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Optimization of fermentation technology for composite fruit and vegetable wine by response surface methodology and analysis of its aroma components

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Fruit wine has certain health care functions, but fruit wine made from a single fruit or vegetable does not have a good enough color, flavor or nutrient composition. Therefore, this study used fresh carrot (*Daucus carota* subsp. *sativus*) and pomegranate (*Punica granatum*) as raw materials to explore the brewing process of carrot and pomegranate compound wine. The fermentation technology of the composite carrot and pomegranate wine was optimized by a single-factor experiment and Box–Behnken design (BBD), which provided a theoretical foundation for the fermentation of this wine. As per the results, the alcohol content of this composite carrot and pomegranate wine was 12.35% vol. under the optimum fermentation conditions of 28 °C initial temperature, 24% initial sugar content, and with the addition of 64 mg L^{-1} sulfur dioxide (SO_2). In the fermented fruit and vegetable wine, a total of 30 aroma components were detected; 21 composites (such as bornyl acetate, caryophyllene and 3-(2-nitrophenylmethyl)-2-thiazolidinone) were newly generated. The relative content of alcohol flavor composites (such as propionic acid 2-methyl-3-hydroxy-2,2,4-trimethylpentan-1-ol, 2-methyl-2-ethyl-3-hydroxycyclohexyl propanoate and terpinene-4-ol) showed an upward trend, and the relative content of alkene components increased significantly after fermentation. The findings of this study provide an experimental foundation for optimizing fermentation technology and for improving the product quality of composite carrot and pomegranate wine.

1. Introduction

The carrot (*Daucus carota* subsp. *sativus*) is commonly known as "glabrous leaf asia bell root".[1] There are various carotenoids and nutritional ingredients in carrots, such as vitamins, anthocyanins, dietary fiber, lignin and minerals.[2] They also have anti-cancer, anti-aging, anti-diabetes, cholesterol lowering, cardiovascular disease prophylaxis, and tumorigenesis reduction effects.[3–5] The pomegranate (*Punica granatum*) is rich in dietary fiber and wholesome nutrients, including vitamins (*i.e.* vitamin C, vitamin A and folic acid) and minerals (such as potassium). Phenolic composites and some alkaloids, triterpenes and sterols are also generated by pomegranates in abundance.[6,7] Additionally, pomegranates can reduce the incidence of chronic diseases,[8,9] and their extract also has positive effects on weight loss.[10]

As is suggested in some studies, fruit wines have certain health care functions.[11–14] In addition to wines made from grapes, those made from cider, green plum, blueberry and other fruits[15–17] are emerging currently, and these wines have gained certain popularity and occupy a huge market.[18] As a new type of beverage, vegetable wines have not only unique vegetable flavors but also contain various vitamins, trace elements and other nutrients from vegetables.[19–22] However, wine made from a single fruit or vegetable will not have good enough color, flavor or nutritional components.[23] The characteristics of both fruits and vegetables can be integrated into composite fruit and vegetable wine, which possesses high nutritional value and unique style.[24] For instance, the composite mango and carrot wine brewed by Meng Jinming[25] is orange and full in color, and sweet and mellow in taste. Carrot wines are rich in carotene and lycopene, which can promote blood circulation, provide energy

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and exert nutrition and health care functions.[26] Pomegranates are abundant in vitamins, inorganic salts, amino acids and other nutrients, which are beneficial for the human body.[27,28] The fermentation of carrot and pomegranate juices mixed in proportion can not only improve the flavor of this wine and enrich its color, but also retain the nutritional components to the maximum extent.[29]

Due to the fact that aroma is one of the qualities appreciated in wine products,[30,31] the analysis of aroma substances is also the basis for realizing flavor control and quality improvement of composite fruit wines. Although some studies and applications have covered many types of composite fruit wines, there has been no exploration into composite carrot and pomegranate wines. Therefore, the purpose of this study was to develop a compound fruit wine with carrot and pomegranate as raw materials, and to optimize the brewing process of this compound fruit wine with rich nutrition and rich aroma, in order to further provide a theoretical reference for the deep processing of compound fruit and vegetable wines and functional health drinks.

2. Materials and methods

2.1 Materials

In this study, fresh carrots and white sugar were purchased from a farmer's market in Zhengzhou; pomegranates were purchased from a market in Xingyang; Angel fruit wine yeast was purchased from Angel Yeast Co., Ltd.; pectinase was purchased from Youyi Food Additives Co., Ltd.; and glucose and tartaric acid were purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd.

2.2 Instruments

The HH-6 digital display constant temperature water bath pot was purchased from Fangke Instrument (Changzhou) Co., Ltd.; the DNP-9272BS-II electric thermostatic incubator was purchased from Shanghai CIMO Medical Instrument Manufacturing Co., Ltd.; the JYZ-D57 Joyang juicer was purchased from Joyang Co., Ltd.; the FA1104 analytical balance (sensitivity 0.0001 g) was purchased from Shanghai Sunny Hengping Instrument Co., Ltd.; and the GCMS-QP2010 Ultra gas chromatograph–mass spectrometer was purchased from Shimadzu Corporation, Japan.

2.3 Experimental methods

2.3.1 Fermentation process of the composite carrot and pomegranate wine. In terms of the pomegranate processing, after the pomegranates were peeled, the pomegranate juice to be used for fermentation was produced.[32] The juice was squeezed out with sterilized gauze to remove the kernel substance. In terms of the carrot processing, after the green covers at the head and the tail of the carrots were removed, the carrots were cut into small square blocks of about 1 cm for the juicing treatment. Subsequently, the raw carrot juice and pomegranate juice were prepared[33,34] and mixed according to the required ratio.

Carrots → Cleaning → Processing and cutting → Juicing → Filtration
Pomegranates → Cleaning and peeling, and diaphragm removal → Juicing and filtration
} Mixing carrot and pomegranate juice

→ Enzymolysis → SO_2 addition → Yeast inoculation → Pre-fermentation → Tank exchange → Post-fermentation → Precipitation and filtration → Package → Composite carrot and pomegranate wine

2.3.2 Activation of yeast. 5% sucrose solution (1000 mL) was prepared and put into a water bath pot at 38 °C for 30 min.

2.3.3 Single-factor experiment. There are many factors affecting the fermentation technology of composite carrot and pomegranate wines. In this experiment, the focus was placed on the effects of six factors that affect the alcohol content and sensory evaluation of composite carrot and pomegranate wines, including the ratio of V(carrot juice) to V(pomegranate juice) (3 : 1, 2 : 1, 1 : 1, 1 : 2 and 1 : 3), fermentation days (3 d, 4 d, 6 d, 7 d and 8 d), yeast addition amount (0.4 g L^{-1}, 0.8 g L^{-1}, 1.2 g L^{-1}, 1.6 g L^{-1} and 2.0 g L^{-1}), SO_2 addition amount (30 mg L^{-1}, 60 mg L^{-1}, 90 mg L^{-1}, 120 mg L^{-1} and 150 mg L^{-1}), initial sugar content (18%, 20%, 22%, 24% and 26%), and temperature (19 °C, 22 °C, 24 °C, 26 °C, 28 °C, 30 °C and 32 °C).

2.3.4 Box–Behnken experiments. The three factors with the most significant influence on the quality of fruit wines were selected by the single-factor experiment. The experimental scheme was designed by response surface software (Box–Behnken design). The BBD for these three factors at three levels was conducted with the alcohol content as the determination indicator, in an attempt to explore the optimum fermentation conditions of the composite carrot and pomegranate wine.

2.3.5 Determination of the main volatile components by GC-MS

2.3.5.1 Sample preparation. The sample preparation was slightly modified based on the method in a published article.[35] After pouring 8 mL of the fruit and vegetable wine sample into a headspace bottle (20 mL), 2 g NaCl was added with appropriate shaking. Wine samples were preheated at 45 °C for 10 min, and then the extraction head was extended into the headspace to perform the adsorption at 45 °C for 40 min, before being pulled out. Subsequently, the extraction head was inserted into the sample injection port of a gas chromatograph and thermally analyzed at 230 °C for 3 min.

2.3.5.2 GC-MS conditions. The GC conditions were slightly modified based on the method in published articles.[36,37] Analytical conditions of GC-MS: the HP-FFAP column was 30 m × 0.32 mm × 0.25 μm; there was no shunt, with a flow rate of 1.21 mL min^{-1}; the temperature at the sample injection port was 250 °C; the heating procedure started at 40 °C for 3 min, followed by heating at 5 °C min^{-1} to 60 °C and 8 °C min^{-1} to 230 °C for 7 min. Conditions of mass spectrometry: the interface temperature was 220 °C, the ionization mode was electron ionization (EI) source, the electron energy was 70 eV, and the ion source temperature was 200 °C.

2.3.6 Quality analysis of fruit wines

2.3.6.1 Sensory scoring method. The sensory scoring method of the composite carrot and pomegranate wine was established according to the sensory scoring method stipulated in Analytical Method of Wine and Fruit Wine (GB/T 15038-2006) (**https://openstd.samr.gov.cn/bzgk/gb/newGbInfo?hcno=4CFF012592D9DE362A765DD3ED1F9C26**). In the experiment, seven professionals with grade I wine taster professional qualification certificates were invited to conduct sensory scoring of the wine samples in the tasting room of the college. The sensory scoring standards of the composite carrot and pomegranate wine are listed in Table 1.

2.3.6.2 Physicochemical indicators. The alcohol content was determined according to the alcohol meter method stipulated in Analytical Method of Wine and Fruit Wine (GB/T 15038-2006). pH was measured using a pH meter. The total sugar (calculated from glucose) was determined according to the direct determination with Fehling's reagent stipulated in Analytical Method of Wine and Fruit Wine (GB/T 15038-2006). The total acid (calculated from tartaric acid) was determined according to the titrimetry stipulated in Analytical Method of Wine and Fruit Wine (GB/T 15038-2006). The volatile acid was determined according to Analytical Method of Wine and Fruit Wine (GB/T 15038-2006). The total SO_2 and free SO_2 were determined according to the direct iodometry stipulated in Analytical Method of Wine and Fruit Wine (GB/T 15038-2006). The soluble solids were determined using a hand-held refractometer.

2.3.6.3 Microbial indicators. The total count of bacterial colonies and coliforms was determined according to Food Microbiological Examination: Aerobic Plate Count (GB 4789.2-2016). The count of pathogenic bacteria was determined according to Limit of Pathogenic Bacteria in Food (GB 29921-2013).

3. Results

3.1 Results of single-factor experiments

3.1.1 Effect of the volume ratio of carrot juice to pomegranate juice on the alcohol content and sensory evaluation of composite fruit and vegetable wines. The effect of the volume ratio of fruit juice on the quality of composite fruit and vegetable wines was explored. The fermentation duration was 7 d, the fermentation temperature was 24 °C, the yeast addition amount was 1.2 g L^{-1}, the initial sugar content was 22 °Bx, the SO_2 addition amount was 120 mg L^{-1}, the pectinase addition amount was 80 mg L^{-1}, and the volume ratios of carrot juice to pomegranate juice were 3 : 1, 2 : 1, 1 : 1, 1 : 2 and 1 : 3. After finishing the fermentation, the alcohol content of the fruit wine was determined and the sensory evaluation was conducted, with the results shown in Fig. 1.

Table 1 Sensory scoring standards of the composite carrot and pomegranate wine

Item	Standard	Score(s)
Color	Light red, clear and transparent	16–20
	Pinkish red, slightly dim in color	10–15
	Dusky in color, with suspended solids	0–9
Smell	Rich pomegranate fruit aroma and mellow wine aroma, fresh smell, without miscellaneous flavor	21–30
	Pomegranate fruit aroma and favorable wine aroma, without miscellaneous flavor	11–20
	With objectionable odors	0–10
Taste	Tasty and mellow	31–40
	Soft, sweet and sour, with slightly light lasting fragrance	21–30
	Incongruous taste, with sour taste	0–20
Typicality	Harmonious composition, perfect typicality, and unique style	9–10
	Light pomegranate and carrot flavors, and favorable typicality	6–8
	Coarse quality, without the typicality of the composite carrot and pomegranate wine	0–5

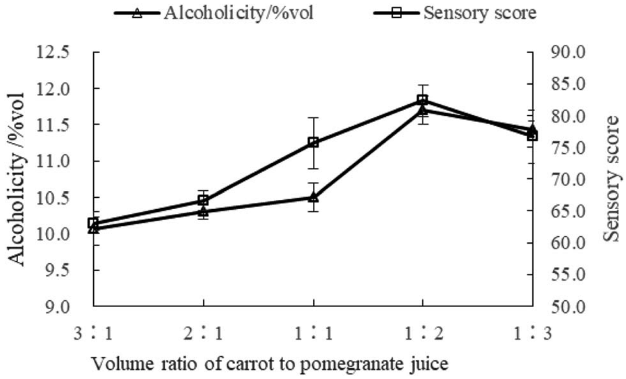

Fig. 1 Effect of the ratio of carrot juice to pomegranate juice on the quality of composite fruit and vegetable wines.

As can be seen from the figure, when the volume ratio of carrot juice to pomegranate juice is 1 : 2, the alcohol content and sensory score reach the maximum, and the wine flavor is harmonious and rich at this time. Subsequently, the alcohol content and sensory score decrease, due to the fact that there is a slight carrot medicinal smell in the wine when the carrot juice ratio is large,[25] which affects the quality of the composite fruit and vegetable wine. Therefore, the optimum fermentation ratio of carrot juice to pomegranate juice is 1 : 2.

3.1.2 Effect of fermentation days on the alcohol content and sensory evaluation of composite fruit and vegetable wines. The effect of fermentation days on the quality of composite fruit and vegetable wines was explored. The fermentation temperature was 24 °C, the yeast addition amount was 1.2 g L^{-1}, the initial sugar content was 22 °Bx, the SO_2 addition amount was 120 mg L^{-1}, the pectinase addition amount was 80 mg L^{-1}, the volume ratio of carrot juice to pomegranate juice was 1 : 2, and the fermentation durations were 3 d, 4 d, 6 d, 7 d and 8 d. After finishing the fermentation, the alcohol content of the fruit wine was determined and the sensory evaluation was conducted, with the results shown in Fig. 2.

As can be seen from the figure, the alcohol content and sensory score of composite fruit and vegetable wines gradually increase with an increase in fermentation days, with their values reaching the maximum on the 6th day of fermentation. With a further increase in fermentation days, the alcohol content and sensory score decrease gradually, and the wine quality is poor. Therefore, the optimum fermentation duration is 6 d.

3.1.3 Effect of the yeast addition amount on the alcohol content and sensory evaluation of composite fruit and vegetable wines. The effect of the yeast addition amount on the quality of composite fruit and vegetable wines was explored. The fermentation duration was 7 d, the fermentation temperature was 24 °C, the initial sugar content was 22 °Bx, the volume ratio of carrot juice to pomegranate juice was 1 : 2, the SO_2 addition amount was 120 mg L^{-1}, the pectinase addition amount was 80 mg L^{-1}, and the yeast addition amounts were 0.4 g L^{-1}, 0.8 g L^{-1}, 1.2 g L^{-1}, 1.6 g L^{-1}, 2.0 g L^{-1} and 2.4 g L^{-1}. After finishing the fermentation, the alcohol content of the fruit wine was determined and the sensory evaluation was conducted, with the results shown in Fig. 3.

As can be seen from the figure, the alcohol content and sensory score of the composite carrot and pomegranate wines gradually increase with the increase of the yeast addition amount. When the yeast addition amount is 1.6 g L^{-1}, the alcohol content and sensory score reach the maximum. As the yeast addition amount continues to increase gradually, the alcohol content and sensory score gradually decrease. This is caused by the fact that when the yeast addition amount is too large, the waste produced by yeast growth and metabolism also increases,[38,39] and the activity of yeast decreases at this time, which also affects the quality of these wines. Therefore, the optimum yeast addition amount is 1.6 g L^{-1}.

3.1.4 Effect of SO_2 addition amount on the alcohol content and sensory evaluation of composite fruit and vegetable wines. The effect of SO_2 addition amount on the quality of composite fruit and vegetable wines was explored. The fermentation duration was 7 d, the fermentation temperature was 24 °C, the initial sugar content was 22 °Bx, the volume ratio of carrot juice to pomegranate juice was 1 : 2, the pectinase addition amount was 80 mg L^{-1}, the yeast addition amount was 1.2 g L^{-1}, and the

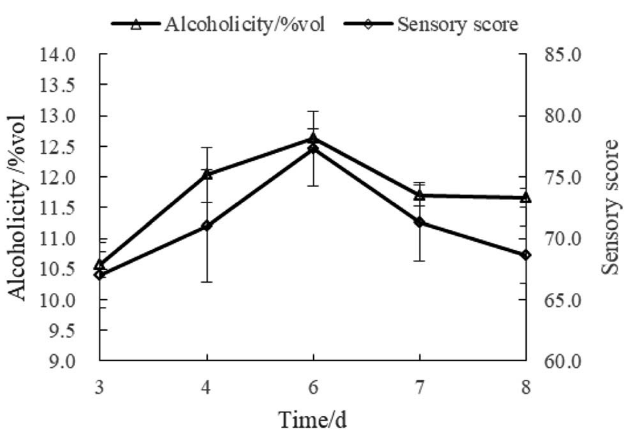

Fig. 2 Effect of fermentation days on the quality of composite fruit and vegetable wines.

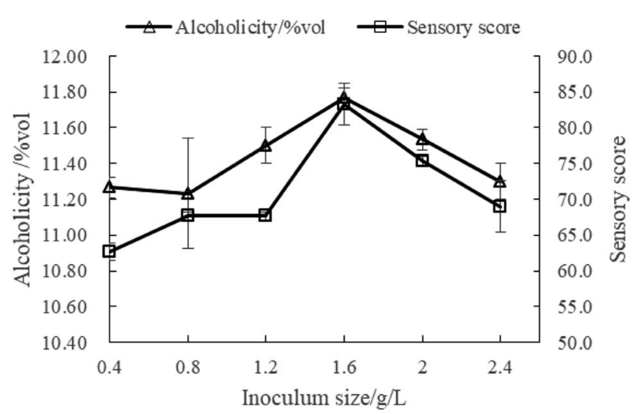

Fig. 3 Effect of the yeast addition amount on the quality of composite fruit and vegetable wines.

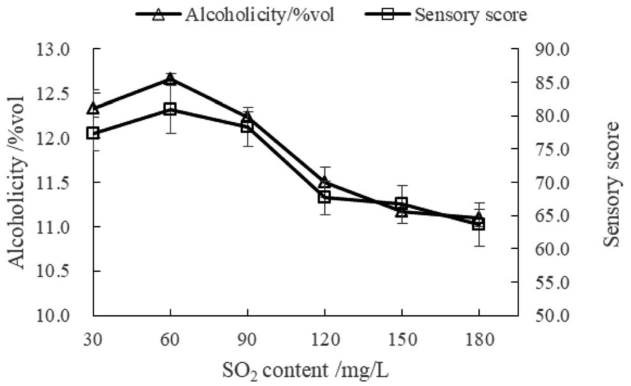

Fig. 4 Effect of SO_2 addition amount on the quality of composite fruit and vegetable wines.

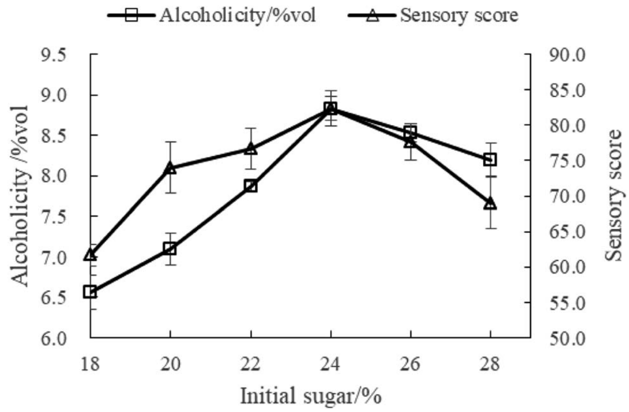

Fig. 5 Effect of the initial sugar content on the quality of composite fruit and vegetable wines.

SO_2 addition amounts were 30 mg L^{-1}, 60 mg L^{-1}, 90 mg L^{-1}, 120 mg L^{-1}, 150 mg L^{-1} and 180 mg L^{-1}. After finishing the fermentation, the alcohol content of the fruit wine was determined and the sensory evaluation was conducted, with the results shown in Fig. 4.

As can be seen from the figure, with the gradual increase in SO_2 addition amount, the alcohol content and sensory score of composite carrot and pomegranate wines show an increasing and then decreasing trend. SO_2 can inhibit the growth of miscellaneous bacteria during the fermentation of fruit wines. When the SO_2 addition amount is 0–60 mg L^{-1}, the growth of other miscellaneous bacteria except yeast is inhibited by SO_2. When the SO_2 addition amount is more than 60 mg L^{-1}, the growth and reproduction of yeast begin to be inhibited, and the alcohol content of the composite fruit wine decreases, while the residual sugar content increases. Therefore, the optimum SO_2 addition amount is 60 mg L^{-1}.

3.1.5 Effect of the initial sugar content on the alcohol content and sensory evaluation of composite fruit and vegetable wines. The effect of the initial sugar content on the quality of composite fruit and vegetable wines was explored. The fermentation duration was 7 d, the fermentation temperature was 24 °C, the yeast addition amount was 1.2 g L^{-1}, the SO_2 addition amount was 120 mg L^{-1}, the volume ratio of carrot juice to pomegranate juice was 1 : 2, and the initial sugar contents were 18%, 20%, 22%, 24%, 26% and 28%. After finishing the fermentation, the alcohol content of the fruit wine was determined and the sensory evaluation was conducted, with the results shown in Fig. 5.

As can be seen from the figure, the sensory score of composite fruit and vegetable wines presents an increasing and then decreasing trend with the increase of the initial sugar content. When the initial sugar content is 24%, the sensory score is at the maximum , and the fermented wine has a pomegranate flavor and mellow wine flavor. However, when the initial sugar content increases, the sensory score decreases, and the remaining residual sugar is excess, thus resulting in a sweet taste. Therefore, the optimum initial sugar content is 24%.

3.1.6 Effect of temperature on the alcohol content and sensory evaluation of composite fruit and vegetable wines. The effect of temperature on the quality of composite fruit and vegetable wines was explored. The fermentation duration was 7 d, the initial sugar content was 24%, the yeast addition amount was 1.2 g L^{-1}, the SO_2 addition amount was 120 mg L^{-1}, the volume ratio of carrot juice to pomegranate juice was 1 : 2, and the fermentation temperatures were 20°C, 22 °C, 24 °C, 26 °C, 28 °C, 30 °C and 32 °C. After finishing the fermentation, the alcohol content of the fruit wine was determined and the sensory evaluation was conducted, with the results shown in Fig. 6.

As can be seen from the figure, the alcohol content and sensory score of composite fruit and vegetable wines also present an increasing and then decreasing trend with an increase in temperature. When the temperature is 28 °C, the alcohol content and sensory evaluation reach the maximum, potentially due to the fact that the optimum fermentation temperature of yeast is 25–28 °C. When the temperature is more than 30 °C, there is a rancid flavor in the wine, due to the fact that such microorganisms as lactic acid bacteria and acetic acid bacteria multiply in large numbers in the environment when

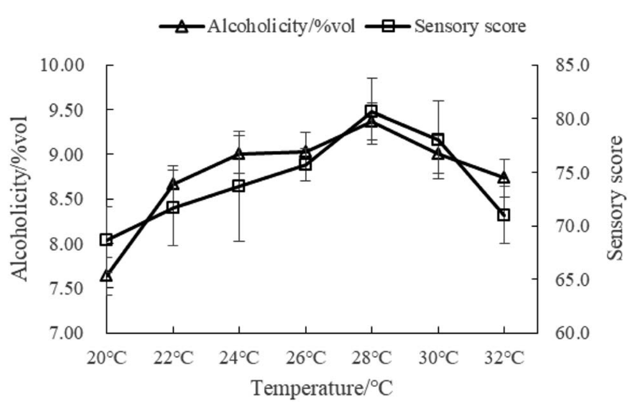

Fig. 6 Effect of temperature on the quality of composite fruit and vegetable wines.

the temperature is more than 30 °C, thus affecting the quality of these wines.

3.2 Results and analysis of response surface methodology

3.2.1 Box–Behnken experiments and results. In order to optimize the fermentation technology of composite carrot and pomegranate wines, the temperature (A), SO_2 addition amount (B) and initial sugar content (C) were selected by a single-factor experiment for the Box–Behnken optimization experiment. The factors and levels of Box–Behnken experiments are listed in Table 2.

The temperature (A), SO_2 addition amount (B) and initial sugar content (C) were selected as the experimental values and the alcohol content was selected as the response value to design Box–Behnken experiments. The experimental results are listed in Table 3 and the variance analysis is listed in Table 4.

3.2.2 Analysis of influencing factors' interaction. As can be seen from the Table 4, the quadratic regression model established by the Box–Behnken design is extremely significant ($P < 0.0001$), which indicates that all experiment points can be described by this model. The determination coefficient R^2 =

Table 2 Factors and levels of Box–Behnken experiments

Level	(A) Temperature/°C	(B) SO_2 addition amount/(mg mL^{-1})	(C) Initial sugar content/%
−1	26	30	22
0	28	60	24
1	30	90	26

Table 3 Design and results of Box–Behnken experiments

Experiment no.	Temperature/°C	SO_2 addition amount/(mg mL^{-1})	Initial sugar content/%	Alcohol content/% vol.
1	30.00	60.00	26.00	11.3
2	28.00	30.00	26.00	11.2
3	28.00	60.00	24.00	12.2
4	26.00	60.00	22.00	10.5
5	30.00	60.00	22.00	11.0
6	28.00	60.00	24.00	12.4
7	28.00	60.00	24.00	12.3
8	28.00	60.00	24.00	12.3
9	26.00	90.00	24.00	11.4
10	28.00	30.00	22.00	10.8
11	30.00	30.00	24.00	11.0
12	28.00	90.00	22.00	10.6
13	30.00	90.00	24.00	11.3
14	26.00	30.00	24.00	11.2
15	26.00	60.00	26.00	11.4
16	28.00	90.00	26.00	11.7
17	28.00	60.00	24.00	12.3

Table 4 Variance analysis of regression model

Source	Sum of squares	Degrees of freedom	Mean square	F value	Prob > F value	Significance
Model	6.22	9	0.69	49.63	<0.0001	**
A	1.250×10^{-3}	1	1.250×10^{-3}	0.090	0.7732	
B	0.08	1	0.08	5.74	0.0477	*
C	0.91	1	0.91	65.42	<0.0001	**
AB	2.500×10^{-3}	1	2.500×10^{-3}	0.18	0.6845	
AC	0.09	1	0.09	6.46	0.0386	*
BC	0.12	1	0.12	8.79	0.0209	*
A^2	1.27	1	1.27	91.44	<0.0001	**
B^2	1.16	1	1.16	83.32	<0.0001	**
C^2	2.06	1	2.06	148.12	<0.0001	**
Residual	0.097	7	0.014			
Lack of fit	0.077	3	0.026	5.17	0.0733	
Pure error	0.02	4	5.000×10^{-3}			
Total	6.32	16				

0.9846 and the adjusted determination coefficient R_{adj}^2 = 0.9647 suggest that this model has good reliability and the regression model can favorably predict the response value. The linear term (C) and the quadratic terms (A^2, B^2 and C^2) are significant ($P < 0.01$), and the interaction terms (AC and BC) are significant ($0.01 < P < 0.05$).

As can be seen from Fig. 7, among the three factors, the interaction between two factors is weak. In contrast, the contour lines between temperature (A) and the initial sugar content (C), and those between the SO_2 addition amount (B) and the initial sugar content (C) show that the highest point of the response surface is elliptical, namely that the interaction between A and C, and between B and C is obvious. Design-Expert 8.0.6 (software) was adopted in combination with the mathematical analysis of the quadratic regression model. It was found that the optimum fermentation parameters of composite carrot and pomegranate wines were as follows: fermentation temperature, 28 °C; SO_2 addition amount, 60 mg L^{-1}; initial sugar content,

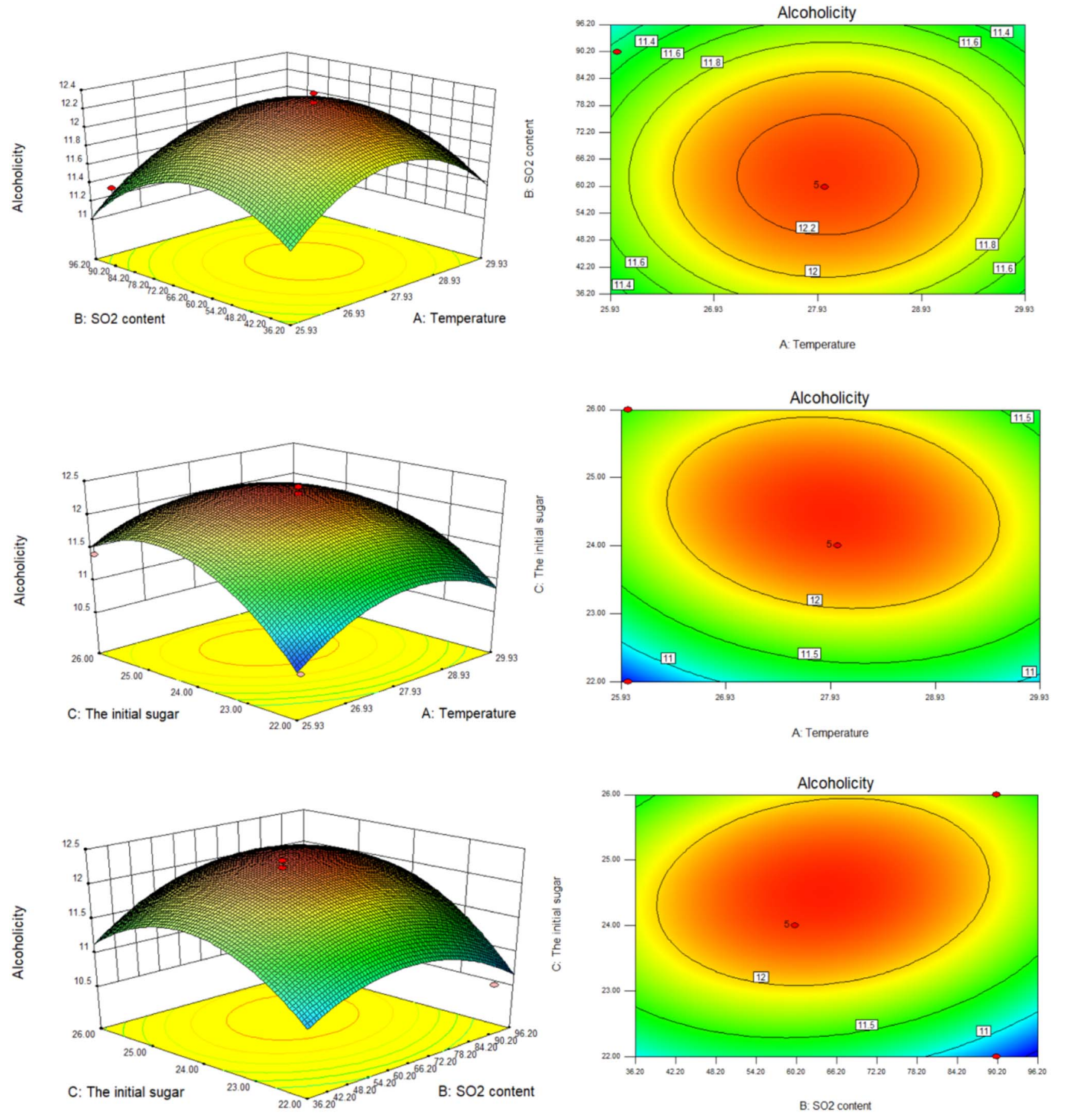

Fig. 7 Response surface diagram.

24%. Under these fermentation conditions, the average alcohol content was 12.35% vol. After three parallel verification tests, the alcohol contents were 12.2% vol., 12.5% vol. and 12.7% vol., respectively, with an average value of 12.47% vol., which was only 0.12% vol. different from the theoretical predicted value. It was verified that the response surface test data were accurate and reliable.

3.3 Analysis of aroma components and principal components of composite fruit and vegetable wines before and after fermentation

3.3.1 Aroma component analysis. The total ion diagrams of aroma components in carrot and pomegranate samples before and after fermentation are shown in Fig. 8 and 9. Through the identification and analysis of each component, a total of 40 main aroma components were detected in the samples before and after fermentation, mainly including 13 esters, 11 alcohols, 2 acids, 4 olefins, 2 alkanes and 9 other components (Table 5). The result was similar to the aroma components of pomegranate wine detected by Lan Y. *et al.*[27] Among the aroma components, there were 9 components (including terpinene-4-ol, alpha-terpineol, isopropyl palmitate, *n*-nonyl alcohol-1, diethyl phthalate, 2-methyl-3-hydroxy-2,2,4-trimethylpentan-1-ol, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, 2-methyl-2-ethyl-3-hydroxycyclohexyl propanoate and 1,2-benzenedicarboxylic acid, bis(2-methylpropyl)ester) detected in the samples before and after fermentation. Additionally, 21 new components were generated during the fermentation, mainly alcohols and olefins.

A total of 19 aroma components were detected in carrot and pomegranate juice before fermentation, among which alcohols (52.42%) and esters (28.49%) had a higher content. Most alcohols had a creamy flavor and fruity flavor, which contributed to the flavor of carrot and pomegranate juice. As the main flavor component of carrot and pomegranate juice, phenethyl alcohol accounted for 49.43% of the total volatile aroma components and endowed carrot and pomegranate juice with a sweet rose-like floral fragrance.

A total of 30 aroma components were detected in composite carrot and pomegranate wines, among which olefins (48.19%) and esters (20.73%) had a higher content. Most olefins had a fruit fragrance and floral fragrance, and their threshold value was low. They played an important role in the flavor of

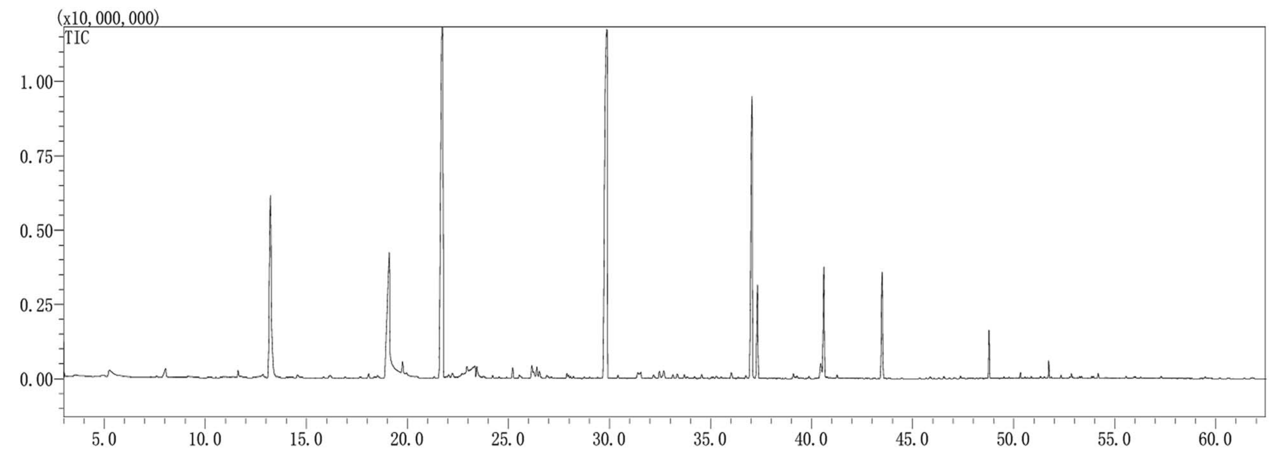

Fig. 8 Total ion diagram of volatile flavor components in samples before fermentation.

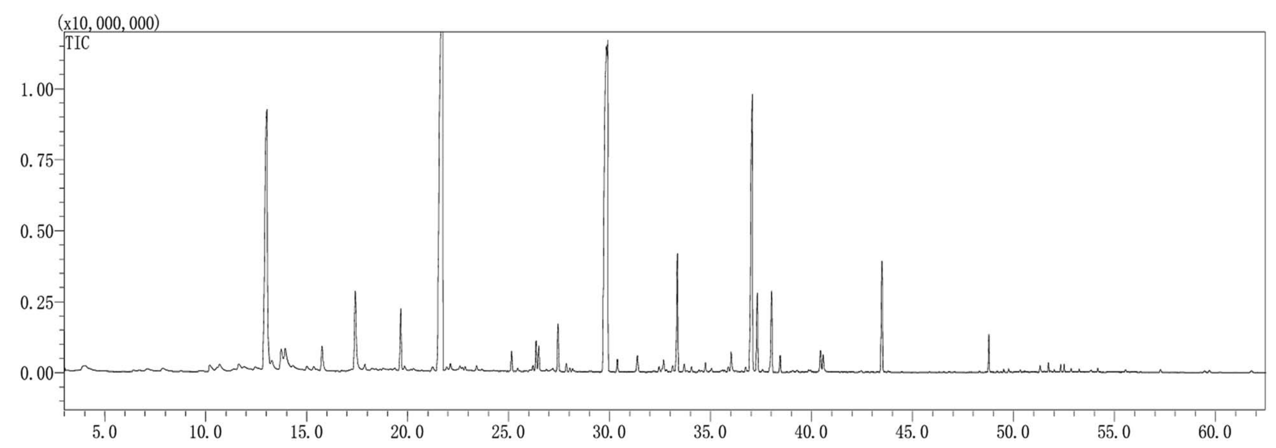

Fig. 9 Total ion diagram of volatile flavor components in samples after fermentation.

composite carrot and pomegranate wines. After fermentation, phenethyl alcohol was almost exhausted. However, the content of olefins was significantly higher than that before fermentation.

Most esters had a distinct floral aroma, which contributed mainly to the aroma formation of composite carrot and pomegranate wines. Among them, bornyl acetate, diethyl phthalate, 2-methyl-3-hydroxy-2 propionate, 2-methyl-3-hydroxy-2,2,4-trimethylpentan-1-ol and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate were the main flavor substances of composite carrot and pomegranate wines. Bornyl acetate, isopropyl palmitate and 3-(2-nitrophenylmethyl)-2-thiazolidinone were newly detected. Bornyl acetate has a cool pine and camphor-like aroma and has antioxidant activity,[39,40] while isopropyl palmitate has a slightly greasy smell.

3.3.2 **Principal component analysis (PCA).** The aroma components in the carrot and pomegranate samples before and after fermentation are shown in Fig. 10. These 26 volatile aroma substances are set as the selection criteria. In the upper right corner of the diagram, caryophyllene is characteristic aroma component of the composite fruit and vegetable wines. In the PCA diagram, the characteristic aroma substance related to composite fruit and vegetable wines is 2-methyl-3-hydroxy-2,2,4-trimethylpentan-1-ol, which occupies the lower right corner of the diagram. As can be seen from the figure, the composite fruit and vegetable wine is composed of many types of aroma substances, with a complex and distinct aroma structure. Esters, aldehydes, ketones, aromatic composites and olefins mainly provide floral, fruity and creamy aromas, which dramatically enrich the flavor of composite carrot and

Table 5 Changes in aroma components of composite carrot and pomegranate wines before and after fermentation

	Number	Composite name	Samples: Carrot and pomegranate juice	Samples: Carrot and pomegranate wine
Esters	a	Diethyl phthalate	2.81	2.73
	b	Acetic acid, 2-phenylethyl ester	2.23	—
	c	Ethyl caprylate	1.93	—
	d	Ethyl caprate	1.12	—
	e	2-Methyl-3-hydroxy-2,2,4-trimethylpentan-1-ol	0.43	2.23
	f	Isopropyl palmitate	0.24	0.52
	g	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	18.41	2.15
	h	2-Methyl-2-ethyl-3-hydroxycyclohexyl propanoate	0.35	1.49
	i	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.11	0.2
	j	Bornyl acetate	—	5.66
	k	3-(2-Nitrophenylmethyl)-2-thiazolidinone	—	0.42
	l	Methyl hydroxybenzoate	—	0.26
Alcohols	m	Terpinene-4-ol	0.71	0.97
	n	Tetrahydro-2,5-dimethyl-2*H*-pyranmethanol	—	0.96
	o	2-(2-Hydroxypropoxy)-1-propyl alcohol	—	0.65
	p	(*S*,*Z*)-Heptathane-1,9-diene-4,6-diyne-3-ol	—	0.48
	q	Alpha-terpineol	0.94	0.48
	r	1-Dodecyl alcohol	—	0.04
	s	Phenethyl alcohol	47.49	—
	t	3-Methylthio-1-propyl alcohol	0.81	—
	u	2-Propyl-1-heptanol	0.28	—
	v	*n*-Nonyl alcohol-1	0.13	0.26
	w	Hydroxyheptane-1	—	0.21
Acids	x	Caprylic acid	2.43	—
	y	*n*-Hexadecanoic acid	—	0.50
Olefins	z	Caryophyllene	—	15.32
	A	(*E*)-1-Methyl-4-(6-methylheptyl-5-en-2-ylidene)cyclohex-1-ene	—	10.48
	B	D-Limonene	—	6.05
	C	Gamma-terpilene	—	4.56
Alkanes	D	Tetradecane	32.60	—
	E	3,7-Dimethyl-nonane	—	0.28
Others	F	2,4-Di-*tert*-butylphenol	—	9.84
	G	*o*-Chlorotoluene	—	4.77
	H	Heptanal	—	0.08
	I	2-Propoxyethanamine	0.11	—
	J	4-Hydroxyacetophenone	—	0.14
	K	Diethylene glycol *tert* butyl ether methyl ether	—	2.25
	L	Humus	—	1.02
	M	4-Isopropoxy-2-butanone	—	0.83
	N	2,4-Bis(1,1-dimethylethyl)-phenol	15.22	—

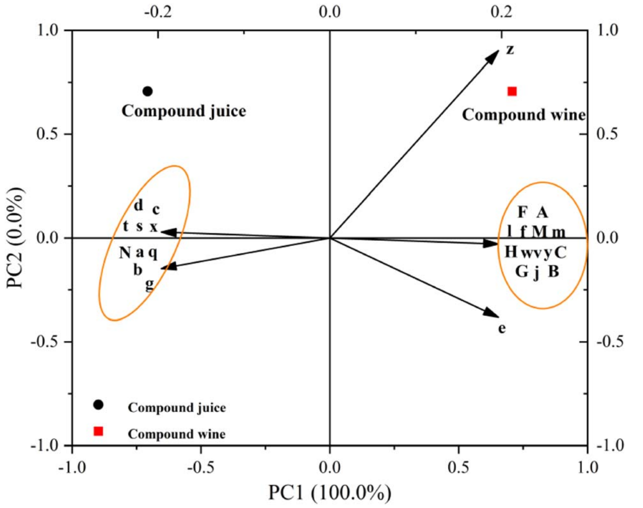

Fig. 10 Principal component analysis diagram.

pomegranate wines. Moreover, there is a significant difference in the intermediate aroma between the carrot and pomegranate juice and the fermented carrot and pomegranate wine, and the results of PCA demonstrate that the fermentation process exerts significant impacts on the flavor of fermented wines.

4. Conclusion

In this study, the fermentation conditions of composite fruit and vegetable wines are optimized by single-factor and response surface experiments. The optimum fermentation conditions of composite carrot and pomegranate wines are identified as follows: temperature, 27.96 °C; initial sugar content, 24.52%; SO_2 addition amount, 64.13 mg L^{-1}. Under these fermentation conditions, the sensory score of the composite carrot and pomegranate wine is 86, and the alcohol content is 12.35% vol. The aroma components of this wine are analyzed and identified by GC-MS. The results indicate that there are 19 main aroma components identified from carrot and pomegranate juice. Among them, alcohols (52.42%) and esters (28.49%) are the main components; phenethyl alcohol accounts for 49.43% of the total aroma components, and it is the main flavor component of carrot and pomegranate juice. Additionally, a total of 28 main aroma components are identified from the composite carrot and pomegranate wine. The main aroma components are caryophyllene, 2,4-di-*tert*-butylphenol, (*E*)-1-methyl-4-(6-methylheptyl-5-en-2-ylidene)cyclohex-1-ene and bornyl acetate. The results of PCA indicate that the fermentation process can exert significant impacts on the flavor of fermented wines. The fermentation conditions of the composite carrot and pomegranate wine identified in this study can provide a reference for the manufacture of composite fruit and vegetable wines in the future. However, this study did not conduct a detailed follow-up study on the changes of nutrients in the fermentation process of compound fruit wine. Therefore, the transformation of nutrients should be paid attention to in the next study, and the function of compound fruit wine should be evaluated.

Data availability

The data presented in this study are available on request from the corresponding author.

Author contributions

Conceptualization, Y. L. and C. P.; writing – original draft preparation, Y. L.; writing – review and editing, supervision, S. H. and C. P.; investigation, Y. W., H. L., F. L. and M. S.; validation, Z. L. and T. Z.; funding acquisition, Y. W. and S. H.; project administration, C. P. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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