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Technological exploration and antioxidant activity determination of purple compound fruit wine

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Abstract

When it comes to processing, purple pepper and purple corn are poorly utilised even though they are rich in anthocyanins and other nutrients. Therefore, studies focusing on their processing are vital for resolving their underutilisation. In the present work, purple pepper and corn were used to prepare a compound fruit wine. The fermentation process parameters were optimised using single factor tests and response surface design. The sensory characteristics of the compound fruit wine were used as an indicator. The antioxidant potential of the purple compound fruit wine was also assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) and hydroxyl radical (·OH) scavenging rates. A purple compound fruit wine with a sensory score of 94.2 and 8.6% alcohol by volume was obtained using a combination of 72.0% purple pepper juice and 28.0% purple corn juice (with an initial sugar content of 23.3%), followed by inoculation with 6.2% *Saccharomyces cerevisiae* and fermentation for 7.8 d at 23°C. The resulting purple compound fruit wine had a typical harmonious fruit aroma and a mellow taste. Furthermore, the content of anthocyanins in purple compound fruit wine was 1.38 ± 0.14 mg/mL, and the half inhibitory concentration (IC_{50}) values against DPPH· and ·OH were 51.31 and 49.08 mg/mL, respectively. This optimised fermentation process could serve as a theoretical basis for the industrial utilisation of purple pepper and corn.

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Introduction

Chili peppers belong to the genus *Capsicum*, which originated in Mexico, South America (Kraft *et al.*, 2014). The *Capsicum* genus includes more than 38 different species. However, only five species (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*) have been domesticated through artificial cultivation (Tripodi and Kumar, 2019; Parisi *et al.*, 2020). Several chili pepper varieties are widely cultivated, processed, and consumed across the globe including Ethiopia, Pakistan, Bangladesh, India, Myanmar, and China (Govindarajan, 1985; 1986a; 1986b; 1987; Govindarajan and Sathyanarayana, 1991). Chili pepper is highly valued for its pungency and unique flavour (Batiha *et al.*, 2020), thus leading to its wide incorporation in the diet, and utilisation in the traditional medicine (Powis *et al.*, 2013). Purple pepper is among the famous breeds of *C. frutescens*, owing to its content of carbohydrates, carotenoids, minerals, antioxidants, fibres, and vitamins (Getahun *et al.*, 2020). In addition, the anthocyanin in ripened purple pepper has high extractable colour intensity (Kasampalis *et al.*, 2022; Ye *et al.*, 2022). The presence of these complex compounds endows *C. frutescens* with antioxidant, chemopreventive, anti-

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inflammatory, cardiovascular disorder preventive, and anti-obesity effects (Villa-Rivera and Ochoa-Alejo, 2020). Although purple pepper is widely consumed as vegetable and spice (Batiha *et al.*, 2020), only a limited number of processed purple pepper products exist currently. Therefore, the preparation of a fermented fruit wine can broaden the application scope of purple peppers.

Previous studies reported that fruit wine brewed using purple pepper alone demonstrated limitations, including a single aroma and an uncoordinated taste. Compound fruit wine is a low alcohol precision beverage brewed using two or more kinds of fermentable fruits. With process optimisation, the compound fruit wine can integrate the aroma, flavour, and nutrients of multiple raw materials into a single product rich in aroma and taste. Our preliminary studies indicated that fruit wine made with a combination of purple pepper and corn possessed better sensory characteristics. However, the brewing process needs to be optimised to obtain a compound fruit wine with excellent sensory characteristics, and rich anthocyanin content and antioxidant properties.

Purple corn (*Zea mays* L.) is an amylaceous and purple-pigmented corn that belongs to the Peruvian race *Kculli*, which means “black” in the “Quechua” native language (Ranilla *et al.*, 2021). Purple corn is grown worldwide, and it is among the most important crops in North America (United States, Canada), South America (Mexico, Brazil), South Africa, and East Asia (India, Japan, China) (Hu *et al.*, 2021). Purple corn is rich in several nutrients including polyphenols, flavonoids, flavonols, flavanols, and anthocyanins (Ramos-Escudero *et al.*, 2012). These bioactive ingredients possess health-promoting effects such as anti-atherosclerosis (Cullen *et al.*, 2020), immunomodulatory (Shakoor *et al.*, 2021), anticancer, antioxidant, anti-inflammatory, antiviral (Ullah *et al.*, 2020), adverse cardiovascular amelioration in diabetes (Leo and Woodman, 2015), cerebral cortical oxygenation and cognition improvement (Gratton *et al.*, 2020), and antiobesity and antiaging activities (Francavilla and Joye, 2020).

In the present work, we optimised the production process of purple compound fruit wine using a single factor-response surface design. We also evaluated the anthocyanin content and antioxidant capacity of the purple compound fruit wine with excellent sensory characteristics.

Materials and methods

Samples, chemicals, and reagents

Fresh purple pepper and corn were purchased from the fruit and vegetable market in Yibin, China. The sucrose was from Ganzhiyuan Sugar Co., Ltd., Nanjing, China. *Saccharomyces cerevisiae* (CICC31482) was from the strain collection centre of Yibin Vocational and Technical College. Glacial acetic acid, sodium acetate, citric acid, sodium citrate, sodium hydrogen phosphate, and sodium hydroxide were purchased from Xinrunde Co., Ltd., Hubei, China. Sodium sulphite was purchased from Nanjing Reagent Co., Ltd., China. Copper sulphate and potassium sodium tartrate were purchased from Yatai Co., Ltd, Wuxi, China. Methylene blue indicator was purchased from Fuchen Chemical Co., Ltd., Tianjin, China. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) and hydroxyl radical (·OH) kits were purchased from Jiancheng Bioengineering Institute in Nanjing, China. Vitamin C was purchased from Chengdu Kelong Chemical Reagent Factory, China. Unless otherwise stated, all chemicals used in the present work were of analytical grade.

Production process flow

For the production of the purple compound fruit wine, a previously described protocol was followed (Zhu *et al.*, 2014) with a slight modification due to the differences in the materials used. The key processing steps in the preparation of the purple compound fruit wine include the preparation of the purple fruit juices by pressing the purple pepper and corn. The juice volume was adjusted to 100 mL in different proportions depending on the experimental design. Sucrose was added at different concentrations. Then, 0.02% sodium sulphite was added to the fruit juice, and the pH was adjusted to 6.0. The juice was pasteurised for 30 min in a 250 mL fermentation bottle. The pasteurised purple fruit juice was inoculated with a yeast suspension. Then it was fermented using different temperatures (°C) and fermentation times (d). It is worth noting that deflating was carried out every day before the 3-d fermentation until no bubbles were observed. After the main fermentation was completed, the barrel was poured, store at low temperature, and filtered to obtain the compound fruit wine (Figure 1).

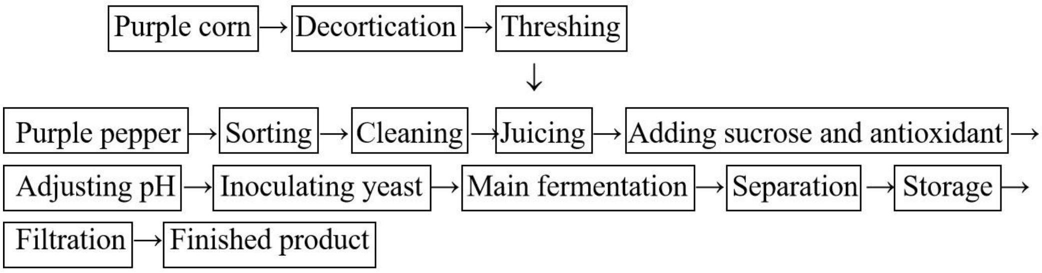

Figure 1. Production process flow of purple compound fruit wine.

Single factor optimisation process

Based on the experimental requirements, each single factor test changed only one factor at a time while keeping all remaining factors constant. The factors were the addition ratio (m/m) of purple pepper and corn (1:9, 3:7, 5:5, 7:3, and 9:1), sucrose addition (15, 17.5, 20.0, 22.5, and 25%), *Saccharomyces cerevisiae* addition (1, 3, 5, 7, and 9%), fermentation temperature (20, 22, 24, 26, and 28°C), and fermentation time (4, 5, 6, 7, and 8 d).

Response surface methodology optimisation process

Based on a single factor test, we used the Box-Behnken design (Design Expert 12.0) to conduct a response surface test for the purple pepper addition ratio (*A*), initial sugar (*B*), *S. cerevisiae* addition (*C*), fermentation temperature (*D*), and fermentation time (*E*). The results are shown in Table 1. The design had a 46-trial experimental setup, where each variable was tested under three different conditions: low (-1), middle (0), and high (+1). The coded values corresponding to purple pepper addition ratio were -1 (50%), 0 (70%), and +1 (90%). For sucrose addition, the values were -1 (20%), 0 (22.5%), and +1 (25%). For the addition of *S. cerevisiae*, the codes were -1 (3.0%), 0 (5.0%), and +1 (7.0%). The coded values for fermentation temperature were -1 (20°C), 0 (22°C), and +1 (24°C). Finally, the values for fermentation time were -1 (6 d), 0 (7 d), and +1 (8 d). Each block contained 5 centre points.

Sensory evaluation

The sensory characteristics of the purple compound fruit wine produced were examined by a sensory evaluation panel consisting of 11 (5 men and 6 women) wine-tasters following the Chinese Standard (NY/T 1508-2017). As shown in Table 2, the four aspects evaluated were appearance (0 - 10 score), aroma (0 - 30 score), taste (0 - 40 score), and typicalness (0 - 20 score). A total sensory score was calculated from the four individual scores.

Physicochemical analysis

The anthocyanin content of the fermented purple compound fruit wine was determined using high-performance liquid chromatography (HPLC) coupled with an ultraviolet detector (UV) (Tang *et al.*, 2020). The total sugar content was determined using a titration method as described in a previous study (Bernstein *et al.*, 2016). The total acid content of the purple compound fruit wine was determined by titration (Tsegay, 2020). The alcohol volume fraction (%) was determined using an alcoholometer (Mettler Toledo, China). The solid dry extract content of the fermented compound fruit wine was determined by filtering the wine through a filter paper (Whatman No. 3) and lyophilising the extract (Biosafer 10 D freezer-dryer; -65°C, 48 h) (Carro-Juarez *et al.*, 2017).

Antioxidant activity

The DPPH· and ·OH free radical scavenging rate of purple compound fruit wine was determined following the instructions of the DPPH· and ·OH kit manufacturers. Vitamin C solution was used as the positive control.

To measure the scavenging activities (SC) of DPPH·, 400 μL of purple 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 was calculated using the following equation: SC (%) = $[(1 - (A_T - A_C)/A_B) \times 100]$.

Table 1. Codes and levels of independent variables in the experimental design.

No.	*A* Purple pepper amount (%)	*B* Initial sugar content (%)	*C* Inoculation amount of *S. cerevisiae* (%)	*D* Fermentation temperature (°C)	*E* Fermentation time (d)	Sensory evaluation
1	-1 (50%)	-1 (20%)	0 (5%)	0 (22°C)	0 (7 d)	61.5
2	1 (90%)	-1 (20%)	0 (5%)	0 (22°C)	0 (7 d)	71.5
3	-1 (50%)	1 (25%)	0 (5%)	0 (22°C)	0 (7 d)	75.1
4	1 (90%)	1 (25%)	0 (5%)	0 (22°C)	0 (7 d)	80.1
5	0 (70%)	0 (22.5%)	-1 (3%)	-1 (20°C)	0 (7 d)	71.3
6	0 (70%)	0 (22.5%)	1 (7%)	-1 (20°C)	0 (7 d)	78.3
7	0 (70%)	0 (22.5%)	-1 (3%)	1 (24°C)	0 (7 d)	81.4
8	0 (70%)	0 (22.5%)	1 (7%)	1 (24°C)	0 (7 d)	90.6
9	0 (70%)	-1 (20%)	0 (5%)	0 (22°C)	-1 (6 d)	71.4
10	0 (70%)	1 (25%)	0 (5%)	0 (22°C)	-1 (6 d)	79.8
11	0 (70%)	-1 (20%)	0 (5%)	0 (22°C)	1 (8 d)	83.3
12	0 (70%)	1 (25%)	0 (5%)	0 (22°C)	1 (8 d)	92.4
13	-1 (50%)	0 (22.5%)	-1 (3%)	0 (22°C)	0 (7 d)	64.1
14	1 (90%)	0 (22.5%)	-1 (3%)	0 (22°C)	0 (7 d)	67.8
15	-1 (50%)	0 (22.5%)	1 (7%)	0 (22°C)	0 (7 d)	68.3
16	1 (90%)	0 (22.5%)	1 (7%)	0 (22°C)	0 (7 d)	80.6
17	0 (70%)	0 (22.5%)	0 (5%)	-1 (20°C)	-1 (6 d)	72.4
18	0 (70%)	0 (22.5%)	0 (5%)	1 (24°C)	-1 (6 d)	80.7
19	0 (70%)	0 (22.5%)	0 (5%)	-1 (20°C)	1 (8 d)	84.1
20	0 (70%)	0 (22.5%)	0 (5%)	1 (24°C)	1 (8 d)	91.2
21	0 (70%)	-1 (20%)	-1 (3%)	0 (22°C)	0 (7 d)	67.9
22	0 (70%)	1 (25%)	-1 (3%)	0 (22°C)	0 (7 d)	84.9
23	0 (70%)	-1 (20%)	1 (7%)	0 (22°C)	0 (7 d)	79.2
24	0 (70%)	1 (25%)	1 (7%)	0 (22°C)	0 (7 d)	87.1
25	-1 (50%)	0 (22.5%)	0 (5%)	-1 (20°C)	0 (7 d)	60.5
26	1 (90%)	0 (22.5%)	0 (5%)	-1 (20°C)	0 (7 d)	72.3
27	-1 (50%)	0 (22.5%)	0 (5%)	1 (24°C)	0 (7 d)	77.1
28	1 (90%)	0 (22.5%)	0 (5%)	1 (24°C)	0 (7 d)	77.5
29	0 (70%)	0 (22.5%)	-1 (3%)	0 (22°C)	-1 (6 d)	70.8
30	0 (70%)	0 (22.5%)	1 (7%)	0 (22°C)	-1 (6 d)	77.9
31	0 (70%)	0 (22.5%)	-1 (3%)	0 (22°C)	1 (8 d)	82.3
32	0 (70%)	0 (22.5%)	1 (7%)	0 (22°C)	1 (8 d)	91.6
33	-1(50%)	0 (22.5%)	0 (5%)	0 (22°C)	-1 (6 d)	61.3
34	1 (90%)	0 (22.5%)	0 (5%)	0 (22°C)	-1 (6 d)	70.9
35	-1(50%)	0 (22.5%)	0 (5%)	0 (22°C)	1 (8 d)	77.1

36	1 (90%)	0 (22.5%)	0 (5%)	0 (22°C)	1 (8 d)	82.7
37	0 (70%)	-1 (20%)	0 (5%)	-1 (20°C)	0 (7 d)	70.4
38	0 (70%)	1 (25%)	0 (5%)	-1 (20°C)	0 (7 d)	85.5
39	0 (70%)	-1 (20%)	0 (5%)	1 (24°C)	0 (7 d)	81.4
40	0 (70%)	1 (25%)	0 (5%)	1 (24°C)	0 (7 d)	88.2
41	0 (70%)	0 (22.5%)	0 (5%)	0 (22°C)	0 (7 d)	89.0
42	0 (70%)	0 (22.5%)	0 (5%)	0 (22°C)	0 (7 d)	89.5
43	0 (70%)	0 (22.5%)	0 (5%)	0 (22°C)	0 (7 d)	88.9
44	0 (70%)	0 (22.5%)	0 (5%)	0 (22°C)	0 (7 d)	92.2
45	0 (70%)	0 (22.5%)	0 (5%)	0 (22°C)	0 (7 d)	90.1
46	0 (70%)	0 (22.5%)	0 (5%)	0 (22°C)	0 (7 d)	90.7

Table 2. Sensory evaluation standards of purple compound fruit wine.

Index	Sensory score	Sensory evaluation standard
Appearance	7 - 10	Bluish violet, transparent clear lustre
	3 - 7	Bluish violet, gloss slightly poor
	0 - 3	Turbidity, lack of lustre
Aroma	20 - 30	Harmonious, mellow, full bodied
	10 - 20	Mellow aroma is not obvious, full bodied but not intense
	0 - 10	Have complex or other unpleasant smells
Taste	30 - 40	Mellow, soft, and refreshing
	20 - 30	Delicious, slightly refreshing, light wine taste
	0 - 20	Tasteless, bitter, sour
Typicalness	15 - 20	Have the unique style of fruit wine, and flavour is generally coordinated
	8 - 15	Style is not obvious, and flavour is generally harmonious
	0 - 8	Flavour is discordant and spicy

For analysis of the SC of ·OH, purple compound fruit wine (8 - 80 ng/µL, 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 purple compound fruit wine (8 - 80 ng/µL, 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 was calculated using the following equation: SC (%) = $[A_B - (A_T - A_C)]/A_B) \times 100$].

Statistical analysis

All experiments were performed in triplicates. IBM SPSS Statistics Version 25.0 was used to analyse the data. Results were expressed as mean ± standard deviation (SD). Single factor experimental results were analysed and plotted with Origin 10.5.109 software. One-way analysis of variance (ANOVA) was used to evaluate the differences among experimental groups. *p*-value < 0.05 was considered statistically significant. Response surface design and result analysis were performed using Design Expert 12.0 software.

Results and discussion

Optimisation of percentages of purple pepper and corn juice

The sensory attributes of the compound fruit wine increased with increasing addition of the purple pepper juice while maintaining the other materials constant (20% initial sugar, 3% *S. cerevisiae*, 22°C fermentation temperature, and 5 d fermentation time). The highest sensory score of 81.4 ± 0.8 was achieved when the added purple pepper juice was 70% (Figure 2A). This sensory score was significantly higher (p < 0.05).

A proper proportion of purple pepper and corn was vital for producing a fermented compound fruit wine with good quality and flavour. The fruit aroma arising from 3-methylbutanoic acid in purple pepper can improve the sensory score (Nguyen *et al.*, 2020; Tsegay and Lemma, 2020). Accordingly, a combination of 30% purple corn juice and 70% purple pepper juice yielded a purple compound fruit wine with the highest sensory score. This combination of purple juices was used to optimise the amount of initial sugar added.

Optimisation of initial sugar content

The sensory score of purple compound fruit wines prepared using initial sugar content of 15, 17.5, 20, 22.5, and 25% was evaluated. The previously optimised 30% purple corn and 70% purple pepper juices were used for preparing the compound fruit wine. The compound juice was inoculated with a 3% *S. cerevisiae* suspension and fermented at 22°C for 5 d. The purple compound fruit wine prepared using 22.5% initial sugar had the highest sensory score of 85.3 ± 0.9 (Figure 2B), which was significantly higher than all other combinations (p < 0.05).

The level of initial sugar content is related to microbial growth, especially lactic acid bacteria and yeasts. Appropriate initial sugar content stimulates *S. cerevisiae* growth and metabolism, thus resulting in increased production of alcohol and other flavour substances in the compound fruit wine (Fiorda *et al.*, 2017). Consequently, low initial sugar content leads to a reduced carbon source for *S. cerevisiae*, thus limiting the production of flavour substances. The insufficient production of flavour substances leads to reduced sensory acceptability. Similarly, the excess use of initial sugar may lead to the overabundance of carbon sources, thus affecting the flavour and quality of compound fruit wine.

Optimisation of S. cerevisiae addition

We evaluated the sensory score of purple compound fruit wine prepared using 1, 3, 5, 7, and 9% *S. cerevisiae* inoculation. The previously optimised proportions of purple corn and pepper juice (30:70), and sucrose (22.5%) were used. The blend was fermented at 22°C for 5 d. A 5% *S. cerevisiae* inoculation yielded a compound fruit wine with the highest sensory score of 85.3 ± 0.9 (Figure 2C), which was significantly higher than the other inoculations (p < 0.05).

The amount of *S. cerevisiae* inoculum used directly influences the fermentation quality of compound fruit wine. Low *S. cerevisiae* inoculum use prolongs the fermentation duration. Low *S. cerevisiae* inoculation also increases the formation of sour substances and reduces the production of flavour compounds, thus leading to a compound fruit wine with poor taste. On the other hand, excessive use of *S. cerevisiae* inoculum leads to the increased production of volatile compounds such as esters, which affects the colour of the compound fruit wine, and causes bitterness (Bindon *et al.*, 2019; Jiang *et al.*, 2020).

Optimisation of fermentation temperature

Previous studies reported the optimum temperature for the growth of *S. cerevisiae* to be within 20 - 30°C. The sensory score of compound fruit wine increased with increasing fermentation temperature, reaching a peak of 88.10 ± 1.2 at 22°C (p < 0.05). This temperature was close to a previously reported fermentation temperature for *Lantana camara* grape wine which was 24.8°C (Tsegay and Lemma, 2020). Raising the fermentation temperature beyond the optimum value influenced the flavour and taste of the compound fruit wine, thus leading to a reduction in sensory score (Figure 2D).

Optimisation of fermentation time

The duration of fermentation is a vital factor for producing a compound fruit wine with a good flavour profile. The sensory score of the compound fruit wine started increasing from the 4th day of fermentation, reaching a peak of 90.2 ± 0.8 on the 7th day (p < 0.05) (Figure 2E). The sensory score started decreasing from the 8th day of fermentation. Therefore, longer fermentation time would not be conducive to the quality formation of compound fruit wine. The decrease in quality arising from a longer fermentation may be associated with the increased content of tannins and alkaloids which can impart astringency (Lisjak *et al.*, 2020). As a result, mastering the appropriate fermentation duration is crucial for producing a compound fruit wine with optimal sensory qualities.

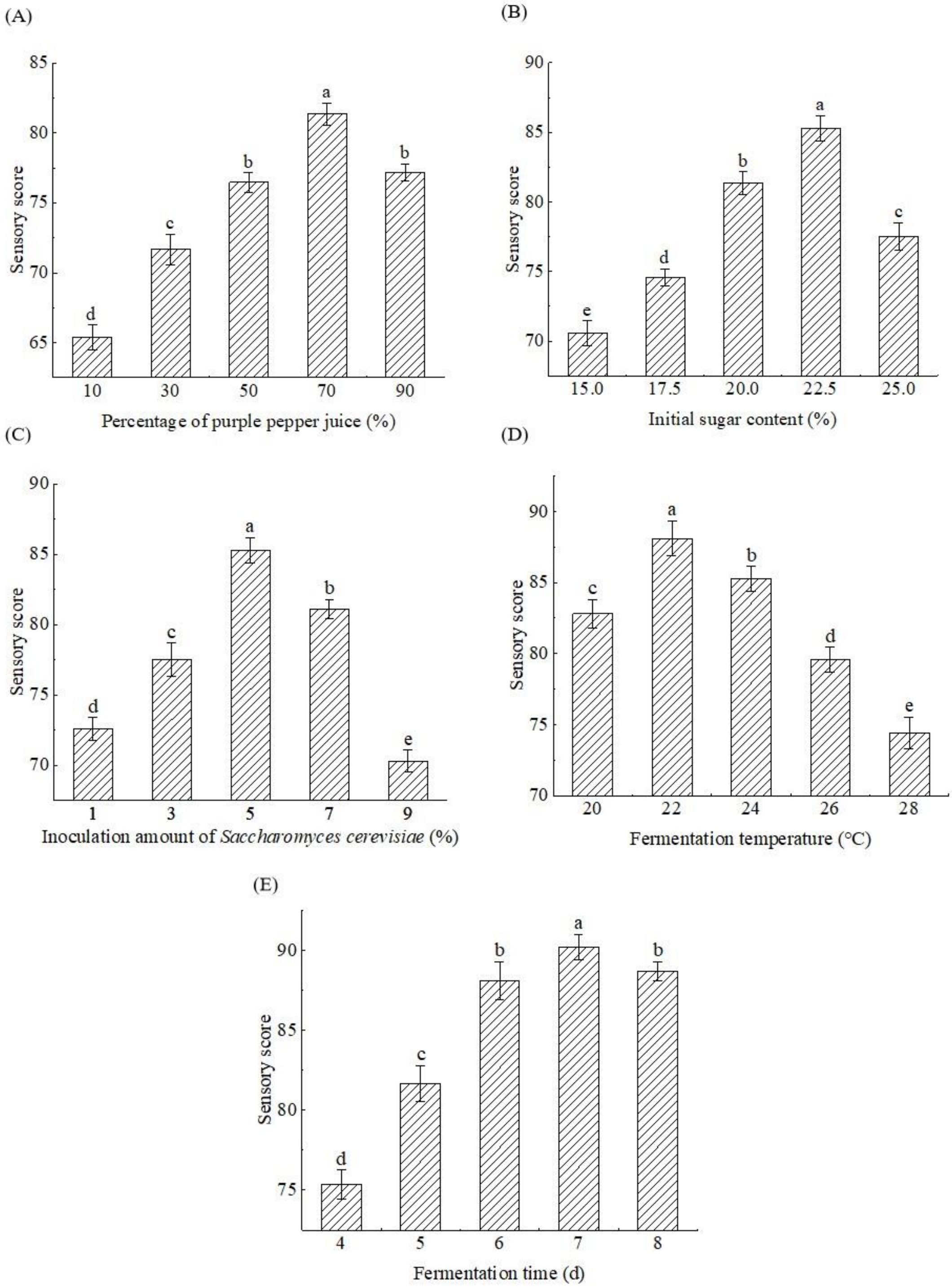

Figure 2. Effect of the percentage of purple pepper juice on the sensory score of purple compound fruit wine **(A)**, effect of the percentage of initial sugar content on the sensory score of purple compound fruit wine **(B)**, effect of the inoculation amount of *S. cerevisiae* on the sensory score of purple compound fruit wine **(C)**, effect of the fermentation temperature on the sensory score of purple compound fruit wine **(D)**, and effect of the fermentation time on the sensory score of purple compound fruit wine **(E)**. Means with different lowercase letters are significantly different ($p < 0.05$).

Response surface methodology optimisation fermentation conditions

The Box-Behnken design (BBD) was used to comprehensively analyse the effects of the interaction between different processing variables on the quality of purple compound fruit wine, and determine the optimal operating conditions. The purple pepper-corn juice ratio (*A*), initial sugar content (*B*), inoculation amount of *S. cerevisiae* (*C*), fermentation temperature (*D*), and fermentation time (*E*) were optimised using 46 experimental runs. Each variable was tested under three different conditions: low (-1), middle (0), and high (+1) (Table 1). The data were analysed by multiple regression analysis using the Design-Expert 12.0 software.

The interaction terms of *A*, *B*, *C*, *D*, E, and *AD* had a significant active effect on the sensory score of the purple compound fruit wine. The quadratic term of B^2 had a significant positive effect on the sensory score ($p < 0.01$). The interaction terms of *AB*, *AC*, *BC*, and *BD* also had a significant effect on the sensory score of the purple compound fruit wine ($p < 0.05$). Therefore, a suitable fermentation process of purple compound fruit wine could be determined using the above regression equation (Table 3). A polynomial equation was derived to express the sensory score of fermented compound fruit wine as a function of the independent variables tested.

$$Y = 90.07 + 3.8A + 4.37B + 4.1C + 3.85D + 5.55E - 1.25AB + 2.15AC - 2.85AD - 1AE - 2.28BC - 2.07BD + 0.175BE + 0.55CD + 0.55CE - 0.3DE - 13.21A^2 - 4.48B^2 - 5.56C^2 - 4.11D^2 - 3.86E^2 + 1.18A^2B + 0.15A^2C + 1.6A^2D + 1.35A^2E - 0.05AB^2 + 0.2AC^2 - 0.75AD^2 - 0.725B^2C - 0.425B^2D + 0.575B^2E + 1.85BC^2 + 1.1BD^2 + 1.75C^2D + 0.75C^2E - 0.05CD^2 - 1.1A^2C^2 - 0.9A^2D^2 - 0.25B^2C^2 - 0.1B^2D^2$$

Table 3. Variance analysis on the sensory evaluation of purple compound fruit wine.

Source of variation	Sum of square	Degree of freedom	Mean square	*F*-value	*p*-value	Significant
Model	3713.65	40	92.84	59.72	0.0001	**
A - Purple pepper amount (%)	57.76	1	57.76	37.15	0.0017	**
B - Initial sugar content (%)	76.56	1	76.56	49.25	0.0009	**
C - Inoculation amount of *S. cerevisiae* (%)	67.24	1	67.24	43.25	0.0012	**
D - Fermentation temperature (°C)	59.29	1	59.29	38.14	0.0016	**
E - Fermentation time (d)	123.21	1	123.21	79.25	0.0003	**
AB	6.25	1	6.25	4.02	0.1013	*
AC	18.49	1	18.49	11.89	0.0183	*
AD	32.49	1	32.49	20.90	0.0060	**
AE	4.00	1	4.00	2.57	0.1696	
BC	20.70	1	20.70	13.32	0.0148	*
BD	17.22	1	17.22	11.08	0.0208	*
BE	0.12	1	0.12	0.08	0.7902	
CD	1.21	1	1.21	0.78	0.4180	
CE	1.21	1	1.21	0.78	0.4180	
DE	0.36	1	0.36	0.23	0.6507	
A^2	364.09	1	364.09	234.19	< 0.0001	**
B^2	41.95	1	41.95	26.98	0.0035	**
C^2	134.81	1	134.81	86.72	0.0002	**
D^2	73.65	1	73.65	47.37	0.0010	**
E^2	64.96	1	64.96	41.78	0.0013	**

*significant difference at $p < 0.05$, and **highly significant difference at $p < 0.01$.

Response surface 3D diagram shown in Figure 3 reflects the comprehensive effect of the interaction between factors on the sensory evaluation of purple compound fruit wine. The visualisation of each fitted model was carried as the function of two independent variables while keeping the other variable at the central value (Tsegay and Lemma, 2020). The interaction effect of *B* and *E* (Figure 3G), *C* and *D* (Figure 3H), *C* and *E* (Figure 3I), and *D* and *E* (Figure 3J) on the sensory properties of purple compound fruit wine was statistically insignificant ($p > 0.05$). On the contrary, the steeper the slope of the response surface graph, the greater the interaction between the two factors (Li *et al.*, 2019). *A* and *D* had a highly significant active effect on the sensory properties of the purple compound fruit wine ($p < 0.01$) (Figure 3C). Besides, the interaction effect of *AB*, *AC*, *BC*, and *BD* showed a significant active effect ($p < 0.05$) on the sensory evaluation of the purple compound fruit wine, which was in agreement with the ANOVA result (Figures 3A, 3B, 3E, and 3F).

As shown in Figures 3A, 3B, 3C, and 3D, when the amount of purple pepper increased from 50 to 72%, the sensory score gradually increased; however, it began to decrease when the amount of purple pepper increased from 72 to 90%. In factor B, when the initial sugar content increased from 20 to 23%, the sensory score increased and then decreased when it was more than 23% (Figures 3A, 3E, 3F, and 3G). Moreover, the sensory score reached the highest value when the amount of *S. cerevisiae* inoculated was 6.2% (Figures 3B, 3E, 3H, and 3I). As shown in Figures 3C, 3F, 3H, and 3J, when the fermentation temperature increased from 20 to 23°C, the sensory score increased, while it began to decrease when the fermentation temperature exceeded 23°C. Furthermore, when the fermentation time was less than 7.8 d, the sensory score increased; however, the sensory score decreased when it was more than 7.8 d (Figures 3D, 3G, 3I, and 3J).

Based on the response surface analysis, a purple compound fruit wine with the highest theoretical sensory score of 94.84 would be attained using a ratio of 72.16:27.84 between purple pepper and corn juice, 23.28% initial sugar, 6.17% *S. cerevisiae* inoculation, 22.91°C fermentation temperature, and 7.78 d of fermentation. Three parallel experiments were carried out to validate the prediction model. Results showed that under the predicted conditions, the sensory score of the purple pepper compound fruit wine was 94.2 ± 0.90, thus indicating reproducibility.

Physicochemical analysis

Five physicochemical indexes were detected in the compound fruit wine using an electronic analytical balance (TS: BS210S), an electric constant temperature incubator (TS: DHP-9162), and a visible spectrophotometer (TS: 723 type). The total sugars, total acids, and alcohol volume fraction of the purple compound fruit wine (Table 4) met the requirements of the Chinese Standard (NY/T 1508-2017). The dry extract was 35.70 ± 0.90 g/L, which was significantly higher than the requirement specified in the standard (≥ 12.0 g/L). The anthocyanin content was 1.38 ± 0.14 mg/mL, which was higher than the 1.1 mg/mL previously reported for grape skin (Li *et al.*, 2019). The total acids and sugars produced in the compound fruit wine have complex health benefits. The indicators of each bioactive compound meet the international daily recommended dose (Kesa *et al.*, 2021).

Antioxidant activity

The DPPH· and ·OH scavenging rates of purple compound fruit wine increased with increasing ethanol content (Figure 4). The antioxidant capacity of the purple compound fruit wine was the strongest when the ethanol content was 67.88 mg/mL, thus leading to a DPPH· and ·OH scavenging rates of 75.33 ± 1.36 and 89.34 ± 0.74%, respectively. However, the radical scavenging rate was lower than the positive control. The half inhibitory concentration (IC_{50}) of DPPH· and ·OH scavenged by compound fruit wine was 51.31 and 49.08 mg/mL, respectively, which were higher than the positive control (0.03 and 0.12 mg/mL, respectively). Coloured peppers (purple or red) have higher water retention and antioxidant activity, and promote yeast number while inhibiting the growth of *E. coli* and harmful fungi (Tang *et al.*, 2021). Based on a previous study, when the purple wheat grains were manufactured into anthocyanin-rich powder, the content of anthocyanin increased from 423 to 13,536 µg/g, while the DPPH scavenging capacity increased from 3.1 to 392.2 µmole Trolox equiv./g (Sharma *et al.*, 2020). Furthermore, since fresh purple waxy corn was rich in anthocyanins, the antioxidant activities showed high correlation (ρ =

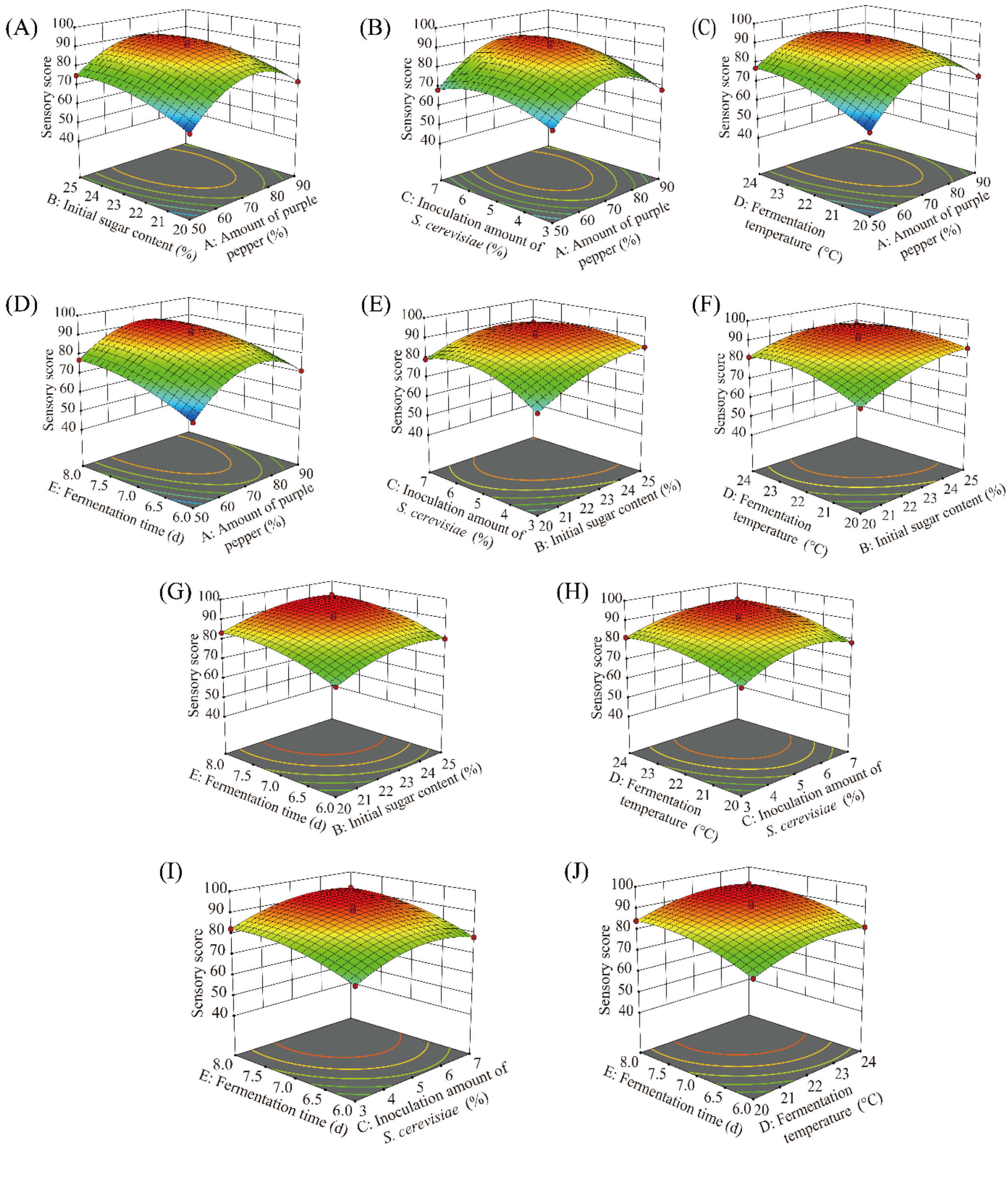

Figure 3. Response surface graph depicting the interaction of the purple pepper amount and initial sugar content **(A)**, purple pepper amount and inoculation amount of *S. cerevisiae* **(B)**, purple pepper amount and fermentation temperature **(C)**, purple pepper amount and fermentation time **(D)**, initial sugar content and inoculation amount of *S. cerevisiae* **(E)**, initial sugar content and fermentation temperature **(F)**, initial sugar content and fermentation time **(G)**, inoculation amount of *S. cerevisiae* and fermentation temperature **(H)**, inoculation amount of *S. cerevisiae* and fermentation time **(I)**, and fermentation temperature and fermentation time **(J)** on the sensory scores of purple compound fruit wine.

Table 4. Physicochemical parameters of purple compound fruit wine.

Parameter	Content
Total sugar (g/L)	10.10 ± 0.30
Total acid (g/100 mL)	0.82 ± 0.21
Alcohol volume fraction (%)	8.60 ± 0.50
Dry extract (g/L)	35.70 ± 0.90
Anthocyanin (mg/mL)	1.38 ± 0.14

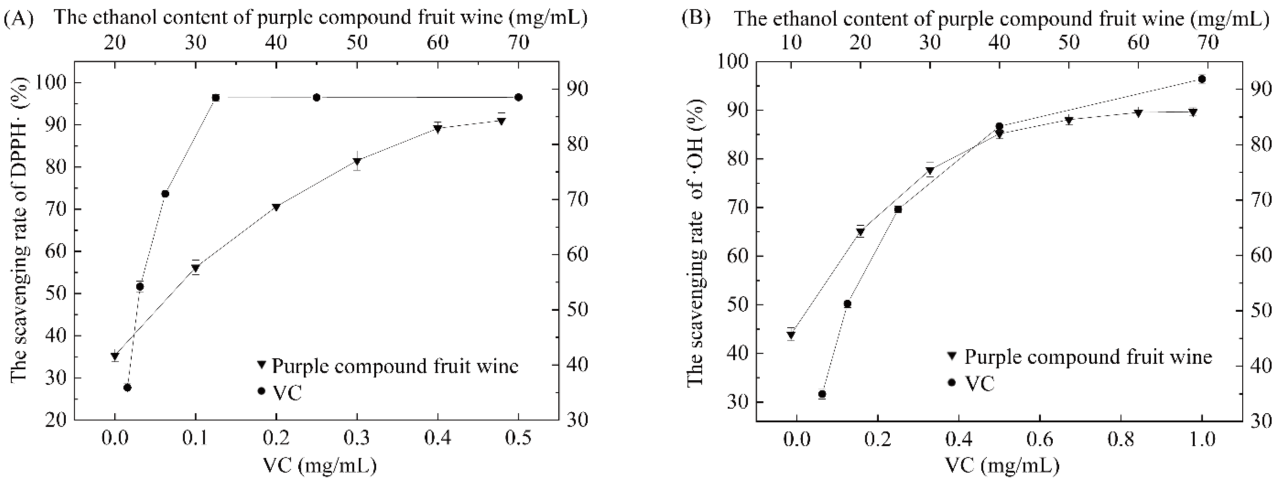

Figure 4. Antioxidant activity of purple compound fruit wine. The DPPH· scavenging rates of purple compound fruit wine **(A)**, and the ·OH scavenging rates of purple compound fruit wine **(B)**. VC was the positive control.

0.77 - 0.89) with the increase in anthocyanins in the fresh purple waxy corn (Harakotr *et al*., 2014). Meanwhile, purple corn pigment has antibacterial, antimutagenic, antioxidant, and anti-inflammatory activities in humans, and protective effects on diabetes, glucose metabolism, lipid metabolism, as well as neuroprotection (Colombo *et al.*, 2021). These beneficial effects may be related to the presence of anthocyanins, carotenoids, polysaccharides, and other bioactive substances.

Conclusion

Purple pepper and corn can be used as raw materials for brewing purple compound fruit wine with *S. cerevisiae* inoculation and sucrose addition. The optimised process conditions were as follows: 72:28 proportion of purple pepper and corn juice; 23.3% initial sugar content; inoculation with 6.2% *S. cerevisiae;* and fermentation at 23.0°C for 7.8 d. The resulting purple compound fruit wine had a sensory score of 94.2, alcohol content of 8.6%, and 1.38 mg/mL anthocyanin. The purple compound fruit wine had a typical harmonious fruit aroma, and a sweet mellow taste. The IC_{50} of purple compound fruit wine on DPPH· and ·OH scavenging was 51.31 and 49.08 mg/mL, respectively. This novel technology could serve as a theoretical basis for the industrial utilisation of purple pepper and corn.

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