf extract in fermented sweet potato beverages. In this study, *Dendrobium officinale* leaf and sweet potato were used as raw materials to produce a compound fermented beverage flavored with *D. officinale* leaf. A sweet potato vinegar folk production method was used, and beneficial microorganisms, including *Rhizopus*, yeast, and lactic acid bacteria, were inoculated into the fermentation. The quantity of *D. officinale* leaves, concentration of the sweet potato koji, fermentation time, and temperature during the fermentation process were optimized using orthogonal experiments and analysis of variance. The results indicated that a *D. officinale* leaf extract of 30%, sweet potato koji concentration of 30%, fermentation time of 5 days, and temperature of 30°C were optimal for beverage formation. Under these conditions, the fermentation broth had a mild flavor of *D. officinale* leaf and a moderately sweet and sour taste, the biofilm was a transparent gelatinous film, and the utilization efficiency of carbohydrates was high. Excellent flavor, color, and stability of the sweet potato beverage were obtained, with a total sugar content of 15.88 mg/mL and a final pH of 2.65.

Keywords: compound beverage; *Dendrobium officinale* leaf; fermentation process; orthogonal experiment; sweet potato koji

1. Introduction

Dendrobium officinale leaf contains various physiologically active substances, such as esters [1], polyphenols, alkaloids, and bibenzyl derivatives [2]. Many studies have indicated that *D. officinale* leaf can inhibit the proliferation of tumor cells, inhibit platelet aggregation, enhance immunity, assist in weight loss, improve cosmetic appearance, and improve health [3, 4].

Sweet potato koji is formed by the mixed fermentation of sweet potatoes by *Rhizopus*, yeast, and lactic acid bacteria. These microbes grow well at appropriate temperatures, converting sucrose into fructose and glucose and then into gluconic acid and acetic acid, forming a sweet–sour, tasty sweet potato koji beverage. Fermented sweet potato koji beverage is a mild and sour-tasting beverage that contains

multiple substances beneficial to the human body. It is purported to reduce swelling and pain, diffuse dampness, dissipate blood stasis, stop bleeding, and assist in skin beautification and lightening and is a natural healthy drink that is enjoyed by people [5–8].

Currently, consumers are increasingly concerned about healthy living. The market demand for natural beverages is growing around the world, and natural beverages can help ensure that consumers meet their nutritional needs. In 2023, the per capita consumption of natural fruit juice reached 4.66 L [9]. In the present study, the folk method was used to prepare sweet potato koji, to which *D. officinale* leaf extract was added. Previous studies have documented the use of traditional fermentation methods for sweet potatoes [10, 11]. To further explore the unique advantages of the fermented combination, we conducted a comparative analysis of the

nutritional components, including polysaccharides, vitamin C, minerals, and antioxidants, between the fermented product, sweet potato koji, and *D. officinale* leaf extract. Additionally, sensory evaluation experiments were carried out to investigate the taste advantages of the fermented beverage. Fermentation of the sweet potato koji and the leaf extract was used to introduce bioactive substances from the leaf extract into the beverage. Through optimizing the recipe and fermentation process conditions, the resulting fermented beverage was sweet-sour and tasty, had the flavor of sweet potato and *D. officinale* leaf, and contained various nutrients. It is a novel beverage with significant development potential. Therefore, we developed a beverage from a mixture of fermented sweet potatoes with the juice of *D. officinale* leaves in this study.

Fermented beverages are formulated through microbial fermentation, with alcohol content typically below 1% [12]. After fermentation, the nutritional value of this type of beverage significantly improves as microorganisms produce new nutrients such as amino acids, minerals, and vitamins during the fermentation process. In addition, fermented beverages also have health benefits, such as regulating gastrointestinal function, lowering blood pressure, and reducing cholesterol [13, 14].

Chwastek et al. [15] studied alternative probiotic foods for consumers who suffer from intolerance to dairy products or who do not consume dairy products as a matter of preference. The use of highbush blueberry-sucrose osmotic syrup for the production of lacto-fermented fruit and vegetable beverages may yield functional products having both nutrition- and health-promoting properties. Vanajakshi et al. [16] studied *Moringa* leaves, which are well known for their nutritional and health benefits and are recommended for malnourished people all over the world. Espirito-Santo et al. [17] believe that fermentation contributes to improving the functional aspects of foods.

On this basis, we developed a *D. officinale* leaf beverage fermented with sweet potato koji. After optimizing the ratio of *D. officinale* leaf juice and sweet potato koji, the stability of the beverage was determined by measuring pH, film weight, and total sugar consumption. Linear regression was used to evaluate the relationship between sensory parameters (color, taste, texture, and overall acceptability) and different sensory evaluators. Our goal was to develop a beverage suitable for adults to enjoy as an attractive alternative, thus meeting the growing consumer demand for healthy compound fermented beverages.

2. Materials and Methods

2.1. Experimental Materials. The *D. officinale* leaves were provided by the Engineering Center of Conservation and Sustainable Utilization of Traditional Chinese Medicine Resources of West Anhui University in Anhui Province. The sweet potato was provided by the Engineering Center of Conservation and Sustainable Utilization of Traditional Chinese Medicine Resources of West Anhui University in Anhui Province. The sweet potato koji was prepared and stored in our laboratory. The yeast and sucrose were food

grade and purchased commercially. The sourdough starter contained *Aspergillus oryzae* and *Saccharomyces cerevisiae*, and the proportion is 1:2.

2.2. Main Instruments and Devices. The instruments used included a tissue ultrafine pulverizer (LG-04, Zhejiang Rui'an Baixin Pharmaceutical Machinery Equipment Factory), far-infrared blast drying oven (DGT-G250, Hefei Huadeli Scientific Equipment Co., Ltd.), electric constant temperature water bath (DK-98-1, Shanghai Keluan Instrument Equipment Co., Ltd.), electric constant temperature incubator (HWS-P29CY, Hefei Dascate Biotechnology Co., Ltd.), electronic balance (AE-124, Shanghai Shunyu Hengping Scientific Instrument Co., Ltd.), and pH meter (PT-11, Lichen Instrument Hardware Store).

2.3. Process Flow. The compound fermented drink was prepared with *D. officinale* leaves and sweet potato as the main raw materials, which were brewed with fungi according to the sweet potato vinegar folk process. The process is shown in Figure 1. The fresh sweet potatoes were cut into pieces, steamed, sterilized, cooled under sterile conditions, and mixed with sourdough starter (concentration of 10%) and prepared red yeast for fermentation. Fresh *D. officinale* leaves were rinsed with running water, sterilized with ultraviolet lamps, and subjected to pulping and filtration under sterile conditions to prepare *D. officinale* leaf juice. The prepared sweet potato koji was inoculated with the juice of *D. officinale* leaves under sterile conditions for fermentation, which eventually produced the *D. officinale* leaf-sweet potato koji compound beverage. The prepared sweet potato koji was mixed with the juice extracted from *D. officinale* leaves under sterile conditions, initiating the fermentation process, which eventually resulted in the production of the *D. officinale* leaf-sweet potato koji compound beverage.

The prepared sweet potato koji is mixed with sterile extracted *Dendrobium officinale* leaf juice to initiate the fermentation process, eventually producing *Dendrobium officinale* leaf-sweet potato koji composite beverage.

2.4. Operational Steps

2.4.1. Preparation of the Sweet Potato Koji. The tools used for incubation, including beakers, gauze, tweezers, and a knife, were first cleaned with water and then soaked in boiling water for sterilization, and oil and other impurities were removed with detergent or a cleaning powder. The cleaning step was to ensure that no mold spores were present. The sweet potatoes were cleaned and steamed to preserve nutrients and then cooled to around 30°C. We used *sourdough starter* as the primary fermentation microorganisms during the fermentation process. After cooling, *sourdough starter* was added at a ratio of 5:1 sweet potato to yeast, corresponding to approximately 0.2 mg *sourdough starter* per kilogram of sweet potato, and the mixture was stirred to a porridge-like consistency, followed by natural

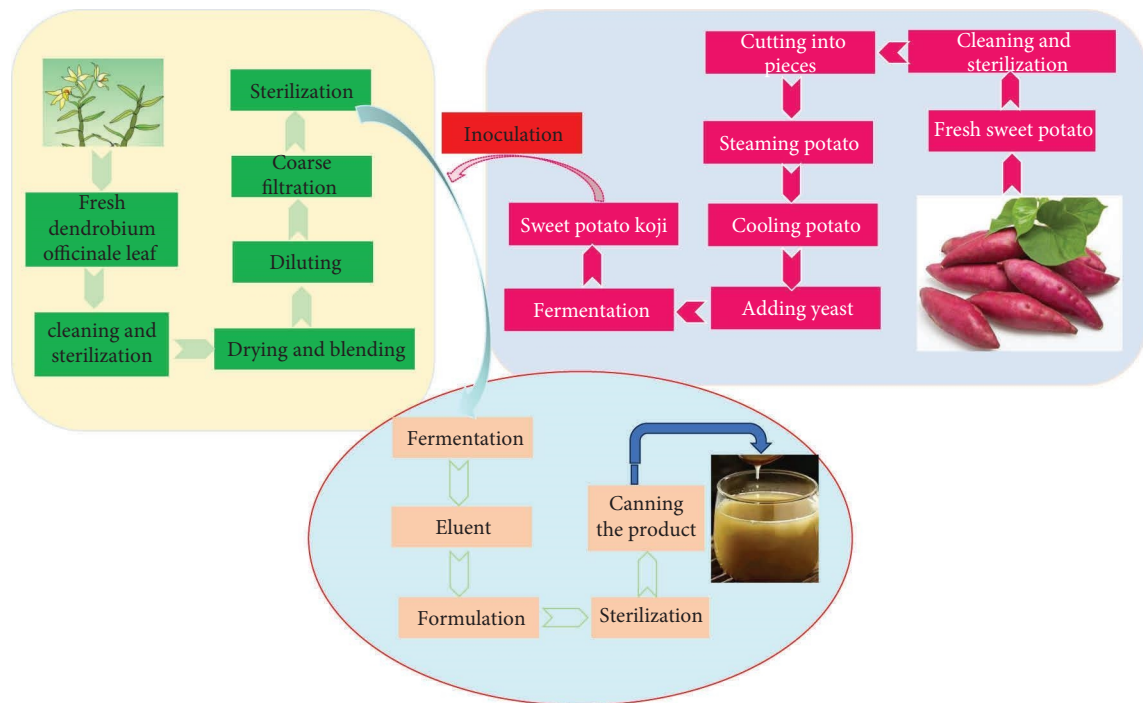


FIGURE 1: Process flow diagram depicting the fermentation technology for preparing the *Dendrobium officinale* leaf-sweet potato koji compound beverage.

fermentation in a tank. The sweet potato koji was stirred once (5–10 min) in the morning and once in the evening. After 6–8 days, when the upper layer of the broth had become watery and showed a golden yellow color with a wine aroma, the initial fermentation was complete, and the sweet potato koji was cooled for future use [18, 19].

2.4.2. Preparation of the *D. officinale* Leaf Extract. Thick, fresh *D. officinale* leaves in good condition and of suitable maturity were selected. Leaf damage was avoided as much as possible during collection. The leaves were cleaned using water, and soil, stones, and other impurities were removed. Subsequently, the sample was soaked in 2% sodium chloride (NaCl) solution for 10–20 min to remove germs, insect eggs, and residual pesticides, as supported by previous studies [20, 21], and then rinsed with water. The cleaned and sterilized *D. officinale* leaves were placed on a sterilized iron plate to let the water on the surface evaporate. The *D. officinale* leaves were crushed with a juicer, resulting in a uniform and fine slurry, and deionized water was used to dilute the slurry to a ratio of *D. officinale* leaves:water = 1:2 to reduce the viscosity and facilitate microbial access to nutrients during fermentation. The diluted slurry was placed into a container and sterilized with 100°C steam for 5 min to ensure microbial decontamination while preserving the integrity of the sample, and the resulting sample was stored in a refrigerator for future use [22, 23].

2.4.3. Preparation of the *D. officinale* Leaf-Sweet Potato Fermented Beverage. To 100 mL of *D. officinale* leaf extract, 35 mL of sweet potato koji stock was added. During

fermentation, the temperature was observed daily, and when it exceeded 42°C, the fermenting liquid was stirred once. After approximately 15 days, the temperature of the fermenting liquid no longer increased, suggesting the end of fermentation. The fermentation endpoint was monitored only by observing the temperature of the fermenting liquid. A tank with an open bottom was prepared, a layer of coarse cloth was placed at the bottom of the tank, and then the mature fermented liquid was placed into the tank. Water at a volume twice that of the fermented liquid was added, and the mixture was stirred once every 2 hours to prevent sedimentation. After stirring five times, the mixture was filtered to obtain the eluent [24, 25]. The microorganisms involved in the fermentation process included lactic acid bacteria, yeast, and *Rhizopus*. Lactic acid bacteria convert sugars into lactic acid, regulating the beverage's acidity, while yeast is responsible for producing flavor compounds that enhance the beverage's taste. *Rhizopus* was used for the preparation of sweet potato koji, converting starches into simple sugars during fermentation.

To determine the optimal fermentation conditions, a two-step optimization process was conducted. First, single-factor experiments were performed to evaluate the effects of critical variables, including sweet potato koji concentration (30%, 35%, and 40%), *D. officinale* leaf extract proportion (20%, 30%, and 40%), fermentation time (3 to 7 days), and fermentation temperature (25°C, 30°C, and 35°C). Each factor was tested individually to identify its impact on fermentation quality, including total sugar consumption, pH, and sensory attributes.

Building on the results of the single-factor experiments, we designed an L9(34) orthogonal experiment. This

experiment was specifically aimed at identifying the optimal combination of factors for the fermentation process. The factors under consideration were the quantity of *D. officinale* leaves, the sweet potato koji concentration, fermentation time, and temperature. Each factor was tested at three levels, and the responses measured included total sugar consumption and pH. The data were then analyzed using analysis of variance (ANOVA) to determine the most effective combination for large-scale production. The ultimate goal of this optimization process was to maximize fermentation efficiency while maintaining high sensory quality and economic feasibility.

2.5. Analytical Procedure. The content of solids was determined using the freeze-drying method [26]. Protein content was determined using the Kjeldahl nitrogen determination method [27]. Microbiological analysis was performed at different stages of fermentation. Samples were collected at 0, 3, 5, and 7 days, and microbial counts were determined using standard plate count methods for lactic acid bacteria, yeast, and molds. Vitamin C was measured using the 2,6-dichlorophenol indophenol titration method [28]. Total minerals were determined using the dry ashing method [29]. Polyphenol content was determined using the Folin phenol method [30, 31]. For the measurement of total sugar and reducing sugar, titration was conducted with Fehling's reagents [32–34]. pH measurements were made with a PT-11 pH meter. The film was weighed using an AE-124 electronic balance after gauze filtration.

The following equation was used to calculate the amount of total sugar consumed during fermentation:

Amount of consumed total sugar (mg/mL) = Initial total sugar concentration (mg/mL) – Final total sugar concentration (mg/mL).

To determine the total sugar consumption, the initial concentration of sugars was measured before the fermentation started. After a specific fermentation period, the final sugar concentration was measured again. The difference between the initial and final sugar levels represents the amount of sugars microorganisms consume during fermentation.

Color and Flavor Evaluation: The color and flavor of the fermentation broth were determined by a panel of 10 trained evaluators. The color was assessed visually under natural light conditions and compared against a standard color chart. The flavor was evaluated through a sensory analysis in which evaluators rated the intensity of sourness, sweetness, and overall flavor profile on a scale from 1 to 10, with 10 being the most intense. The aroma of the fermentation broth was consistent with a typical aroma of fermentation. The biofilm was a transparent gelatinous film, and there was a small amount of precipitate. The fermentation broth was relatively clear, with a light yellow or light brown-yellow color.

To accurately assess the sensory properties of the fermented combination beverage, a 100-point scoring system was used, focusing on factors such as color, aroma, taste, and

overall acceptability. The evaluation standards, which are of paramount importance, were referenced from the study by Barros et al. [28].

Color: The clarity, brightness, and hue of the beverage were evaluated, with a focus on consistency and appeal. A higher score indicated better color characteristics.

Aroma: Our panelists, with their extensive experience and training, assessed the intensity and freshness of the aroma, with attention to any undesirable odors such as sourness or fermentation by-products.

Taste: The balance between sweetness and sourness was evaluated. Beverages with a harmonious balance and pleasant aftertaste received higher scores.

Overall Acceptability: This attribute considered the overall impression of the beverage, including mouthfeel, flavor complexity, and whether the panelists would recommend it to consumers.

Each attribute was scored on a 100-point scale, with the following benchmarks:

- 90–100 points: Excellent quality, with outstanding characteristics in the respective attribute.
- 75–89 points: Good quality, desirable characteristics, and minor deviations.
- 60–74 points: Acceptable quality, with some noticeable imperfections, but still acceptable for consumption.
- Below 60 points: Poor quality, with significant issues in one or more sensory attributes.

The average scores for each attribute were calculated, and the results were used to determine the overall sensory quality of the beverage.

Sensory evaluation was conducted following the guidelines of international sensory analysis standards [35, 36].

The antioxidant capacity measurement was performed according to the method of He et al. [37]. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging rate was calculated using the following equation:

$$\text{DPPH radical scavenging rate (\%)} = \frac{[A_0 - (A_1 - A_2)]}{A_0} \times 100, \quad (1)$$

where A_0 is the value measured of the sample tube (DPPH + extraction reagent + sample), A_1 is the value measured of the sample reference tube (anhydrous ethanol + extraction reagent + sample), and A_2 is the value measured of the control (DPPH + extraction reagent).

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical scavenging rate was calculated according to the method of Sun et al. [38], using the following equation:

$$\text{ABTS radical scavenging rate (\%)} = \frac{A_2 - A_1}{A_2} \times 100, \quad (2)$$

TABLE 1: Effect of the concentration of sweet potato koji on fermentation biofilm.

Concentration of the sweet potato koji (%)	Physical and optical characteristics	Thickness characteristics	Film weight (g·500 mL ⁻¹)
30	Transparent gelatinous film	Thin film	3.78 ± 0.21 ^a
35	Translucent gelatinous film	Thick film	5.43 ± 0.11 ^b
40	Gelatinous film, a small milky white area in the center	Thick film	7.65 ± 0.12 ^c
45	Gelatinous film, milky white	Thick film	7.94 ± 0.13 ^b
50	Gelatinous film, milky white	Thick film	8.13 ± 0.14 ^a

Note: ± indicates standard deviation.

^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

where A_2 represents the measured value of deionized water + ABTS and A_1 represents the measured value of sample solution + ABTS.

2.6. Cost-Benefit Analysis. To evaluate the economic feasibility of different koji concentrations (30% vs. 40%), a preliminary cost-benefit analysis was conducted. The analysis considered the costs of raw materials (koji and other fermentation ingredients), energy consumption (fermentation time and temperature), and labor costs. Production costs were calculated for 30% and 40% koji concentrations based on ingredient costs per kilogram and the overall energy usage required for fermentation. The analysis also included the cost of equipment usage, particularly for maintaining fermentation conditions. The sensory qualities of the final products were compared, and the results were analyzed to determine the most cost-effective fermentation concentration.

2.7. Statistical Analysis and Orthogonal Experimental Design. The fermentation process was optimized using an L9(3⁴) orthogonal experimental design, focusing on three key factors: (A) sweet potato koji concentration, (B) *D. officinale* leaf extract proportion, and (C) fermentation time. Each factor was tested at three levels:

- Factor A (sweet potato koji concentration): 20%, 30%, and 40%.
- Factor B (*D. officinale* leaf extract proportion): 20%, 30%, and 40%.
- Factor C (fermentation time): 4 days, 5 days, and 6 days.

The primary responses measured were total sugar consumption, pH, and sensory attributes (color, taste, aroma, and overall acceptability).

An orthogonal experimental design was used for the sensory analysis. All experiments were performed independently with at least three determinations. Mean values ± standard deviations for all quantitative parameters were calculated using Microsoft Excel 2003 (Microsoft Co., Ltd., WA, USA). Paired ANOVA and F-test were used to compare the effect of a specific treatment with without treatment. A probability value ≤ 0.05 was considered to be statistically significant.

3. Results and Discussion

At the beginning of the experiment, the sweet potato koji solution was prepared, and the culture was expanded. The *D. officinale* leaves were treated, and the sweet potato koji solution and *D. officinale* leaves at different ratios were used for fermentation for a specific time and at a specific temperature.

Typically, fermented sweet potato beverages rely on self-starch as the primary carbon source. However, adding *D. officinale* leaves, which are rich in polysaccharides, cellulose, and pectin [39], provides microbes with a diverse array of sugars to utilize during fermentation. This results in the production of significant amounts of organic acids. The beverage showed a rapid decrease in pH, and a large quantity of sugar was consumed. The changes in the total sugar level and acidity during fermentation were measured periodically, and the fermentation conditions, such as the color, fragrance, film conditions, and film weight, were observed and recorded [40].

Using single-factor experiments based on the quantity of *D. officinale* leaves added to the fermentation, the sweet potato koji concentration, fermentation time, and temperature, we determined the factors and levels of the orthogonal experiment. The L₉(3⁴) orthogonal experiment and ANOVA were performed to screen the optimal combination of each factor and level [41]. In addition, a cost-benefit analysis was conducted to compare the economic feasibility of using 30% and 40% koji concentrations. The results showed that while the 40% koji concentration produced slightly better sensory characteristics, the increased raw material costs and extended fermentation time made it less economically viable. The 30% koji concentration resulted in lower production costs, primarily due to reduced ingredient and energy consumption, while maintaining satisfactory fermentation quality and sensory attributes. Therefore, the 30% koji concentration was the most cost-effective option for large-scale production. Orthogonal experimental design refers to a method of experimental design that studies multiple factors and levels. Representative points from a comprehensive experiment based on orthogonality are selected for experimentation. Orthogonal experimental design is the main method of fractional factorial design [42].

Finally, through a confirmatory experiment [43], the optimal ratio and fermentation conditions during the formation process were determined,

TABLE 2: Effect of the concentration of sweet potato koji on fermentation liquid.

Concentration of the sweet potato koji (%)	Color	Flavor	pH	Total sugar consumed (mg·mL ⁻¹)
30	Relatively clear fermentation broth, light yellow color	Light sour taste	298 ± 1.32 ^a	10.83 ± 0.56 ^a
35	Relatively clear fermentation broth, light yellow color	Strong sour taste	2.65 ± 1.11 ^a	14.10 ± 0.34 ^b
40	Relatively clear fermentation broth, light yellow color	Strong sour taste	2.38 ± 1.16 ^b	14.67 ± 0.21 ^a
45	Relatively clear fermentation broth, small amount of precipitate, light yellow color	Strong sour taste	2.07 ± 1.31 ^c	15.03 ± 0.54 ^a
50	Relatively clear fermentation broth, small amount of precipitate, light yellow color	Strong sour taste	1.75 ± 0.87 ^a	14.84 ± 0.32 ^c

Note: ± indicates standard deviation.

^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 3: Effect of the amount of added *Dendrobium officinale* leaf extract on fermentation biofilm.

<i>D. officinale</i> leaf extract (%)	Physical and optical characteristics	Thickness characteristics	Film weight (g·500 mL ⁻¹)
20	Transparent gelatinous film	Thin film	5.25 ± 0.14 ^a
25	Translucent gelatinous film, a small milky white area in the center	Thick film	6.66 ± 0.21 ^b
30	Translucent gelatinous film, milky white	Thick film	7.61 ± 0.21 ^b
35	Translucent gelatinous film, milky white	Thick film	7.86 ± 0.14 ^a
40	Gelatinous film, white	Thick film	7.56 ± 0.21 ^a

Note: ± indicates standard deviation.

^{a,b}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 4: Effect of the amount of added *Dendrobium officinale* leaf extract on fermentation liquid.

<i>D. officinale</i> leaf extract (%)	Color	Flavor	pH	Total sugar consumed (mg·mL ⁻¹)
20	Relatively clear fermentation broth, light yellow color	Light sour taste	3.01 ± 0.23 ^b	12.12 ± 0.34 ^a
25	Relatively clear fermentation broth, light yellow color	Light sour taste	2.96 ± 0.56 ^a	14.02 ± 0.11 ^c
30	Clear fermentation broth, small amount of precipitate, light yellow color	Sour taste	2.79 ± 0.11 ^a	15.44 ± 0.31 ^c
35	Clear fermentation broth, small amount of precipitate, light yellow color	Moderate sour taste	2.77 ± 0.13 ^b	14.46 ± 0.45 ^a
40	Clear fermentation broth, appearance of precipitate, light yellow color	Strong sour taste	2.85 ± 0.15 ^c	14.91 ± 0.32 ^a

Note: ± indicates standard deviation.

^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 5: Effect of fermentation time on fermentation quality liquid.

Fermentation time (d)	Color	Flavor	pH	Total sugar consumed (mg.mL ⁻¹)
3	Slightly turbid fermentation broth, light yellow color	Sweet-sour taste	3.14 ± 0.03 ^b	10.23 ± 0.01 ^c
4	Relatively clear fermentation broth, light yellow color	Moderate sour taste	3.02 ± 0.01 ^a	13.67 ± 0.02 ^c
5	Relatively clear fermentation broth, light yellow color	Strong sour taste	2.71 ± 0.03 ^c	14.87 ± 0.06 ^a
6	Clear fermentation broth, small amount of precipitate, light yellow color	Strong sour taste	2.67 ± 0.03 ^a	15.47 ± 0.04 ^a
7	Clear fermentation broth, large amount of old microbe precipitate, light yellow color	Strong sour taste	2.66 ± 0.03 ^b	15.67 ± 0.01 ^a

Note: ± indicates standard deviation.

a~Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 6: Effect of fermentation time on fermentation quality biofilm.

Fermentation time (d)	Physical and optical characteristics	Thickness characteristics	Film weight (g·500 mL ⁻¹)
3	Transparent gelatinous film	Thin film	3.13 ± 0.14 ^a
4	Translucent gelatinous film, a small milky white area in the center	Relatively thin film	5.58 ± 0.08 ^c
5	Gelatinous film, milky white	Thick film	6.15 ± 0.08 ^b
6	Gelatinous film, white	Thick film	6.51 ± 0.11 ^a
7	Gelatinous film, yellow-white	Thick film	6.84 ± 0.09 ^b

Note: ± indicates standard deviation.

^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 7: Effect of temperature on fermentation liquid.

Temperature (°C)	Color	Flavor	pH	Total sugar consumed (mg.mL ⁻¹)
25	Clear fermentation broth, light yellow color, small amount of precipitate	Strong sour taste	2.84 ± 0.02 ^a	13.83 ± 0.12 ^c
30	Clear fermentation broth, light yellow color, small amount of precipitate	Strong sour taste	2.68 ± 0.01 ^c	15.92 ± 0.11 ^b
35	Clear fermentation broth, light brown-yellow color, small amount of precipitate	Strong sour taste	2.66 ± 0.03 ^a	15.43 ± 0.13 ^a

Note: ± indicates standard deviation.

^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 8: Effect of temperature on fermentation biofilm.

Temperature (°C)	Physical and optical characteristics	Thickness characteristics	Film weight (g·500 mL ⁻¹)
25	Semigelatinous film	Thick film	5.52 ± 0.01 ^b
30	Milky white gelatinous film	Thick film	7.83 ± 0.02 ^a
35	Yellow-white gelatinous film	Thick film	7.85 ± 0.01 ^a

Note: ± indicates standard deviation.

^{a,b}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 9: Factors and levels of the $L_9(3^3)$ test for fermentation of the *Dendrobium officinale* leaf-sweet potato koji compound beverage.

Level	A (%)	B (%)	C (days)
1	20	20	4
2	25	30	5
3	30	40	6

Note: A, sweet potato koji concentration; B, *Dendrobium officinale* leaf extract; C, fermentation time.

TABLE 10: Results and range analysis of the orthogonal experiment for fermentation of the *Dendrobium officinale* leaf-sweet potato koji compound beverage.

	Experiment	A (%)	B (%)	C (day)	Total sugar consumed (mg·mL ⁻¹)	pH
Total sugar consumed	1	1	1	1	8.06	3.20
	2	1	2	2	11.02	2.96
	3	1	3	3	14.29	2.84
	4	2	1	2	12.10	2.93
	5	3	2	2	15.55	2.66
	6	2	3	1	9.74	3.00
	7	3	1	3	15.14	2.75
	8	3	2	1	10.36	2.97
	9	2	3	2	13.02	2.68
	K ₁	33.37	35.30	28.16		
	K ₂	37.39	36.93	36.14		
	K ₃	38.52	37.05	44.98		
	k ₁	11.12	11.77	9.37		
	k ₂	12.66	12.32	13.05		
	k ₃	12.84	12.35	14.43		
	R	1.72	0.58	5.06		
pH	K ₁	9.00	8.88	9.23		
	K ₂	8.65	8.59	8.57		
	K ₃	8.41	5.58	8.25		
	k ₁	3.00	2.96	3.10		
	k ₂	2.82	2.86	2.79		
	k ₃	2.80	2.86	2.77		
	R	0.12	0.10	0.33		

Note: A, sweet potato koji concentration; B, *D. officinale* leaf extract; C, fermentation time.

TABLE 11: Variance analysis of the orthogonal experiment.

Source of variation	df	Total sugar consumed (mg·mL ⁻¹)			pH			F _{0.05} (2, 4)	F _{0.01} (2, 4)
		SS	MS	F	SS	MS	F		
A	2	4.89	2.45	12.54*	0.06	0.03	4.00	6.94	18
B	2	0.64	0.32	1.64	0.02	0.01	1.33		
C	2	47.20	23.60	121.03**	0.16	0.08	10.67*		
Error e	2	0.14	0.07		0.01	0.005			
Error e	4	0.78	0.195		0.03	0.0075			
Sum	8	52.87	52.87		0.25				

“*”, $F_{0.01} > F > F_{0.05}$, the effects of different factor levels on the treatment result were significantly.

“***”, $F > F_{0.01}$, the effects of different factor levels on the treatment result were extremely significant.

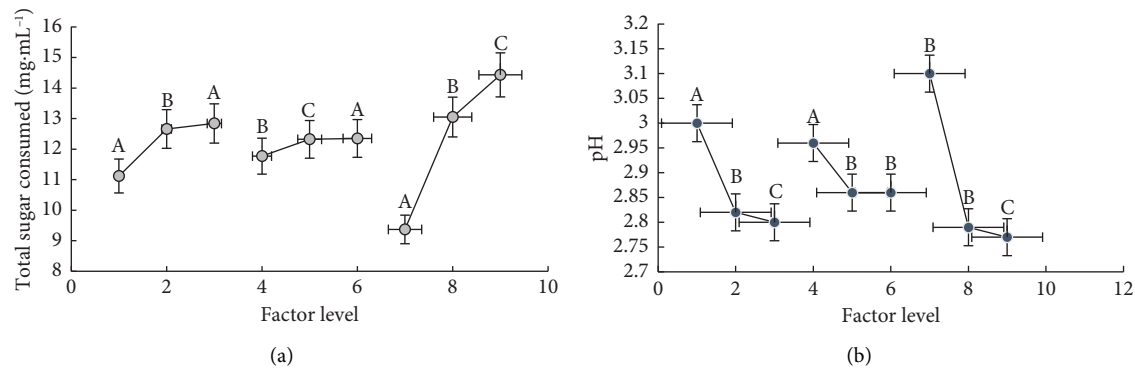


FIGURE 2: The trend of the effect of each factor level on the total sugar consumed (a) and pH (b). Note: ^{A-C}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

$$\text{Amount of consumed total sugar} \left(\frac{\text{mg}}{\text{mL}} \right) = \text{Amount of total sugar before fermentation} - \text{Amount of total sugar after fermentation.} \quad (3)$$

3.1. Single-Factor Experiments. At the beginning of the experiment, the sweet potato koji solution was prepared, and the culture was expanded. The *D. officinale* leaves were treated and then mixed with the sweet potato koji solution at different ratios for fermentation under controlled time and temperature conditions. The broth developed a typical aroma during fermentation, consistent with natural fermentation processes—a transparent, gelatinous biofilm formed on the surface, accompanied by a small amount of precipitate. The fermentation broth appeared relatively straightforward, exhibiting a light yellow to light brown-yellow color.

3.1.1. Effect of Different Inoculum Concentrations of the Sweet Potato Koji on the Fermentation Broth. The effects of different sweet potato koji concentrations on the fermentation of the beverage were investigated by adding *D. officinale* leaf extract (40% of the total volume) and fermenting at 30°C for 5 days. As can be seen from Table 1, on the surface of the fermentation broths using five different concentrations of the sweet potato koji, the growth conditions of the biofilm were not considerably different. However, as the concentration of the sweet potato koji increased, the thickness, weight, and pH of the biofilm increased accordingly (Tables 1 and 2). When an inoculum concentration of 40% was compared to a sweet potato koji concentration of 50%, the changes in the weight of the biofilm did not show large differences. The color, fragrance, and taste of the fermentation broth with a 40% inoculum concentration were better than those with a 50% sweet potato koji concentration, and the amount of consumed total sugar was not significantly different when the inoculum concentration was over 45%. Considering the economics, a sweet potato koji concentration below 40% was determined to be feasible. If the fermentation time must be shortened, the sweet potato koji concentration can be increased.

3.1.2. Effects of Different Proportions of *D. officinale* Leaf Extract in the Fermentation Broth. To investigate the effects of adding different quantities of *D. officinale* leaf extract to the fermentation broth, the fermentation solution was inoculated with sweet potato koji (30%) and fermentation was carried out at 30°C for 5 days. As can be seen from Table 3, the film conditions of the five quantities of *D. officinale* leaf extract added to the fermentation differed substantially. As the percentage of *D. officinale* leaf extract increased, the texture and thickness of the biofilm improved, and the amount of consumed total sugar and the pH of the fermentation liquid broth improved to some extent (Tables 3 and 4). Optimal values of film weight and pH were obtained when the proportion of *D. officinale* leaf extract was 30%. The fermentation broths with *D. officinale* leaf extract at 35% and 40% were compared, and the pH of the fermentation liquid and the amount of consumed total sugar did not exhibit large differences. Furthermore, with an increasing proportion of *D. officinale* leaf extract, large quantities of insoluble solids could not be utilized by the microorganisms and, therefore, were present in the fermentation broth in the form of precipitates. Therefore, the amount of *D. officinale* leaf extract should not exceed 30%.

3.1.3. Effect of Different Fermentation Times on the Fermentation Broth. With a sweet potato koji concentration of 30% and a *D. officinale* leaf extract concentration of 30%, fermentation was conducted for different durations at a fermentation temperature of 30°C. The results are shown in Tables 5 and 6. As a fermented beverage, the fermentation time directly influences the economic benefit; the longer the fermentation time, the higher the consumption of raw materials, labor, and other costs. After 3 days of fermentation, the acidity decreased, a unique sweet-sour taste was present, and the beverage could be directly consumed. After 5 days of fermentation, the flavors of the *D. officinale* leaf and

TABLE 12: Results of the orthogonal experiment.

Experiment	Film conditions	Color and flavor of the fermentation broth	Film weight (g·500 mL ⁻¹)	pH	Total sugar consumed (mg·mL ⁻¹)
1	Transparent, gelatinous film	Appearance of a fermentation flavor, light sour taste, light yellow fermentation broth, small amount of precipitate in the fermentation broth	7.38 ± 0.02 ^a	2.63 ± 0.02 ^a	15.91 ± 0.01 ^a
2	Transparent, gelatinous film	Appearance of a fermentation flavor, light sour taste, light yellow fermentation broth, clear fermentation broth	6.79 ± 0.01 ^b	2.68 ± 0.01 ^b	15.86 ± 0.03 ^b
3	Transparent, gelatinous film	Appearance of a fermentation flavor, light sour taste, light yellow fermentation broth, clear fermentation broth	7.63 ± 0.03 ^c	2.64 ± 0.02 ^c	15.88 ± 0.01 ^c

Note: ± indicates standard deviation.
^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

sweet potato were very strong, and the sour taste was relatively strong. The pH values at 5 days of fermentation and 6 days of fermentation were not notably different, and the amount of total consumed sugar was not considerably different between 6 days and 7 days of fermentation. Thus, a suitable fermentation time of 5 days was established.

3.1.4. Effect of Different Temperatures on the Fermentation Broth. With a sweet potato koji concentration of 30%, a *D. officinale* leaf extract concentration of 30%, and a fermentation time of 5 days, three temperatures (25°C, 30°C, and 35°C) were selected for fermentation. The fermentation results are shown in Tables 7 and 8. The consumed total sugar at 30°C and 35°C was higher than at 25°C, and there was no notable difference in pH, suggesting that the temperature required to produce the sweet potato beverage was flexible. Furthermore, 25°C–35°C is a standard fermentation temperature condition that can be easily controlled, thereby meeting the conditions for large-scale production. Based on the findings of this experiment, 30°C was determined to be the most suitable temperature for fermentation.

3.2. Orthogonal Experiment. Based on the single-factor tests, appropriate ranges of sweet potato koji concentration, the *D. officinale* leaf extract proportion, and fermentation time were selected. At 30°C, an orthogonal experiment was used to determine the optimal ratio. Each factor and level are shown in Table 9, and the results and analysis of the orthogonal experiments are shown in Table 10.

As can be seen from Table 6, the effects of all factors on the amount of consumed total sugar and pH were in the order of $C > A > B$. That is, the fermentation time (factor C) showed the largest impact on the amount of consumed total sugar and pH, followed by the sweet potato koji concentration of the sweet potato koji (factor A), while the effect of the amount of added *D. officinale* leaf extract (factor B) exhibited the lowest impact on the two indicators.

The above results indicated that the growth and the material conversion of the sweet potato koji symbiont exhibited a stepwise and orderly process, which is consistent with the general pattern of the growth and metabolism of microorganisms. As can be seen from the range analysis, the optimal combination obtained for the highest amount of consumed total sugar was $C_2A_3B_2$, that is, the sweet potato koji concentration of the sweet potato koji was 30%, the proportion of *D. officinale* leaf extract was 30%, and the fermentation time was 5 days. The optimal combination obtained for the lowest pH was $C_2A_3B_2$, that is, the sweet potato koji concentration of the sweet potato koji was 30%, the proportion of *D. officinale* leaf extract was 30%, and the fermentation time was 5 days.

As indicated in the orthogonal experimental results (Table 11), when the main influencing factor C (fermentation time) was 5 days, the consumed total sugar was the highest, while the pH was the lowest. Considering the economics, the ideal fermentation time was set to 5 days. When the sweet potato koji concentration was 30%, the changes in the amount of consumed total sugar and pH were optimal, and thus, 30%

TABLE 13: Physical and chemical analyses.

Factor	Fermented beverage
Solid matter (g/L)	0.87 ± 0.01^a
pH	3.76 ± 0.01^a
Reducing sugar (% dry weight)	19 ± 0.02^b
Protein (% dry weight)	0.69 ± 0.02^b
Vitamin C (mg/g)	0.1421 ± 0.01^c
Total minerals (%)	0.94 ± 0.01^c
Polyphenols (%)	0.33 ± 0.01^a

Note: $\pm$ indicates standard deviation.

^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

TABLE 14: Sensory evaluation.

Sensory factor	Sensory score
Color	65.78 ± 0.56^a
Taste	78.43 ± 0.43^b
Texture	80.32 ± 0.81^a
Overall acceptability	86.67 ± 0.26^c

Note: $\pm$ indicates standard deviation.

^{a-c}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

was selected as the sweet potato koji concentration. Excessive quantities of *D. officinale* leaf extract could not be completely utilized by the microorganisms. The trend shown in Figure 2 also indicated that the highest quantity of added *D. officinale* leaf extract (30%) was the most suitable.

Based on the above analysis, the optimal ratio and fermentation process for the compound beverage was $C_2A_3B_2$, that is, the sweet potato koji concentration of the sweet potato koji was 30%, the proportion of the *D. officinale* leaf extract was 30%, and the fermentation time was 5 days.

3.3. Confirmatory Experiment. To further verify the optimal combination $C_2A_3B_2$, a confirmatory experiment using this combination was performed, and the experimental results are shown in Table 12. After 5 days of fermentation with a sweet potato koji concentration of 30% and a *D. officinale* leaf extract quantity of 30%, the average pH of the beverage decreased to 2.65, the amount of consumed total sugar reached 15.88 mg/mL, and the average film weight was 7.27 g/500 mL.

3.4. Formulation of the Beverage. Most of the sugar in the fermentation broth after fermentation was consumed by lactic acid bacteria. Acetic acid and lactic acid were produced, the acidity increased, the pH decreased, the sour taste became strong, and the flavor became unpleasant. To improve the flavor of the beverage, 5 g sucrose was mixed into the 100 mL beverage, and finally, 0.5 g of vitamin C and 20 mL honey were added to improve the palatability of the beverage. The addition of sucrose can neutralize the fermented beverage's sour taste and enhance its sweetness; vitamin C, as an antioxidant, can prolong the shelf life, while honey not only improves the flavor but also provides additional nutrition and health benefits.

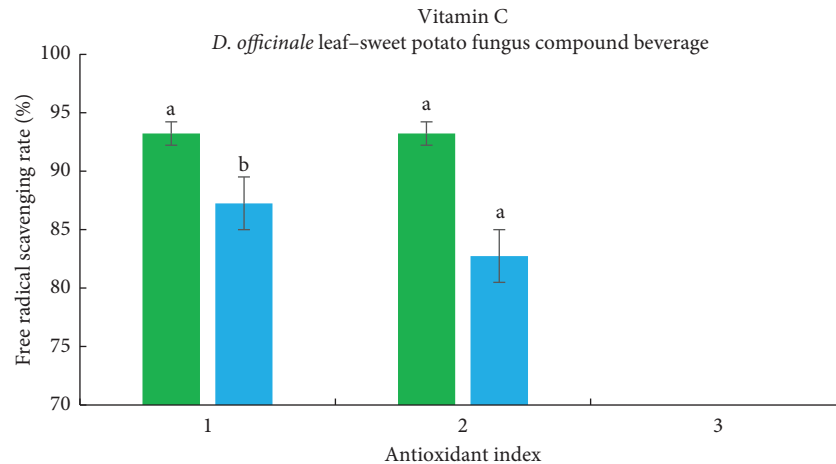


FIGURE 3: Comparison of the free radical scavenging rates. Note: ^{a,b}Mean values with different letters in the same column are statistically different ($p \leq 0.05$).

To verify the quality of the blended beverage, physical and chemical analyses of the blended beverage were performed, as shown in Table 13.

According to the results shown in Table 9, the mixed beverage had a solids content of 0.87 g/L, pH of 3.76, reducing sugar content of 19%, protein content of 0.69%, vitamin C content of 0.1421%, total mineral content of 0.94%, and polyphenol content of 0.33%. This set of physicochemical data confirmed that the compound fermented beverage of *D. officinale* and sweet potato koji complied with the requirements specified in GB/T 31326-2014 for plant beverages.

3.5. Sensory Properties. A total of 20 sensory evaluators were selected for sensory analysis of the composite fermented beverage of *D. officinale* and sweet potato koji. Scoring of the color, taste, texture, and overall acceptability of the beverage was conducted using a percentage system.

The evaluation of the blended beverage by 20 sensory evaluators is shown in Table 14, with a color evaluation score of 65.78 points, a taste evaluation score of 7.43 points, a texture evaluation score of 80.32 points, and an overall acceptable score of 86.67 points. According to the experiments by Barros [44], Goel [45], and Mohsin [46] on physical, chemical, and sensory experimental data of beverages formed by blending different plant juices, we believe that the optimal sensory performance of the composite fermented beverage of *D. officinale* and sweet potato koji was produced using 30% *D. officinale* leaf juice and a 30% sweet potato koji inoculation per 100 mL, with a fermentation time of 5 days, a temperature of 30°C, 5% sucrose water at a 10% concentration, 1% vitamin C at a 1% concentration, and 5 mL of *Sophora japonica* honey. The main objective of this study is to enhance the taste and nutritional value of the beverage by optimizing the fermentation process. After fermentation, we added sucrose, vitamin C, and honey further to enhance the flavor and nutritional content of the beverage. Relevant literature indicates that post-fermentation optimization through formulation helps

improve the beverage's sensory quality and nutritional functionality [47]. Through the dual effect of optimizing the formulation, the beverage's sensory acceptance and nutritional functionality have been further enhanced.

3.6. Antioxidant Activity. As shown in Figure 3, compared with the clearance rate of DPPH free radicals and ABTS cationic radicals by vitamin C ($93.23 \pm 0.14\%$), the clearance rates of DPPH and ABTS by the compound fermented beverage of *D. officinale* and sweet potato koji were $87.26 \pm 0.63\%$ and $82.75 \pm 0.29\%$, respectively. The free radical scavenging rate by the compound fermented beverage was 5.97% and 10.48% lower, respectively, relative to that by vitamin C.

The compound fermented beverage of *D. officinale* and sweet potato koji may have different structures of phenolic compounds due to the fermentation treatment, thus possessing antioxidant activity. Therefore, the beverage scavenged free radicals, but the scavenging effect was slightly lower than that of vitamin C. These results were similar to those of Huang et al. [48].

4. Conclusions

The optimal production process of a beverage from the fermentation of *D. officinale* leaf extract and sweet potato koji was $A_3B_2C_2$, that is, the sweet potato koji concentration of the sweet potato koji was 30%, the proportion of *D. officinale* leaf extract was 30%, the fermentation time was 5 days, and the temperature was 30°C. This process resulted in a beverage with a desirable balance of sweet and sour flavors, excellent color, and good stability. The optimal production process for a beverage from the fermentation of *D. officinale* leaf extract and sweet potato koji was determined to be a 30% concentration of sweet potato koji, a 30% proportion of *D. officinale* leaf extract, and a fermentation time of 5 days at a temperature of 30°C. The sweet potato koji concentration had the greatest impact on fermentation speed and outcomes, with 30% being optimal for

achieving a high sugar conversion efficiency and desirable pH levels. A *D. officinale* leaf extract of 30% provided the best flavor and characteristics without excessive precipitation. A fermentation time of 5 days produced the best balance of sweetness and acidity. The beverage demonstrated significant antioxidant activity, with DPPH and ABTS scavenging rates of 87.26% and 82.75%, respectively. This study demonstrates that the *D. officinale* leaf-sweet potato koji compound beverage is a promising product with potential health benefits and market appeal. Further research and development could enhance its commercial viability and consumer acceptance.

Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Chunlei Zhang: conceptualization, resources, supervision; Zhenlin Zhang: methodology, software, writing—original draft, review, and editing; Yanhong Wang: validation, writing—review, and editing; Zhichao Yin, Xingtang Fang, and Xi Chen: writing—review and editing; Qingqing Xue, Weiwei Cheng, Meihua Hu, Yixuan Zhu, and Yin Yao: methodology, investigation, and software.

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