Development of *Brassica* Vegetable Juice Drink

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Abstract [**Objectives**] This study was condcuted to investigate the formula optimization and nutritional components of *Brassica* vegetable juice beverage. [**Methods**] *Brassica* was selected as the raw material to optimize the formula of the vegetable juice beverage. The vitamin C content and flavor components were analyzed in both sterilized and non-sterilized samples. [**Results**] Based on water, the optimal formula for the *Brassica* vegetable juice beverage was determined as: 20% *Brassica* juice, 5% erythritol, and 0.1% citric acid. The highest vitamin C content was observed in unsterilized samples (12.167 mg/100 g sample), followed by samples sterilized at 71 ℃ for 15 s (9.864 mg/100 g sample). The most significant loss of vitamin C occurred under sterilization conditions of 68 ℃ for 30 min. GC-MS analysis detected a total of seven volatile components in the *Brassica* vegetable juice beverage, including siloxanes, alcohols, aldehydes, and methoxyphenyl oxime. Before sterilization, siloxane compounds (D3, D4, D5) showed the highest content in the *Brassica* vegetable juice, accounting for 63.606%, followed by methoxyphenyl oxime at 24.802%. After sterilization, siloxane compounds (D3, D4, D5) exhibited the highest content reaching 81.963%, while methoxyphenyl oxime taking the second place decreased to 14.276%. [**Conclusions**] This study provides new insights and methodologies for the development and utilization of *Brassica* crops and other agricultural products, offering a theoretical foundation for accelerating the integrated development of *Brassica* processing and sales.

Key words *Brassica*; Vegetable juice drink; GC-MS; Flavor ingredients

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Brassica, also known as rapeseed shoot, refers to the roots, stems, and leaves of the rapeseed plant in the *Brassica*ceae family. It is rich in nutrients such as vitamin C, Ca, Se, and Zn[1], and possesses medicinal properties including cooling blood, resolving stasis, removing toxic materials, and reducing swelling. The tender stems and leaves of *Brassica* crops are consumed as a kind of vegetable, known for its unique flavor, refreshing taste, and high nutritional value[2]. However, its use as a vegetable is limited by a short shelf life, and traditional cooking methods often lead to significant nutrient loss[3]. To achieve higher-value utilization of *Brassica* crops, vegetable juice extracted from it has been developed. In recent years, with the continuous development and evolution of the food industry, high-quality vegetable juices have gradually gained prominence in the Chinese beverage market. Due to their pleasant taste, rich nutrition, green and natural attributes, and portability, they have attracted significant consumer attention[4]. With the improvement of living standards, health awareness has increased, leading to a growing recognition of the dietary and medicinal value of compounds such as flavonoids, dietary fiber, vitamin C, carotenoids, glucosinolates, and their degradation products found in *Brassica* vegetables. Consequently, consumer interest in food products derived from *Brassica* vegetables as raw materials has also risen. However, current research on the processing technologies for *Brassica* vegetable juice remains limited globally, and the product varieties are relatively single. Further studies are needed to optimize the formulation and nutritional composition of *Brassica* vegetable juice beverages[5-6].

Brassica vegetable juice beverages are not only convenient for consumption and suitable for all age groups, but also rich in nutritional components, offering various health benefits and holding broad development prospects. In this study, *Brassica* was selected as the raw material to optimize the formulation of *Brassica* vegetable juice beverages, and the flavor components and vitamin C content of the beverage were analyzed before and after sterilization. The study aimed to further advance research in the field of *Brassica* vegetable juice, and accelerate the integration of *Brassica* processing and sales and promote the advancement of modern *Brassica* processing technologies and the thriving development of the *Brassica* processing industry according to China's "13th Five-Year Plan" policy guidelines[7-9].

Materials and Methods

Instruments and materials

Instruments The instruments used in this study are listed in Table 1.

Materials Fresh *Brassica* stalks (commercially available); erythritol and citric acid (food grade, commercially available); NaCl and vitamin C (analytically pure, Sinopharm Chemical Reagent Co., Ltd); HCl and NaOH (analytically pure, Tianjin Tianli Chemical Reagent Co., Ltd.).

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Table 1 Main instruments and equipment

Name	Model	Manufacturers
Joyoung Juicer	D05	Joyoung Co., Ltd.
Digital constant-temperature water bath	HH-6	Changzhou Guohua Electric Appliance Co., Ltd.
GC-MS (Gas Chromatography-Mass Spectrometer)	7890A-5975C	Agilent Technologies, USA
UV-Vis Spectrophotometer	UV-3600	Shimadzu Corporation, Japan

Process flow

Brassica stalks were treated through sequential steps including pretreatment, blanching, cooling, juicing, filtration, blending, clarification, filtration, and sterilization to obtain the final product.

Preparation of *Brassica* juice

Fresh, undamaged *Brassica* stalks were selected as the raw material. After soaking and washing in clean water, the material was cut into sections. Blanching was conducted at 85 ℃ for 90 s, followed by juicing using a blender at a solid-to-liquid ratio of 1 : 2. The mixture was thoroughly homogenized and filtered, and the filtrate was collected and stored under refrigeration.

Optimization of *Brassica* vegetable juice beverage formula

Single-factor experiments

(1) Addition ratio of *Brassica* juice: After pretreatment, *Brassica* juice was added at ratios of 10%, 15%, 20%, 25%, and 30%, respectively. Sensory evaluation was conducted to determine the optimal solid-to-water ratio for the vegetable beverage.

(2) Addition ratio of erythritol: Using the prepared *Brassica* vegetable juice as the base and sensory evaluation as the primary indicator, erythritol was added at ratios of 3%, 4%, 5%, 6%, and 7% respectively to determine the optimal erythritol addition level.

(3) Addition ratio of citric acid: Using the prepared *Brassica* vegetable juice as the base and sensory evaluation as the primary indicator, citric acid was added at ratios of 0.05%, 0.10%, 0.15%, 0.20%, and 0.25% respectively to determine the optimal citric acid addition level[10-11].

Orthogonal test Based on the single-factor experiments, an $L_9(3^4)$ orthogonal test design[12] was adopted to investigate the effects of *Brassica* juice addition ratio, erythritol addition ratio and citric acid addition ratio on the sensory score of *Brassica* vegetable juice, so as to determine the optimal formula for the *Brassica* vegetable juice beverage. The factor-level table for the orthogonal test is shown in Table 2.

Table 2 Orthogonal test factor level table

Level	Factor		
	A Addition ratio of *Brassica* juice//%	B Addition ratio of erythritol//%	C Addition ratio of citric acid//%
1	10	3	0.1
2	15	4	0.2
3	20	5	0.3

Sensory evaluation criteria According to the sensory evaluation standards, ten laboratory personnel were randomly selected to form a taste panel. The panel evaluated the *Brassica* vegetable juice beverage based on four aspects: color, texture, taste, and aroma[13]. The specific scoring criteria are detailed in Table 3.

Table 3 Sensory scoring criteria for *Brassica* vegetable juice

Scoring Items	Scoring criteria	Score points
Texture (30 points)	Clear and uniform texture without turbidity or separation	21-30
	A small amount of sediment, no layering	11-20
	A significant amount of sediment, obvious layering	0-10
Taste (30 points)	Well-balanced sweet and sour, palatable	21-30
	Poor sweet-sour ratio, too sour or too sweet	11-20
	Rough texture with a bitter and astringent taste	0-10
Color (20 points)	Pure color, light green	16-20
	Relatively even color, presence of some minor discoloration in parts	11-15
	Yellowish and uneven color	0-10
Aroma (20 points)	*Brassica* special flavor with a fresh scent	16-20
	No obvious *Brassica* scent, no off-odor	11-15
	No characteristic *Brassica* odor, presence of an off-odor	0-10

Sterilization

Pasteurization was adopted in this experiment. The formulated and filtered *Brassica* vegetable juice beverage was filled into glass bottles with lids, leaving a headspace of 5-7 mm, and glass bottles were sealed. A water bath was preheated to a required temperature, and the bottled beverage was sterilized in the water bath. The pasteurization conditions were set at 71 ℃ for 15 s and 68 ℃ for 30 min. After sterilization, the bottles were rapidly cooled.

Determination of vitamin C content

Vitamin C exhibits its maximum absorption peak at 243 nm. The absorbance of three experimental samples (before sterilization, after sterilization at 71 ℃ for 15 s, and after sterilization at 68 ℃ for 30 min) was measured at 243 nm. The vitamin C concentration of each sample was determined by referencing a standard curve, and the vitamin C content was calculated accordingly[14-15]. The specific steps are given below.

(1) Preparation of the vitamin C standard curve.

Different volumes (0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 ml) of a standard vitamin C solution (concentration: 0.1 mg/ml) were transferred into separate 50 ml brown volumetric flasks. Each flask was diluted to volume with distilled water, and the absorbance of each solution at 243 nm was measured and recorded. A standard curve was plotted with vitamin C concentration on the x-axis and absorbance at 243 nm on the y-axis.

(2) Absorbance measurement of *Brassica* vegetable juice

beverage.

A 5 ml aliquot of the supernatant from the *Brassica* beverage was transferred into a beaker, followed by the addition of 2 ml of 10% HCl. The mixture was then diluted to 50 ml with distilled water in a volumetric flask. Using distilled water as a blank reference, the absorbance at 243 nm was measured and recorded as A_1. Another 5 ml aliquot of the supernatant was combined with 10 ml of distilled water and 4 ml of 1 mol/L sodium hydroxide solution. After thorough mixing and standing for 20 min, 4 ml of 10% HCl was added, and the solution was diluted to volume with distilled water. Using distilled water as a blank reference, the absorbance at 243 nm was measured and recorded as A_2.

(3) Calculation.

The absorbance value of the *Brassica* vegetable juice beverage was determined according to $A = A_1 - A_2$. The corresponding vitamin C concentration in the *Brassica* vegetable juice beverage solution was calculated based on the standard curve based on the absorbance at 243 nm and corresponding vitamin C concentration. The vitamin C content was calculated using the following formula:

$$V_c \text{ content(mg/100 g sample)} = 25 \times \frac{C}{V}$$

In the equation, C is the vitamin C concentration calculated from the standard curve prepared in step (1), in μg/ml; V is the volume of the supernatant of *Brassica* vegetable juice beverage used for absorbance measurement, in ml.

Analysis of Flavor Components

Gas chromatography-mass spectrometry (GC-MS)

(1) Analytical method: GC-MS was employed to determine the volatile components of the *Brassica* vegetable juice[16-19].

(2) Sample preparation: Exactly 2 g of sodium chloride was weighed into a 10 ml headspace vial, followed by the addition of 5 ml of vegetable juice and a magnetic stirring rotor. The vial was sealed with a sealing gasket. The sample was incubated by the headspace method in a water bath at 60 ℃ for 50 min, and then directly injected and desorbed for 5 min.

(3) Analytical conditions: Chromatographic column: DB-WAX (30 m × 320 μm × 0.25 μm); carrier gas: helium; injection port temperature: 250 ℃; flow rate: 1.3 ml/min; splitless mode; GC temperature program: initial temperature 50 ℃ held for 3 min, increased at 3 ℃/min to 70 ℃ held for 2 min, and then raised at 3 ℃/min to 170 ℃ held for 0 min, followed by an increase at 8 ℃/min to 240 ℃ held for 4 min; ionization mode: EI; ionization energy: 70 eV; transfer line temperature: 280 ℃; ion source temperature: 220 ℃; solvent delay time: 1.5 min; scan mode: full scan/selected ion scanning (SIS); scan range: 30 – 500 amu.

(4) Data analysis: Preliminary analysis was conducted using the NIST08 database. The relative contents of flavor components in the *Brassica* vegetable juice beverage before and after sterilization were analyzed and calculated.

Results and Analysis

Results of single-factor experiments

The results of the single-factor experiments are shown in the figure below.

Effect of *Brassica* juice content on sensory score As shown in Fig. 1, under constant conditions of other factors, the sensory score of the vegetable juice beverage initially increased and then decreased with the increasing proportion of *Brassica* juice. The bar chart peaked at a *Brassica* juice content of 20%, indicating the highest sensory score at this level, and the vegetable juice berverage was characterized by a rich product flavor and moderate viscosity at this level. When the *Brassica* juice content was too low, the beverage lacked sufficient fruit pulp concentration, resulting in a weak *Brassica* flavor and thin mouthfeel. Conversely, higher *Brassica* juice content led to an excessive thick and dense mouthfeel.

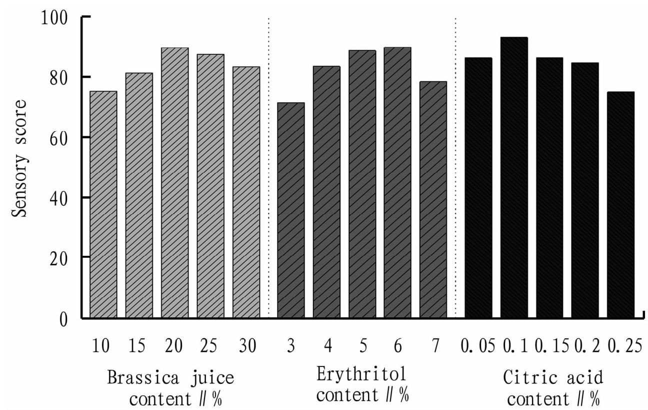

Fig. 1 Effects of *Brassica* juice content, erythritol content and citric acid content on sensory score of beverages

Effect of erythritol content on sensory score Under constant conditions of other factors, Fig. 1 shows that as the erythritol content increased, the sensory score of the vegetable juice beverage initially increased and then decreased , peaking at an erythritol content of 6%. At this level, the beverage achieved the highest sensory score, with a well-balanced and pleasant sweet-sour mouthfeel. When the erythritol content was too low, the product exhibited high acidity and insufficient sweetness, resulting in poor taste. Conversely, excessively high erythritol content led to overly low acidity and excessive sweetness, similarly compromising the beverage's palatability.

Effect of citric acid content on sensory score As shown in Fig. 1, under constant conditions of other factors, the sensory score of the vegetable juice beverage initially increased and then decreased with increasing citric acid content, reaching its peak at a citric acid concentration of 0.10%. At this level, the beverage achieved the highest sensory score, exhibiting a well-balanced and pleasant sweet-sour taste. When the citric acid content was too low, the product lacked sufficient acidity and was excessively sweet, resulting in poor palatability. Conversely, when the citric acid content was too high, the beverage became overly acidic with inadequate sweetness, leading to an unfavorable taste.

Results of orthogonal test

As shown in Table 4, the order of factors affecting the sensory score of the *Brassica* vegetable juice beverage was A (*Brassica* juice content) > B (erythritol content) > C (citric acid content).

The Brassica juice content was the primary factor, showing the greatest effect on the sensory score of the vegetable juice beverage, while the erythritol content was the secondary factor. The optimal combination identified through the orthogonal test was $A_3B_3C_1$, specifically: 20% *Brassica* juice, 5% erythritol, and 0.1% citric acid. Since the optimal formula combination did not appear in the orthogonal test groups, a validation test was conducted. The optimal formula $A_3B_3C_1$ was compared with the highest-scoring combination $A_3B_2C_1$ from the orthogonal test table in terms of sensory evaluation. The results showed that the $A_3B_3C_1$ combination achieved a higher sensory score, thus confirming it as the optimal combination in this orthogonal test.

Table 4 Orthogonal test results of $L_9(3^4)$

No.	Factor			Comprehensive score/points
	A (*Brassica* juice)	B (Erythritol)	C (Citric acid)	
1	1	1	1	70
2	1	2	3	67
3	1	3	2	76
4	2	1	3	71
5	2	2	2	77
6	2	3	1	91
7	3	1	2	78
8	3	2	1	93
9	3	3	3	83
k_1	71.0	73.0	83.7	
k_2	79.7	79.0	77.0	
k_3	84.7	83.3	73.7	
R	13.7	10.3	10.0	

Determination results of vitamin C content

The standard curve for vitamin C was calculated based on the measured absorbance values and the aforementioned standard curve, with the results presented in Table 5.

As shown in Table 5, the unsterilized *Brassica* vegetable juice beverage had the highest vitamin C content at 12.167 mg/100 g sample, followed by the sample sterilized at 71 ℃ for 15 s with a content of 9.864 mg/100 g sample. The sterilization condition of 68 ℃ for 30 min resulted in the greatest loss of vitamin C. Thus, short-time sterilization could minimize the thermal degradation of vitamin C. Consequently, the sterilization condition for the *Brassica* vegetable juice beverage was determined as 71 ℃ for 15 s. For subsequent analysis of volatile components, samples sterilized under the condition of 68 ℃ for 30 min were excluded.

Table 5 Results of Vc assays under different sterilization conditions

Condition	Unsterilized	71 ℃, 15 s	68 ℃, 30 min
Absorbance	0.061	0.048	0.043
Concentration/μg/ml	2.433	1.973	1.796
Content//mg/100g sample	12.167	9.864	8.978

Analysis results of flavor components

Table 6 and Table 7 present the GC-MS analysis results of volatile components in the *Brassica* vegetable juice beverage before and after sterilization, respectively. The GC-MS analysis detected seven volatile components in the beverage both before and after sterilization, including siloxanes, alcohols, aldehydes, and methoxyphenyl oximes. Before sterilization, siloxane compounds (D3, D4, D5) were the most abundant volatile components in the *Brassica* vegetable juice beverage, accounting for 63.606%, followed by methoxyphenyl oxime at 24.802%. After sterilization (71 ℃, 15 s), siloxane compounds remained the dominant volatile components, with their content increasing to 81.963%, while methoxyphenyl oxime decreased to 14.276%. Additionally, the aroma component 1-octen-3-ol disappeared after sterilization, and a new aroma component, hexanal, was detected. These findings indicated that temperature significantly affected volatile components in the *Brassica* vegetable juice beverage. After heating, not only did the concentrations of the original volatile compounds change, but some alcohols were lost, and new volatile components were generated.

Among these compounds, hexamethylcyclotrisiloxane exhibits a distinct odor. Octamethylcyclotetrasiloxane is colorless and odorless. Decamethylcyclopentasiloxane carries a faint silicone-like scent. Hexanal presents a raw oily, grassy, and apple-like aroma. 1-Pentanol has a pleasant fragrance. Heptanol emits a floral aroma. 1-Octen-3-ol features a mushroom, rose, and hay-like aroma. The characteristic odor of methoxyphenyl oxime has not been documented to date.

Table 6 Analysis results of volatile components in *Brassica* vegetable juice beverage before sterilization

No.	CAS	Name of matching item	Relative content//%
1	000541-05-9	Cyclotrisiloxane, hexamethyl-	29.395
2	000556-67-2	Cyclotetrasiloxane, octamethyl-	15.115
3	000541-02-6	Cyclopentasiloxane, decamethyl-	19.096
4	000071-41-0	1-Pentanol	7.543
5	003391-86-4	1-Octen-3-ol	1.897
6	000111-70-6	1-Heptanol	2.152
7	1000222-86-6	Oxime-, methoxy-phenyl-_	24.802

Table 7 Analysis results of volatile components in *Brassica* vegetable juice beverage after sterilization

No.	CAS	Name of matching item	Relative content//%
1	000541-05-9	Cyclotrisiloxane, hexamethyl-	42.697
2	000556-67-2	Cyclotetrasiloxane, octamethyl-	22.777
3	000541-06-2	Cyclopentasiloxane, decamethyl-	16.489
4	000066-25-1	Hexanal	1.403
5	000071-41-0	1-Pentanol	2.022
6	000111-70-6	1-Heptanol	0.335
7	1000222-86-6	Oxime-, methoxy-phenyl-_	14.276

Most alcohols possess pleasant aromas, typically characterized by fresh plant-like aromas. Unsaturated alcohols can significantly contribute to product flavor even at low concentrations, whereas saturated alcohols have minimal impact on the flavor even at higher levels. However, when present alone, saturated alcohols with longer carbon chains exhibit plant-like or floral-fatty aromas[20]. Analysis indicates that 1-pentanol and heptanol are saturated alcohols, while 1-octen-3-ol is an unsaturated alcohol. Before sterilization, 1-octen-3-ol significantly contributed to the flavor of the *Brassica* vegetable juice beverage. After sterilization, the overall contribution of alcohols to the flavor became smaller. Since aldehydes are characterized by low thresholds and intense aromas, hexanal contributed significantly to the beverage's flavor after sterilization.

Conclusions and Discussion

In this study, based on the rich nutritional components and health benefits of *Brassica* crops, drawing on existing processing parameters and formulations for fruit and vegetable juice beverages, the formula of the *Brassica* vegetable juice beverage was optimized through single-factor and orthogonal experiments, so as to determine the most suitable ratio. As a result, in a uniquely flavored and nutrient-rich beverage product was prepared. Additionally, the vitamin C content of the prepared *Brassica* vegetable juice beverage was determined using ultraviolet-visible spectrophotometry, and its flavor components were analyzed by gas chromatography-mass spectrometry (GC-MS). This study provides new insights and methodologies for the development and utilization of *Brassica* and other agricultural products.

The experimental results demonstrated that: (1) using water as the base solvent, the optimal formula for the *Brassica* vegetable juice beverage was 20% *Brassica* juice, 5% erythritol, and 0.1% citric acid. (2) The highest vitamin C content was observed in unsterilized samples at 12.167 mg/100 g sample, followed by samples sterilized at 71 ℃ for 15 s at 9.864 mg/100 g sample, while sterilization at 68 ℃ for 30 min resulted in the greatest loss of vitamin C. Thus, to minimize vitamin C degradation, the recommended sterilization condition for the *Brassica* vegetable juice beverage was 71 ℃ for 15 s. And (3) GC-MS analysis revealed changes in both the content and variety of flavor components in the *Brassica* vegetable juice beverage before and after sterilization. After sterilization, the samples showed an increase in siloxane content, a decrease in methoxyphenyl oxime, a reduction in alcohols, and the new detection of hexanal. These findings indicated that temperature significantly affected the volatile components in the *Brassica* vegetable juice beverage.

Based on the experimental results, further studies can be designed to investigate the specific effects of sterilization on the characteristic flavor components of *Brassica* and related reactions involved, as well as to explore methods for optimizing the production process of *Brassica* vegetable juice beverages.

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