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Optimization of Main Ingredient Ratio, Metabolomics Analysis, and Antioxidant Activity Analysis of Lycopene-Enriched Compound Fruit Wine

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Abstract: To find the optimal main ingredient ratio of compound fruit wine for enriching the varieties of lycopene-enriched fruit products and improving their economic value, fuzzy mathematics sensory evaluation and the D-optimal mixture design were considered. Under the main ingredient ratios of tomato juice, papaya juice, carrot juice, and gac fruit juice of 27.2%, 27.5%, 10.0%, and 35.3%, respectively, a clear and transparent compound fruit wine with a full-bodied fruit and wine aroma and mellow taste can be obtained. Meanwhile, a total of 406 metabolites were identified in the compound fruit wine, which were classified into nine superclasses including lipids and lipid-like molecules (150), organic acids and derivatives (69), and others. The relative levels of 54 metabolites after optimization were decreased significantly (VIP > 1.0, $p < 0.05$, FC < 0.5), while the relative levels of 106 metabolites including lycopene and (13Z)-lycopene were increased significantly (VIP > 1.0, $p < 0.05$, FC > 2). Furthermore, the EC_{50} values of this compound fruit wine after optimization of the main ingredient ratio for scavenging $ABTS^{+}\cdot$, DPPH·, $O_2^{-}\cdot$, and ·OH were 78.62%, 57.74%, 42.85%, and 59.91%, respectively. Together, a compound fruit wine rich in lycopene with antioxidant activities was manufactured, which has application potential in the development of functional foods.

Keywords: antioxidant activity; compound fruit wine; fuzzy mathematics; lycopene; sensory score

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1. Introduction

Lycopene is a kind of natural red open-chain hydrocarbon carotene with a strong antioxidant capacity in nature, and its antioxidant activity is higher than that of vitamin E [1–4]. And lycopene has physiological functions such as preventing cancer [5–9], protecting the cardio-cerebrovascular system [10–12], and improving immunity [13–15]. Since neither humans nor animals can produce natural lycopene, food is the only source of acquisition, which is widely distributed in tomatoes, soft-shelled turtles, carrots, watermelons, papaya, mangoes, grapes, guava, citrus, and other fruits [16–18], while tomato is an important, healthy, and functional vegetable food, which can supplement a large number of elements needed by the human body. In edible fruits and vegetables, it is widely used in food and health products [19,20]. Papaya is rich in lycopene, which can effectively control blood lipids and can be used as a natural substitute for expensive cholesterol-lowering drugs [21,22]. Carrots are rich in nutrients, including carotenoids, phenols, and flavonoids, and are very healthy functional vegetables [23,24]. Gac fruit is known to have the highest content of β-carotene and lycopene [25,26]. Therefore, how to develop these fruits into lycopene-rich products is particularly important.

Compound fruit wine is made from fruits commonly eaten in daily life. It contains a variety of nutrients, antioxidants, and organic acids, which can regulate the metabolism of the human body, and it occupies a place with fierce competition in the wine market with its unique flavor and quality [27,28], while compound fruit wine is a low-alcohol beverage made from two or more fruits. After optimization of the fermentation process, it can synthesize the aroma, flavor substances, and nutrients of several raw materials to enrich the aroma and taste of the product [27,29]. However, few studies have focused on the lycopene content in compound fruit wine and the correlation between antioxidant activity and sensory characteristics of lycopene-enriched compound fruit wine.

Applying the calculation method of fuzzy mathematics to the sensory evaluation of food can effectively avoid errors generated during traditional sensory evaluation, thereby improving the accuracy of the results [30]. To enhance the reliability of experiments, researchers often use the D-optimal mixture design to optimize the material formula of food [31], while previous research has found that the compound fruit wine fermented from tomato juice (25%), papaya juice (25%), carrot juice (25%), and gac fruit juice (25%) had better sensory characteristics. Therefore, using the sensory evaluation method of fuzzy mathematics and the D-optimal mixture design to explore compound fruit wine with good sensory quality and antioxidant activity is of both scientific and commercial interest.

To provide a certain reference value for the development and utilization of lycopene-enriched fruit, e.g., tomato, papaya, carrot, and gac fruit, and to provide data support and guidance for the research and development of new compound fruit wine, we used tomato, papaya, carrot, and gac fruit as raw materials, the sensory evaluation method of fuzzy mathematics, and the D-optimal mixture design to explore the optimal main ingredient ratio of compound fruit wine when sensory score and lycopene content were highest, while the metabolites and antioxidant activities of lycopene-enriched compound fruit wine were analyzed.

2. Materials and Methods

2.1. Materials and Reagents

Fresh and ripe tomato, papaya, carrot, gac fruit, and sucrose were purchased in the Wal-Mart supermarket in Yibin City, Sichuan Province, China; *Saccharomyces cerevisiae* CICC31482 was preserved in the Strain Preservation Center of Yibin Vocational and Technical College.

Pectinase (10^5 U/mL) was purchased in Shanghai Jie Rabbit Co., Ltd. (Shanghai, China); potassium metabisulfite (food grade) was purchased in Guilin Bao Diamond Food Co., Ltd. The commercial kits for in vitro antioxidant activity analysis including scavenging activities of scavenging diammonium 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radicals ($ABTS^+\cdot$), 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH·), superoxide anion radicals ($O_2^-\cdot$), and hydroxyl radicals (·OH) were purchased from Grace Biotechnology Co., Ltd. (Suzhou, China).

2.2. The Fermentation Process of Compound Fruit Wine

The fermentation process of compound fruit wine is shown in Figure 1.

The main points of operation were as follows: Fresh and ripe tomato, papaya, carrot, and gac fruit were juiced separately, then 20 U/g of pectinase was added for clarification treatment at 40 °C for 3 h and then filtered and mixed according to the volume ratio needed in the experiment. Sucrose was added to adjust the soluble solid content to 22 °Brix, potassium metabisulfite was added to adjust the total acid to 0.90%, and 5% yeast (*Saccharomyces cerevisiae* CICC31482) suspension (1.32×10^7 cfu/mL) was inoculated by volume fraction. After static fermentation at 23 °C for 7 days, the finished product was obtained after the filtration and clarification of diatomite, and pasteurization (65 °C, 30 min).

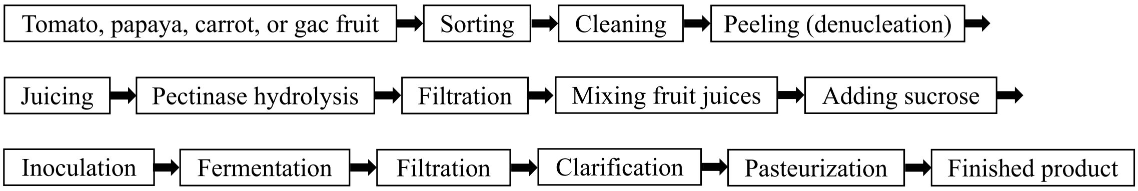

Figure 1. The fermentation process of compound fruit wine.

2.3. *Response Surface Experiment on the Main Ingredient Ratio of Compound Fruit Wine*

Taking the additional amount of tomato juice (A), papaya juice (B), carrot juice (C), and gac fruit juice (D) as the independent variable, A, B, C, and D were set as four parameters, in which $A + B + C + D = 100\%$. The content of lycopene and sensory score were used as response values by the D-optimal mixture design test. The range of the four juices was 10% to 70%.

2.4. *Determination of Lycopene Content in Compound Fruit Wine*

The extraction process of lycopene was as follows: 10 mL of compound fruit wine or fruit juice was accurately absorbed into a 100 mL triangle flask, and 15 mL of 95% ethanol was added for pretreatment for 30 min. The precipitate was centrifuged at 13,000$\times$ g/min for 10 min (4 °C), and the obtained precipitate was extracted with petroleum ether in a thermostatic oscillator at 30 °C and 150$\times$ g/min for 4 h without light. After extraction and standing for 10 min in the dark, the organic phase was lycopene extract. The lycopene content was determined according to the previously established method [32].

2.5. *Sensory Evaluation of Compound Fruit Wine*

The sensory evaluation of the compound fruit wine was examined by a sensory evaluation panel consisting of 10 (5 men and 5 women) wine-tasters according to the Chinese standard (GB/T 15038—2006) [33], as described in a previous study [34]. As shown in Table 1, the sensory evaluation of compound fruit wine was divided into four parts with a full score of 100. This study was reviewed and approved by the Yibin Vocational and Technical College IRB and informed consent was obtained from each subject prior to their participation in the study.

Table 1. Sensory evaluation standard of compound fruit wine.

Index	Evaluation Standard			
	Excellent	**Good**	**General**	**Worse**
Appearance (20)	Orange red, clear and transparent (16–20)	Orange yellow, relatively clear, no suspended matter (10–15)	Brown, slightly turbid (6–10)	Brown, turbid with obvious suspended matter (0–5)
Aroma (25)	The aroma of fruit and wine is full-bodied and harmonious (19–25)	The aroma of fruit and wine is rich, general harmonious (13–18)	The aroma of fruit and wine is not enough, with a slight peculiar smell (7–12)	The aroma of fruit and wine is insufficient and the aroma is not harmonious (0–6)
Taste (35)	The wine is full-bodied, refreshing (28–35)	The wine is soft, refreshing (19–27)	The wine is softer, less refreshing and has heterogenous taste (10–18)	The wine is light, tasteless, and has obvious heterogenous taste (0–9)
Typicality (20)	Elegant and unique style (16–20)	Good style (10–15)	General style (6–10)	Style is not typical (0–5)

2.6. Establishment of Sensory Evaluation Model of Fuzzy Mathematics

Let the evaluation grade be V (Equation (1)).

$$V = (V_1, V_2, V_3, V_4) \tag{1}$$

V_1 represented excellent, V_2 represented good, V_3 represented general, and V_4 represented worse, and the corresponding scores were 100, 75, 50, and 25.

Appearance, aroma, taste, and typicality were the factors that constitute the sensory evaluation of compound fruit wine. The evaluation weight (X) (Equation (2)) was obtained according to the subjective weighting method [30] and the sensory evaluation of compound fruit wine (Table 1).

$$X = (X_1, X_2, X_3, X_4) = (0.20, 0.25, 0.35, 0.20) \tag{2}$$

Ten sensory evaluators evaluated the compound fruit wine one by one according to the four factors of appearance, aroma, taste, and typicality; and the votes of each quality factor were counted in each grade. The sensory evaluation fuzzy relation matrix (R) was obtained by normalization; the membership matrix was then established according to the index data of each group; and the sensory score (Y) (Equation (3)) was obtained according to the principle of fuzzy transformation [30].

$$Y = XRV^T \tag{3}$$

2.7. Physicochemical Indexes and Metabolomics Analysis of Compound Fruit Wine

The contents of alcohol by volume (ABV), total sugar, total acid, and dry extract in the compound fruit wine before optimization (BO) and after optimization (AO) of the main ingredient ratio were performed according to Chinese standard (GB/T 15038—2006) [33]. Metabolites in BO and AO were extracted and analyzed using an ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS)-based metabolomics approach performed by Shanghai Meiji Biomedical Technology Co., Ltd., Shanghai, China. Meanwhile, quality control (QC) samples were prepared by mixing sample extracts to examine the repeatability of the analysis.

The extraction of samples, UPLC separation and ESI-MS/MS monitoring (UPLC, Shim-pack UFLC Shimadzu CBM30A system, MS, Applied Biosystems 4500 Q-Trap), and data processing were performed as described previously [35]. Metabolites were characterized by searching internal and public databases (MassBank, KNApSAcK, HMDB, MoTo DB, and METLIN) and comparing their m/z values, retention times, and fragmentation patterns with those of the standards [36]. The chromatographic peak area of each was calculated. Positive and negative data were combined to obtain a combined data set.

2.8. Antioxidant Activity Assays

The antioxidant activity in vitro of BO and AO was evaluated using the following assays: scavenging activities of $ABTS^{+}\cdot$, $DPPH\cdot$, $O_2^{-}\cdot$, and $\cdot OH$. These activities were determined using commercial kits according to the manufacturer's instructions, as described previously [34].

For analysis of the scavenging activities (SC) of $ABTS^{+}\cdot$, 50 μL of compound fruit wine was mixed with ABTS solution (950 μL) and ethanol (950 μL) to produce test (T) and control solutions (C), respectively. The blank solutions (B) contained a mixture of ABTS solution (950 μL) and ethanol (50 μL). These solutions were held for 30 min in the dark at room temperature and the absorbance at 734 nm was measured. The SC of $ABTS^{+}\cdot$ was calculated using the following equation: SC (%) = $[(1 - (A_T - A_C)/A_B) \times 100]$.

To measure the SC of $DPPH\cdot$, 400 μL of compound fruit wine was mixed with DPPH solution (600 μL) or ethanol (600 μL) to produce test (T) and control solutions (C), respectively. The blank solution (B) contained a mixture of DPPH solution (600 μL) and ethanol (400 μL). These solutions were held for 30 min in the dark at room temperature and centrifuged for 5 min at 12,000 rpm. The absorbance at 517 nm of the supernatant was then measured. The SC of DPPH· was calculated using the following equation: SC (%) = $[(1 - (A_T - A_C)/A_B) \times 100]$.

To measure the SC of $O_2^-\cdot$, solution I (260 μL), solution II (320 μL), solution III (40 μL), and solution IV (60 μL) were mixed with compound fruit wine (40 μL) or ddH_2O (40 μL) to produce test (T) and control solutions (C), respectively. The blank solution (B) contained a mixture of ddH_2O (80 μL), solution I (260 μL), solution II (320 μL), and solution IV (60 μL). The mixtures were held for 10 min at 37 °C and their absorbances at 570 nm were measured. The SC of $O_2^-\cdot$ was calculated using the following equation: SC (%) = $(A_C - A_T)/(A_C - A_B) \times 100$.

For analysis of the SC of ·OH, compound fruit wine (125 μL) was mixed with ddH_2O (500 μL), solution I (125 μL), solution II (125 μL), and solution III (125 μL) to produce the test solution (T). The control solution (C) contained a mixture of compound fruit wine (125 μL), ddH_2O (625 μL), solution I (125 μL), and solution II (125 μL). The blank solution (B) contained a mixture of ddH_2O (625 μL), solution I (125 μL), solution II (125 μL), and solution III (125 μL). These mixtures were held for 20 min at 37 °C and their absorbances at 510 nm were measured. The SC of ·OH was calculated using the following equation: SC (%) = $[A_B - (A_T - A_C)]/A_B) \times 100]$.

2.9. Data Processing

One-way analysis of variance (ANOVA) with the least-significant difference (LSD) method ($p < 0.05$) was applied to compare the data by SPSS Statistics 20.0, and response surface analysis was performed using Design Expert 12.0 [37]. Orthogonal partial least square discriminant analysis (OPLS-DA) results were generated by SIMCA 14.1 to visualize the metabolic differences between the experimental groups, after normalization and standardization processing. Variable importance in projection (VIP) analysis ranked the overall contribution of each variable to the OPLS-DA model, and those variables with VIP > 1.0, $p < 0.05$, and fold change (FC) > 2 or < 0.5 were classified as differentially changed metabolites (DCMs) [38].

3. Results and Discussion

3.1. Comprehensive Sensory Evaluation Results of Fuzzy Mathematics

Ten sensory evaluation experts evaluated the appearance, aroma, taste, and typicality of BO. According to the test results, 5 experts in the 10 sensory evaluation groups thought that the appearance grade of BO was excellent, 2 experts thought good, 2 experts thought general, and 1 expert thought worse. So, $R_{\text{Appearance}}$ = (0.5,0.2,0.2,0.1). Similarly, R_{Aroma} = (0.6,0.1,0.2,0.1), R_{Taste} = (0.5,0.3,0.1,0.1), and $R_{\text{Typicality}}$ = (0.7,0.1,0.1,0.1). Convert the above indicators into a matrix, namely: R_{BO}. According to the principle of fuzzy change $Y = XRV^{\text{T}}$, the evaluation result of Y_{BO} was as follows:

$$Y_{BO} = XR_{BO}V^T = (0.20, 0.25, 0.35, 0.20) \times \begin{pmatrix} 0.5, 0.2, 0.2, 0.1 \\ 0.6, 0.1, 0.2, 0.1 \\ 0.5, 0.3, 0.1, 0.1 \\ 0.7, 0.1, 0.1, 0.1 \end{pmatrix} \times \begin{pmatrix} 100 \\ 75 \\ 50 \\ 25 \end{pmatrix} = 80.5$$

The score of the single-valued fuzzy vector of BO was 80.5. The comprehensive fuzzy evaluation of 20 experimental groups was carried out by this method, and the results are shown in Table 2.

Table 2. Sensory evaluation standard of compound fruit wine.

Test No.	*A*: Tomato Juice/%	*B*: Papaya Juice/%	*C*: Carrot Juice/%	*D*: Gac Fruit Juice/%	Lycopene Content/ (μg/mL)	Sensory Evaluation
1	10.0	10.0	70.0	10.0	16.28	81.8
2	10.0	10.0	40.0	40.0	17.92	91.0
3	17.5	17.5	17.5	47.5	17.99	91.8
4	40.0	40.0	10.0	10.0	16.84	85.5
5	10.0	40.0	10.0	40.0	17.80	90.4
6	40.0	40.0	10.0	10.0	16.93	85.9
7	10.0	10.0	40.0	40.0	17.77	90.2
8	70.0	10.0	10.0	10.0	17.15	87.1
9	10.0	40.0	40.0	10.0	15.88	80.6
10	17.5	47.5	17.5	17.5	16.39	83.3
11	10.0	10.0	10.0	70.0	17.96	92.5
12	47.5	17.5	17.5	17.5	17.66	89.6
13	17.5	17.5	47.5	17.5	16.48	83.7
14	40.0	10.0	10.0	40.0	17.60	89.3
15	40.0	10.0	40.0	10.0	17.34	88.1
16	10.0	70.0	10.0	10.0	15.73	79.9
17	40.0	10.0	10.0	40.0	17.37	88.2
18	10.0	40.0	40.0	10.0	15.92	80.8
19	30.0	30.0	30.0	10.0	16.61	84.4
20	10.0	40.0	10.0	40.0	17.55	89.1

3.2. Response Surface Results of Lycopene Content and the Sensory Score of Compound Fruit Wine with Different Main Ingredient Ratios

Taking lycopene content and the sensory score of compound fruit wine as the response value, according to the D-optimal mixture design principle, a response surface test was designed by DesignExpert 12.0 software to investigate the effects of tomato juice (*A*), papaya juice (*B*), carrot juice (*C*), and gac fruit juice (*D*) on lycopene content and the sensory score of compound fruit wine. The results of the response surface test are shown in Table 2 and the analysis of variance is shown in Table 3.

Table 3. Analysis of variance of regression model by response surface methodology.

Source	Sum of Squares		Degree of Freedom		Mean Square		*F* Value		*p* Value		Significance	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Model	10.55	295.66	13	13	0.81	22.74	42.7	46.7	<0.001	<0.001	**	**
Linear Mixture	8.3	241.06	3	3	2.77	80.35	145.58	165.01	<0.001	<0.001	**	**
AB	0.21	5.04	1	1	0.21	5.04	10.83	10.34	0.017	0.018	*	*
AC	0.26	8.64	1	1	0.26	8.64	13.7	17.74	0.01	0.006	*	**
AD	<0.01	1.15	1	1	<0.01	1.15	0.29	2.36	0.61	0.176		
BC	<0.01	0.03	1	1	<0.01	0.03	0.49	0.05	0.509	0.829		
BD	0.68	12.46	1	1	0.68	12.46	35.9	25.58	0.001	0.002	**	**
CD	0.52	11.42	1	1	0.52	11.42	27.35	23.45	0.002	0.003	**	**
ABC	0.04	1.09	1	1	0.04	1.09	2.19	2.25	0.19	0.185		
ABD	0.13	3.03	1	1	0.13	3.03	6.87	6.23	0.04	0.047	*	*
ACD	0.02	0.52	1	1	0.02	0.52	0.93	1.06	0.371	0.343		
BCD	0.3	6.49	1	1	0.3	6.49	15.65	13.32	0.008	0.011	**	*
Residual	0.11	2.92	6	6	0.02	0.49						
Lack of fit	0.04	1.05	1	1	0.04	1.05	2.73	2.81	0.16	0.154		
Pure terror	0.07	1.87	5	5	0.02	0.37						
Cor total	10.66	298.59	19	19								

**: highly significant difference at the 0.01 level, *: significant difference at the 0.05 level.

The data in Table 2 were fitted by multiple regression by DesignExpert software, and the regression equations of lycopene content (Y_1 (μg/L)) and sensory score (Y_2) of compound fruit wine were obtained:

$$Y_1 = 17.13A + 15.71B + 16.26C + 18.00D + 1.81AB + 2.50AC - 0.30AD - 0.39BC + 3.30BD + 2.88CD - 6.51ABC + 41.13ABD + 15.32ACD - 62.07BCD \quad (4)$$

$$Y_2 = 87.01A + 79.81B + 81.81C + 92.71D + 8.97AB + 14.40AC - 4.29AD - 0.63BC + 14.11BD + 13.51CD - 33.42ABC + 198.14ABD + 82.70ACD - 289.86BCD \quad (5)$$

According to the analysis of variance, the interaction items *AB*, *AC*, and *ABD* had significant effects on lycopene content in compound fruit wine ($p < 0.05$), while *BD*, *CD*, and *BCD* had extremely significant effects on lycopene content ($p < 0.01$). The effects of interaction items *AB*, *ABD*, and *BCD* on the sensory score of compound fruit wine were significant ($p < 0.05$), while the effects of interaction items *AC*, *BD*, and *CD* on the sensory score of compound fruit wine were extremely significant ($p < 0.01$). The results showed that the lycopene and the sensory score of compound fruit wine could be determined by using the above two regression equations.

Meanwhile, the lycopene contents of the four raw materials were also analyzed; among them, the lycopene contents of tomato juice, papaya juice, carrot juice, and gac fruit juice were (110.38 ± 0.75) μg/L, (31.46 ± 0.40) μg/L, (29.32 ± 0.29) μg/L, and (134.53 ± 1.04) μg/L, respectively. Interestingly, papaya juice, carrot juice, and gac fruit juice, which were composed of the top two absolute values of the difference between the lycopene content of the two juices (*CD* and *BD*), had extremely significant effects on lycopene content ($p < 0.01$), while *BCD* had extremely significant effects on lycopene content ($p < 0.01$).

The contour lines formed by the interaction of different main ingredient ratios on lycopene content and sensory score are shown in Figure 2 and Figure 3, respectively.

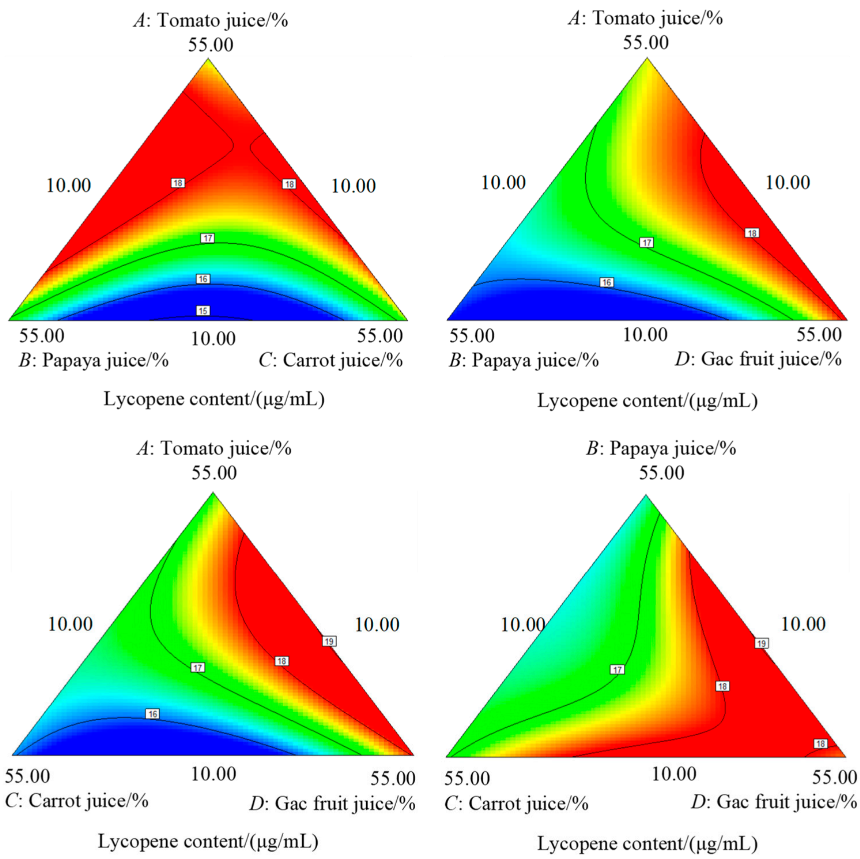

Figure 2. Contour map of the interaction of main ingredient ratio on the lycopene content.

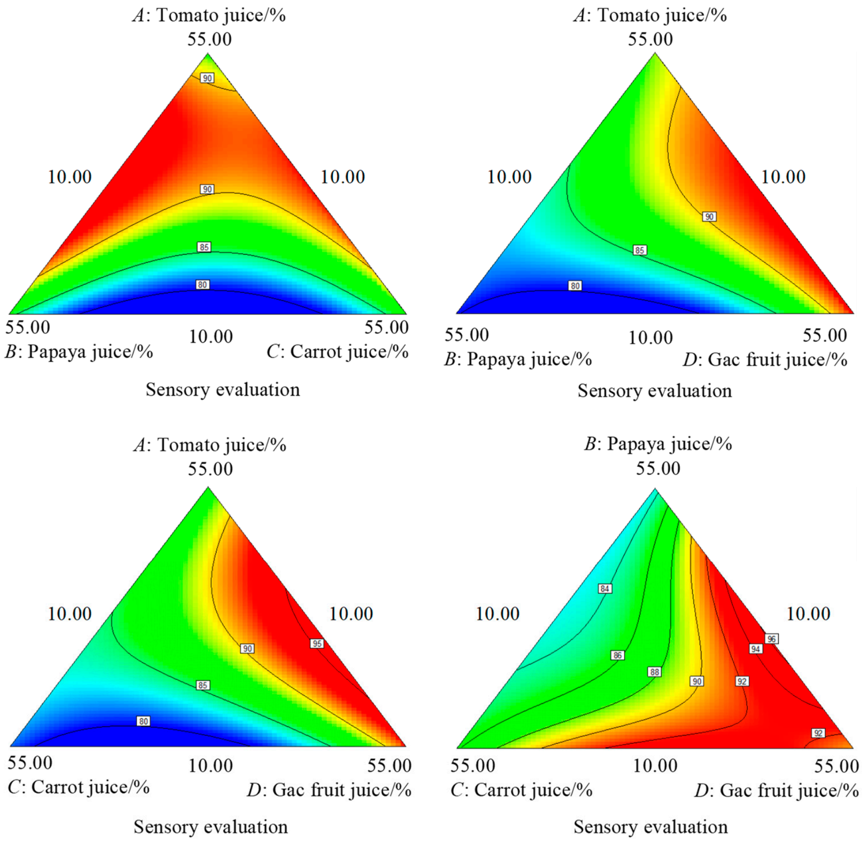

Figure 3. Contour map of the interaction of main ingredient ratio on the sensory score.

The greater the amount of elliptical contour lines of the response surface graph, the greater the interaction between these factors [39]. And the contour lines formed by the interaction between factors *B* (papaya juice), *C* (carrot juice), and *D* (gac fruit juice) on lycopene content (Figure 2) and sensory score (Figure 3) were more elliptical than the interaction of other factors. The results showed that the interaction of *B*, *C*, and *D* had the greatest effect on lycopene content and the sensory score of compound fruit wine. The results were consistent with the results of the analysis of variance in Table 3. The effects of interaction term *BCD* on lycopene content and the sensory score of compound fruit wine were extremely significant ($p < 0.01$) and significant ($p < 0.05$), respectively.

3.3. Optimization and Verification Test of Main Ingredient Ratio of Lycopene-Enriched Compound Fruit Wine

According to the response surface software analysis, the highest theoretical value of lycopene content was 19.0597 μg/L under the conditions of tomato juice at 27.747%, papaya juice at 27.909%, carrot juice at 10.000%, and gac fruit juice at 34.344%. Under the conditions of 27.188% tomato juice, 27.539% papaya juice, 10.000% carrot juice, and 35.273% gac fruit juice, the highest theoretical sensory score of 96.2711 was obtained.

For the operability of the experiment, three groups of parallel experiments were carried out on 27.8% tomato juice, 27.9% papaya juice, 10.0% carrot juice, 34.3% gac fruit juice; and 27.2% tomato juice, 27.5% papaya juice, 10.0% carrot juice, and 35.3% gac fruit juice. The results showed that the highest lycopene content and sensory score were obtained under the conditions of 27.2% tomato juice, 27.5% papaya juice, 10.0% carrot juice, and 35.3% gac fruit juice. The highest lycopene content and sensory score were (19.01 ± 0.77) μg/L and 95.2 ± 0.2, respectively.

Although the lycopene content of this compound fruit wine was lower than that of the four fruit juices, it formed a product with clear and transparent appearance, full-bodied fruit and wine aroma, and mellow taste, while a lycopene-enriched compound fruit wine with excellent sensory characteristics was first reported. Due to the easy oxidation of lycopene [40,41], the polymerization or copigmentation reactions of the coloring compounds in wine [42], and the association of the coloring compounds with the cellular walls of yeasts [43], we should try to protect it in the fermentation process of compound fruit wine in the later stage.

3.4. Physicochemical Indexes and Metabolites in Compound Fruit Wines by Metabolomics Approach

As can be seen from Table 4, the physicochemical indexes of AO were not significantly different from those of BO ($p > 0.05$), except for the dry extract. And the physicochemical indexes met the requirements of the Chinese standard (GB/T 15038—2006) [33], indicating that this fermentation technology was relatively stable.

Table 4. Physicochemical indexes of compound fruit wine in AO and BO.

	AO	BO
ABV/%	8.71 ± 0.18	8.69 ± 0.21
Total sugar/(g/L)	7.66 ± 0.13	7.70 ± 0.16
Total acid/(g/L)	6.03 ± 0.10	6.07 ± 0.14
Dry extract/(g/L)	36.89 ± 0.86 ^{b} [1]	39.44 ± 0.91 ^{a}

[1] Different lowercase superscripts in a row indicate significant differences at $p < 0.05$.

Metabolomics is broadly applied in food science and has tremendous potential for establishing correlations between wine metabolites and quality characteristics [44,45]. Therefore, the changes in metabolites of the compound fruit wine before and after optimization of the main ingredient ratio should be analyzed.

A total of 406 metabolites were identified (Figure 4A and Table S1), which were classified into nine superclasses, while the major metabolites included lipids and lipid-like molecules (150 metabolites), organic acids and derivatives (69 metabolites), and organic oxygen compounds (51 metabolites); these were followed by organoheterocyclic compounds (39 metabolites), benzenoids (25 metabolites), phenylpropanoids and polyketides (18 metabolites), organic nitrogen compounds (7 metabolites), nucleosides, nucleotides, and analogs (2 metabolites) and others (45 metabolites). They were further grouped into 40 classes, including fatty acyls (84 metabolites), carboxylic acids and derivatives (57 metabolites), organooxygen compounds (51 metabolites), prenol lipids (37 metabolites), and benzene and substituted derivatives (18 metabolites).

To gain an overview of the DCMs between the AO and BO, we developed a new OPLS-DA of metabolites (Figure 4B). In the comparison of AO to BO, the relative levels of 54 DCMs decreased significantly (VIP > 1.0, $p < 0.05$ and FC < 0.5); among them, the relative levels of 2 DCMs decreased more than 10-fold, e.g., 3-hydroxydodecanedioic acid and [2-hydroxy-5-(3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-1-benzopyran-2-yl) phenyl] oxidanesulfonic acid. Meanwhile, the relative levels of 106 DCMs including lycopene and (13Z)-lycopene increased significantly (VIP > 1.0, $p < 0.05$ and FC > 2); among them, the relative levels of 21 DCMs increased more than 10-fold, e.g., 3,4-dimethyl-5-propyl-2-furantridecanoic acid, N-ethyl trans-2-cis-6-nonadienamide, 3′-hydroxyhexobarbital, oxoglutaric acid, ipomeatetrahydrofuran, phenylethylamine, alpha-D-galacturonic acid, galactosylglycerol, PS(18:1(9Z)/0:0), 13,14-dihydro PGF-1a, alanyl-proline, glycylleucine, 9-pentadecenoic acid, natamycin, 3-isopropylmalate, 4-oxoretinol, tyramine, 1-oleoylglycerophosphoinositol, serylisoleucine, threoninyl-valine, and 5-methyl-2-furaldehyde. Interestingly, the relative levels of five dipeptides, including alanyl-proline, serylisoleucine, threoninyl-valine, phenylalanyl-valine, and tyrosyl-valine, increased significantly (VIP > 1.0, $p < 0.05$ and FC > 2) in AO/BO. Dipeptide is a biologically active peptide with antioxidant activity, anti-inflammatory properties, and fatigue resistance [46,47]. Therefore, the metabolites in the optimized compound fruit wine changed significantly, resulting in changes in sensory characteristics, while optimized compound fruit wine significantly increased the relative levels of lycopene and dipeptides with antioxidant activity. And the antioxidant activity of this compound fruit wine should be further subjected to analysis.

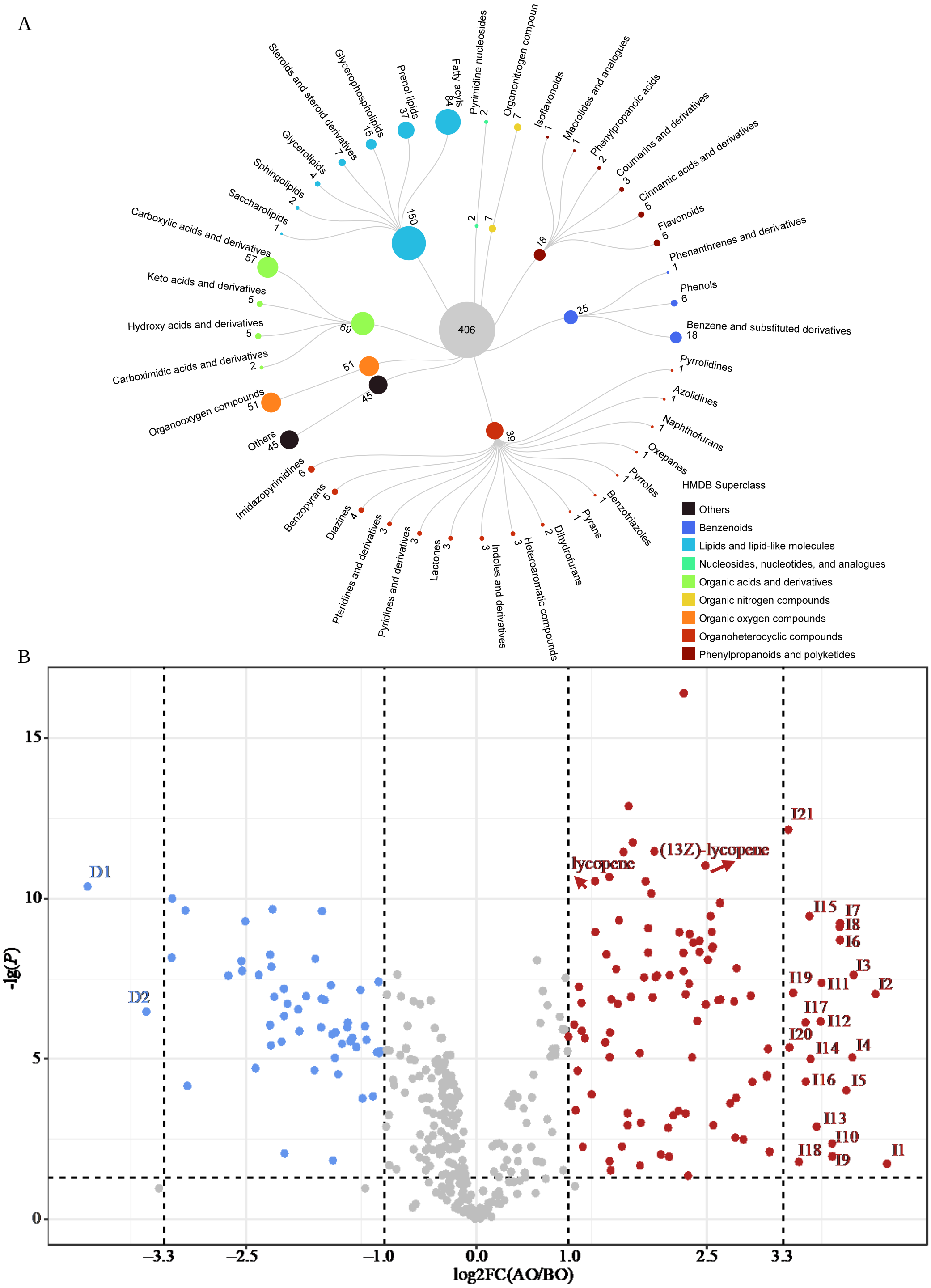

Figure 4. The classification of identified metabolites in AO, BO, and QC (**A**), and DCMs in the compound fruit wine after optimization of main ingredient ratio (**B**). D1-2: 3-hydroxydodecanedioic acid, [2-hydroxy-5-(3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-1-benzopyran-2-yl)phenyl] oxidanesulfonic acid; I1-21: 3,4-dimethyl-5-propyl-2-furantridecanoic acid, N-ethyl trans-2-cis-6-nonadienamide, 3′-hydroxyhexobarbital, oxoglutaric acid, ipomeatetrahydrofuran, phenylethylamine, alpha-D-galacturonic acid, galactosylglycerol, PS(18:1(9Z)/0:0), 13,14-dihydro PGF-1a, alanyl-proline, glycylleucine, 9-pentadecenoic acid, natamycin, 3-isopropylmalate, 4-oxoretinol, tyramine, 1-oleoylglycerophosphoinositol, serylisoleucine, threoninyl-valine, 5-methyl-2-furaldehyde.

3.5. Antioxidant Activity Analysis of Lycopene-Enriched Compound Fruit Wine

In vitro assays showed good scavenging activities for $ABTS^{+}$·, DPPH·, O_2^{-}·, and ·OH of compound fruit wine with a dose-dependent behavior (Figure 5).

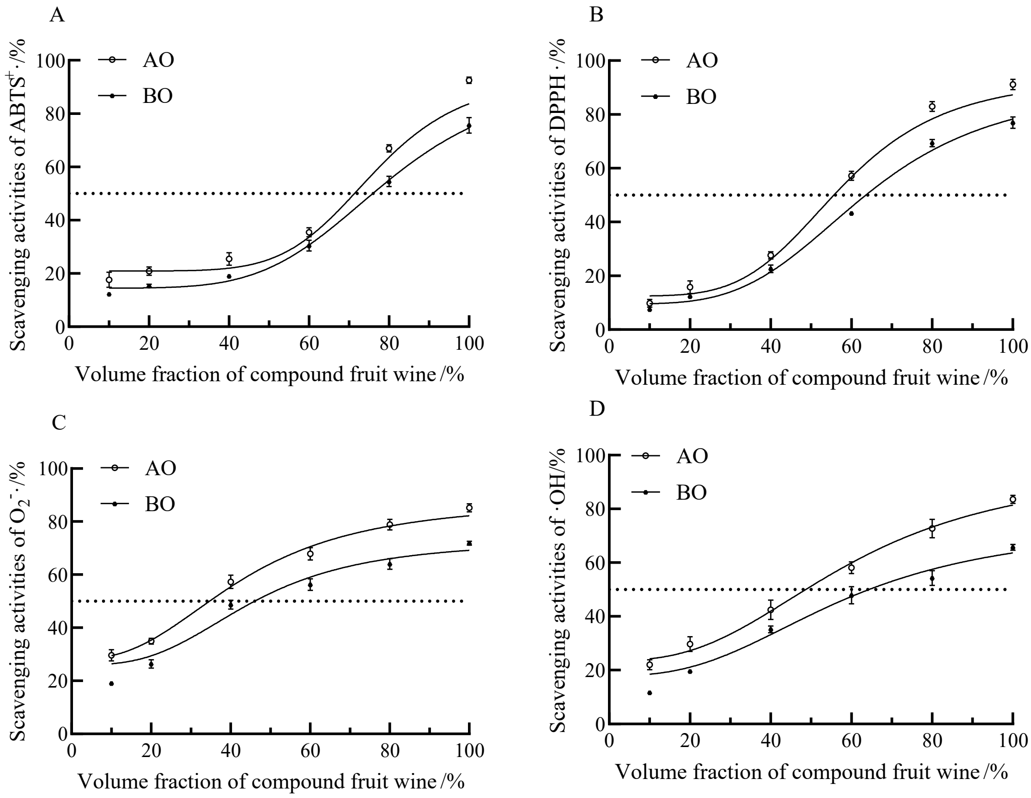

Figure 5. Scavenging activities of $ABTS^{+}$· (**A**), DPPH· (**B**), O_2^{-}· (**C**), and ·OH (**D**) in the compound fruit wine.

Meanwhile, the 50% effective concentrations (EC_{50}) of AO for scavenging $ABTS^{+}$·, DPPH·, O_2—·, and ·OH were 78.62%, 57.74%, 42.85%, and 59.91%, respectively, lower than that of BO (Figure 5). Through contrastive analysis, it was found that the scavenging activities in vitro of this compound fruit wine after optimization of the main ingredient ratio were better than those of mulberry wine, Ethiopian honey wine, and another kind of compound fruit wine (blueberry-grape wine) [48–50]. From the above results, it can be seen that the metabolites in the optimized compound fruit wine changed significantly; especially, lycopene and (13Z)-lycopene increased significantly (VIP > 1.0, $p < 0.05$ and FC > 2), so the antioxidant activity of this compound fruit wine had been improved. Indeed, the antioxidant activities primarily originate from the lycopene [51–53].

4. Conclusions

Through fuzzy mathematics sensory evaluation and the response surface test, the optimal main ingredient ratio for fermenting compound fruit wine from 27.2% tomato juice, 27.5% papaya juice, 10.0% carrot juice, and 35.3% gac fruit juice was obtained. This compound fruit wine was clear and transparent, had a full-bodied fruit and wine aroma, and had a mellow taste; lycopene content and sensory score were (19.01 ± 0.77) µg/L and 95.2 ± 0.2, respectively. Meanwhile, the physicochemical indexes met the requirements of the Chinese standard (GB/T 15038—2006). Furthermore, the relative levels of 54 metabolites after optimization decreased significantly (VIP > 1.0, $p < 0.05$ and FC < 0.5); the relative levels of 106 metabolites including lycopene and (13Z)-lycopene increased significantly (VIP > 1.0, $p < 0.05$ and FC > 2); and the compound fruit wine after optimization showed good antioxidant activities in vitro. Therefore, significant changes in metabolites, especially lycopene and (13Z)-lycopene, improved sensory characteristics and the antioxidant activity of compound fruit wine. The test results can be used as a reference for the standardization and industrial production of the same kind of compound fruit wine.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9070591/s1, Table S1: The metabolites in BO, AO, and QC.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The experimental data provided in this work are available in articles and Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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