RESEARCH ARTICLE

Optimization of sugar removal of purple carrot pigment and fermentation process of purple carrot vinegar

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The pigment extract from purple carrot with high sugar is prone to microbial contamination and anthocyanidin deterioration. Effective desugar of natural pigment extracts is essential for improving product quality. This work aimed to design a yeast fermentation process to reduce sugar in the concentrated juice from purple carrot. The effects of fermentation parameters such as temperature, initial pH, and initial sugar content on the sugar-removing rate were analyzed *via* single-factor and response surface analysis. Results showed that the maximum desugar rate of 91.03% was obtained under the optimal conditions of initial pH 3.2, temperature 30℃, and initial sugar content of 160 g/L, which was similar with model predictions. Subsequently, to broaden the application of purple carrots, the fermentation process of purple carrot vinegar was investigated using the resulting fermented juices with low sugar as raw material. The fermentation broth was treated and inoculated with *Acetobacter pasteuriana* AS1.41. The optimized conditions were found as temperature of 30℃, pH 4.2, and inoculation amount of 8%. The corresponding acetic acid content was 35.62 g/L. The work has thus provided new insights into the development of natural food additives.

Keywords: purple carrot pigment; sugar reduction; wine active dry yeast; purple carrot vinegar; fermentation.

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Introduction

Purple carrots belong to a special category of carrots with a purple black exterior and interior and much higher nutritional value than other ones. It has been reported that purple carrots represent an excellent dietary source of anthocyanins, which are the prominent component of water-soluble purple carrot pigments [1-3]. Anthocyanins, namely 2-phenylbenzopyrrilium or flavylium salts, are the glycosylated forms of anthocyanidins belonging to flavonoid derivatives with basic C6-C3-C6 skeleton (Figure S1) [4, 5]. Cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin are the most prevalent anthocyanidins (aglycones) in nature [4, 6], in which cyanidin is the predominant one in purple carrots [7]. The color and stability of anthocyanins are susceptible to light, metal ions, structure, especially pH and temperature [8, 9]. The structures of anthocyanins undergo reversible pH-dependent transitions in aqueous solutions, resulting in color-shift from red to blue with changes in solution pH from acidic to alkaline [6, 10]. In addition, a higher solution temperature is

not conducive to the stability of anthocyanin, leading to a shift from a stable flavylium cation to an unstable chalcone structure of anthocyanin, increasing its color loss [4, 11].

At present, anthocyanins have been widely used in industries such as food, medicine, and cosmetics due to the advantages of antioxidant, anti-inflammatory, anticancer, as well as safety and environmentally friendly [4, 12]. Among anthocyanin products from different sources, purple carrot anthocyanin is one of the widely used water-soluble red pigments, mainly supplied to the market in the form of concentrated juice without preservatives and other adjuvants. However, concentrated pigment extracts with high sugar content are susceptible to microbial contamination, and more importantly, sugars accelerate the browning and degradation of anthocyanins [13, 14]. Therefore, it is crucial to remove sugar in the concentrated juice from purple carrots to improve the product quality.

Many methods including membrane separation, macroporous resin adsorption, and aqueous two-phase extraction have been applied for the separation and purification of anthocyanins [15-18]. However, the similar molecular weights of pigments and sugars often lead to incomplete sugar removal by membrane separation, associated with membrane fouling. Macroporous resin adsorption can remove sugars from the pigment extract with other nutrients including inorganic salts, proteins, and organic acids, which is inconsistent with the requirement of the retention of the original juice components to the maximum. In contrast, aqueous two-phase extraction, especially for polyethylene glycol (PEG)- or ethanol-salts systems, is more suitable for removing sugars from natural extract with less loss of other components. However, the unresolved issues such as sugar residues and the removal of phase-forming substances restrict the large-scale application of this desugar process [19, 20]. Therefore, efforts have been made to develop more efficient and scalable processes for sugar removal. Inspired by the conversion of sugar into ethanol and carbon dioxide during the ethanol fermentation with wine active dry yeast, this work was proposed to develop a yeast fermentation process to reduce sugar in the concentrated juice from purple carrot. Due to glucose consumption at different rate with different yeasts, the concentrated juices from purple carrot were allowed to ferment with six types of wine active dry yeasts to evaluate the fermentability of yeasts using the desugar rate and pigment loss as indicators. On this basis, the conditions were optimized in terms of initial pH, fermentation temperature, and initial sugar content. Subsequently, to further utilize the fermentation products except for acting as color additives, the concentrated juice after fermentation was sterilized and inoculated with *Acetobacter pasteuriana* AS1.41 for acetic acid fermentation, in which the ethanol in the juices was oxidized to acetic acid, resulting in a novel product related to purple carrot, namely purple carrot vinegar, which provided more possibilities for the application of purple carrots.

Materials and Methods

Sugar removal by yeast fermentation

The original concentrated juice of purple carrot pigment (32% of solid content) (Pengyuan Kanghua Natural Products, Co. Ltd, Qingdao, Shandong, China) was diluted to a sugar content of 100-450 g/L, and then adjusted to different pH values of 2.0, 3.0, 4.0, 5.0, and 6.0 with hydrochloric acid or sodium hydroxide solution. Six wine active dry yeasts including Selectys® La Raffinée (RA) (Sofralab, Magenta, France), RMS2, F33, CEREVISIAE (CERE), and RX60 (Laffort, Bordeaux, France), and Fermentis W-34/70 (Weihenstephan, Munich, Germany) were employed in this study. The suspension of the activated yeast (Glucose and yeast of the same quantity were added to 10 mL of deionized water, followed by incubating in a thermostatic water bath at 30℃ for 30 mins) was added to 100 mL of the above juice to obtain final yeast content of 0.05-0.25‰ in a conical flask. After sealing treatment, the mixture was fermented in

the ZWY-2102C shaking thermostatic incubator (ZHI CHENG, Shanghai, China) at 30℃, 120 rpm, for 6 days. Aliquots of the samples were withdrawn daily and centrifuged, followed by measuring the sugar and pigment contents. After fermentation, the mixture was adjusted to a strong acidic nature, separated by centrifugation, and finally the solution was sterilized for further use.

The sugar removing rate was calculated from the ratio of the difference of sugar content in the initial and final juices to that of the initial one following the Fehling reagent (ketone sulfate solution and sodium hydroxide solution containing potassium sodium tartrate) titration method [21], in which reducing sugar can reduce divalent copper ions in Fehling reagent to monovalent copper. The content of anthocyanins was determined by pH differential method [22, 23]. The absorbances of the solutions at pH of 1.0 (0.025 mol/L Potassium chloride buffer) and 4.5 (0.4 mol/L sodium acetate buffer) were measured at 520 nm and 700 nm, respectively, using a UV-1285 spectrophotometer (Shimadzu, Nakagyō-ku, Kyoto, Japan).

Preparation of purple carrot vinegar

300 mL of the fermentation broth of concentrated purple carrot pigment juice after sterilization treatment was adjusted to pH 2.0, 3.0, 4.0, 5.0, and 6.0 with hydrochloric acid or sodium hydroxide solution, respectively. The seed solution of *Acetobacter Pasteurianus* AS1.41 (from the fermentation laboratory of the College of Life Sciences, Yantai University, Shandong, China) with different concentrations of 6, 8, 10, 12, and 14% were added into the solution. After sealing treatment with gauzes, the mixture was fermented in the shaking thermostatic at different temperatures of 22, 26, 30, 34, and 38℃. The samples were taken every day to determine the acetic acid content in fermentation broth by acid-base titration [24].

Response surface design

Response surface methodology (RSM) was used to optimize the effects of operating parameters. For the sugar removal from the concentrated juices, a Box-Behnken design was conducted by using the sugar removing rate as the response value with three critical variables including initial pH (A), fermentation temperature (B), and initial sugar content (C) (Table S1). For the fermentation of purple carrot vinegar, three different factors including temperature (D), pH (E), and inoculation amount (F) were chosen as the independent factors, and the acetic acid yield was designated as the dependent variables (Table S2).

Statistical analysis

The alcohol fermentation and acetic acid fermentation included 17 trials for each fermentation and triplications for each experiment. The experimental designs were shown in Tables S3 and S4, respectively. The resulting data were reported as the average values of three replicate experiments. Data were analyzed using Design-Expert 13.0 software (Stat-Ease, Inc., Minneapolis, MN, USA) including statistical analysis and regression model. The fit of the model was evaluated using the coefficients of determination (R^2) and the analysis of variance (ANOVA).

Results and discussion

The fermentation ability in different yeasts to sugar-removing from purple carrot extract

The effects of sugar removal by fermentation of six wine active dry yeast were shown in Figure 1. The sugar content in alcoholic fermentation by the three yeasts (RA, RMS2, and RX60) decreased at a faster rate than those of W-34/70, F33, and CERE, with the final desugar rate of over 91% (Figure 1A and 1C). However, the more serious anthocyanin loss was observed from RMS2 and RX 60 (Figure 1B and 1C). In contrast, RA strain performed best with a final sugar-removing rate of 93.2% and a pigment retention rate of 83.5%. Subsequently, the changes in sugar and pigment contents over time during fermentation with RA were evaluated, and the results showed that a sharp decrease in sugar content was observed at

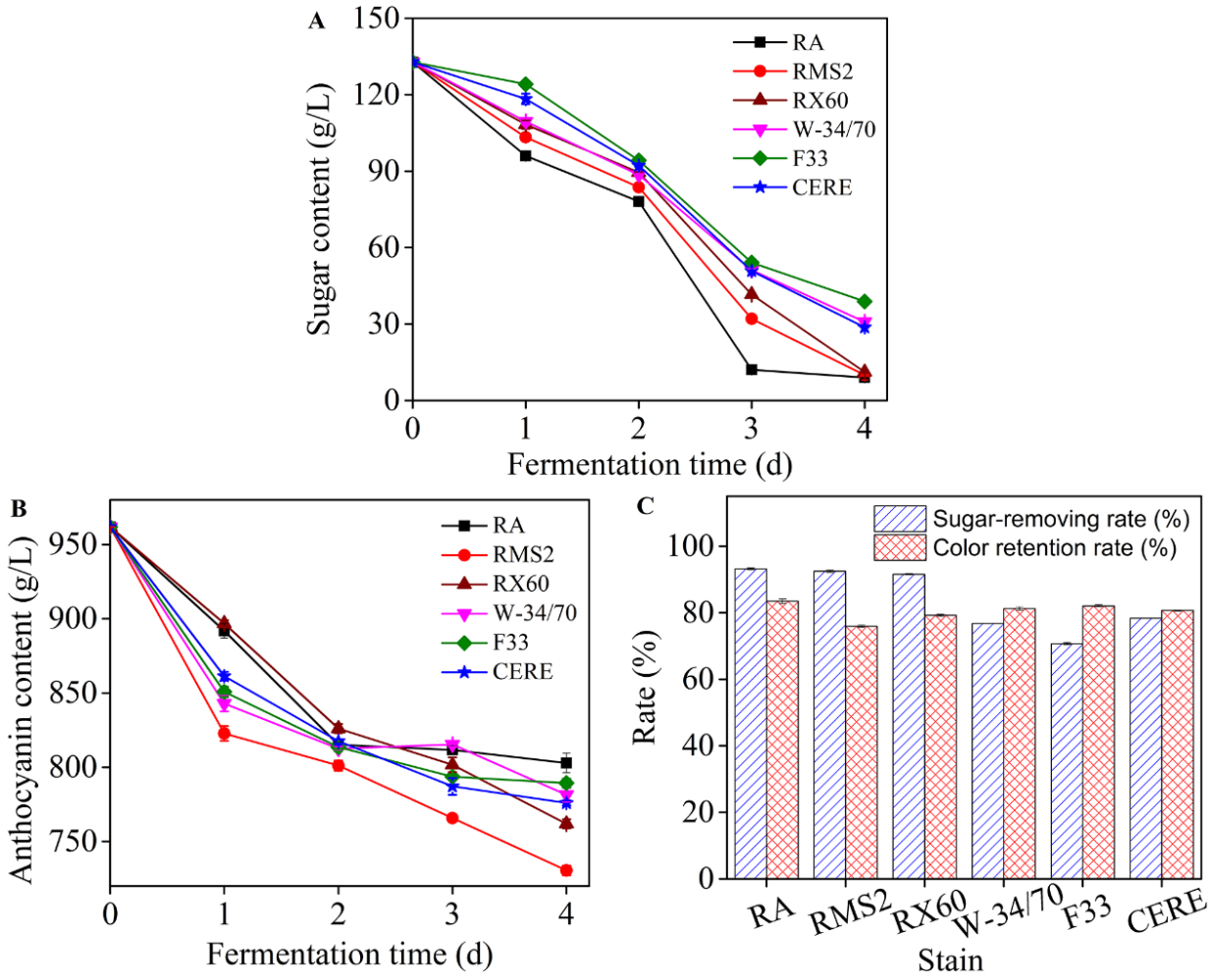

Figure 1. The fermentation characteristic of different yeasts in sugar-removing of purple carrot concentrated juices. **A.** The trend of sugar content. **B.** The trend of anthocyanin content. **C.** Final sugar-removing rate and anthocyanin retention rate. The concentrated juices were fermented by 0.15‰ of yeasts with 200 g/L sugar content at pH 3.0, 30℃ for 4 days.

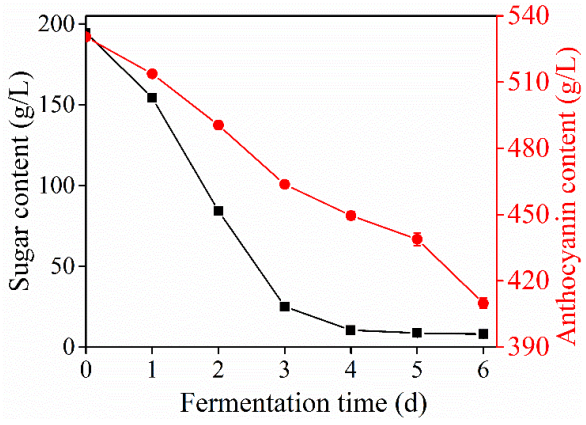

Figure 2. The changes in sugar and pigment contents over time. The concentrated juices were fermented by 0.15‰ of RA with 200 g/L sugar content at pH 3.0 and 30℃.

the first 3 days, followed by a slower down in the next day and a slight subsequent decline, owing to the dynamics of yeast growing (Figure 2). In the early stage of fermentation, the rapid growth of yeast using sugar in the concentrated juice as a substrate resulted in continuous consumption of sugar. With the increase of fermentation time, the residual sugar was not sufficient to meet the nutrient requirements of growing yeasts, manifested as inhibition of yeast growth along with the consumption of sugar at a slower rate by yeast. Until the later stage, trace sugar cannot maintain the yeast growth, leading to yeast death, and thus almost constant sugar content, indicating the end of fermentation. However, the

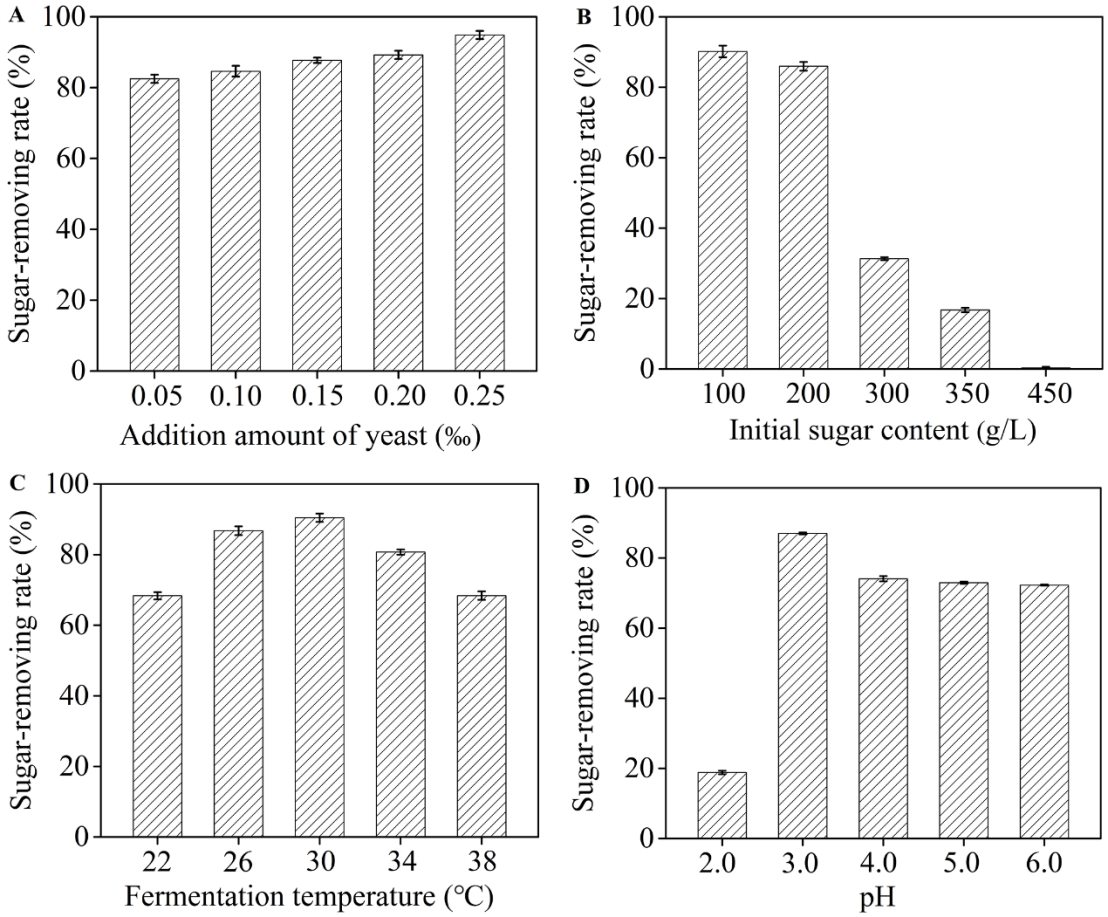

Figure 3. Optimization condition for sugar removal by yeast fermentation. **A.** Strain addition amount. **B.** Initial sugar content. **C.** Fermentation temperature. **D.** Initial pH.

content of anthocyanidin in the juice showed a decreasing trend over time due to the hydrolysis-prone nature of anthocyanin by its unstable structure (Figure 2). Hence, to reduce pigment loss while maintaining better sugar removal, the fermentation time for 4 days by RA was used in the following studies on optimizing the fermentation condition including the yeast addition amount, initial sugar content, fermentation temperature, and initial pH for sugar removal of the concentrated juice from purple carrot taking the sugar-removing rate as an indicator.

Effect of various factors on sugar-removing by yeast fermentation

The trends of sugar contents with the addition amount of yeast during fermentation were demonstrated in Figure 3A. The results showed that the sugar-removing rate increased with the increase of yeast addition amount, which might be related to the accelerated growth rate of yeast with increasing the added amount, leading to an increase in the consumption of sugar in the concentrated juice. However, in the light of economic benefits, the sugar content decreased rapidly under 0.15‰ of yeasts addition and a final reduction rate could fit in with the requirement of industrial production. Thus, 0.15‰ was considered the optimal addition quantity of yeast. The influence of initial sugar content on the sugar removal were shown in Figure 3B. The sugar-removing rate of the concentrated juice from purple carrot presented a sharp decline trend with increasing the initial sugar content, which decreased from 86% to 31%

Table 1. ANOVA results of response surface quadratic model for the sugar-removing rate.

Source [a]	Sum of squares	Degree of freedom	Mean square	*F* value	*P* value
Model	11,449.98	9	1,272.22	101.88	< 0.0001
A	339.56	1	339.56	27.19	0.0012
B	11.12	1	11.12	0.8901	0.3769
C	9,830.12	1	9,830.12	787.17	< 0.0001
AB	20.12	1	20.12	1.61	0.2450
AC	0.3306	1	0.3306	0.0265	0.8753
BC	0.3249	1	0.3249	0.0260	0.8764
A^2	250.16	1	250.16	20.03	0.0029
B^2	266.82	1	266.82	21.37	0.0024
C^2	607.38	1	607.38	48.64	0.0002
Residual	87.42	7	12.49		
Lack of fit	57.86	3	19.29	2.61	0.1884
Pure error	29.55	4	7.39		
Total	11,537.39	16			
R^2		0.9924			
R^2_{adj}		0.9827			

Notes: A: initial pH. B: temperature (°C). C: initial sugar content (g/L). A^2, B^2, and C^2 were the quadratic terms of the corresponding variables (A, B, C).

with an increase of the initial sugar content from 200 to 300 g/L. The sugar content in the juice almost unchanged under 450 g/L of initial sugar exposure with a final desugar rate of less than 0.5%, which might be attributed to the high osmotic pressure environment caused by high sugar levels, inducing cell dehydration and rupture, leading to the imbalance of its physiological metabolism and function, further inhibiting cell growth and reproduction, and finally to cell death. Although a higher desugar rate was obtained at a lower initial sugar content, problems would occur that the lower sugar levels might result in a significant increase in the volume of the concentrated juice from purple carrot, and improving the cost of equipment and subsequent processes, which were not applicable to plant production. Taken together, the initial sugar content was chosen as 200 g/L for further experiments. The fermentation temperature is a critical variable during alcohol fermentation process. Too low or high fermentation temperature will inhibit the growth rate and metabolic activity of yeast, inducing a slow decrease in the sugar content. The sugar-removing rate exhibited a trend of first increasing and then decreasing with the increase of fermentation temperature, reaching a maximum value (>90%) at 30℃ (Figure 3C). Furthermore, the sugar content of the juice during yeast fermentation with different initial pH were also measured (Figure 3D). An apparent peak in the desugar rate was detected for fermentation at pH 3.0, which could be attributed to the reached maximum for the growth and metabolic activity of yeast (RA) under the optimal pH. On the contrary, the excess acidic or basic condition could lead to the growth and metabolic rate of yeast being inhibited, promoting the cell death. Overall, the initial pH value of 3.0 was optimal for sugar removal by yeast fermentation.

Response surface analysis for sugar removal

To further optimize the level of the reaction factors for sugar removal and evaluate the interactions between the three variables and the sugar-removing rate, the Box-Behnken design of RSM was performed with three-level-three-factor including initial pH (A), temperature (B), and initial sugar content (C). The 17 designed experiments and the corresponding results were listed in Table S3. The experimental data were analyzed using multiple regression to establish a quadratic polynomial equation for explaining the

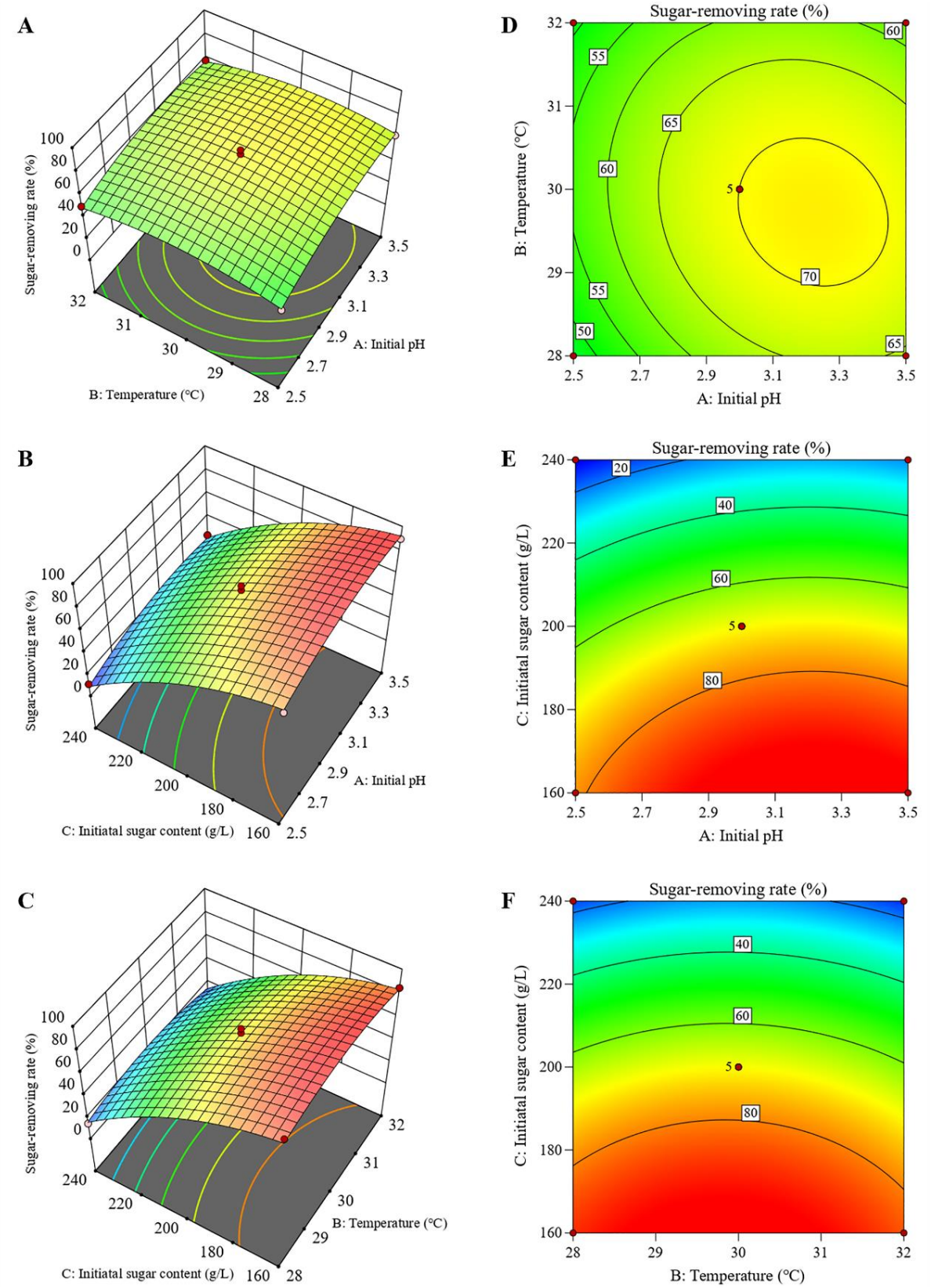

Figure 4. Response surface plots (A, B, and C) and contour plots (D, E, and F) presented the influence of initial pH (**A**), temperature (**B**), and initial sugar content (**C**) on the sugar-removing rate for the purple carrot concentrated juices by yeast fermentation.

mathematical relationship between the three independent variables (A, B, C) and the dependent response (sugar-removing rate, designed as Y_1). The regression model was obtained as $Y_1 = 70.02 + 6.51A - 1.18B - 35.05C - 2.24AB - 0.2875AC + 0.285BC - 7.71A^2 - 7.96B^2 - 12.01C^2$ (Equation 1). The ANOVA for the response surface quadratic model was presented in Table 1. The model of the equation was significant at 1% level, demonstrated by the *P* value less than 0.0001. The lack of fit with *P* value of 0.1884 was insignificant at 5% level. The results indicated that the model fit well with the experimental values. The coefficient of determination ($R^2 = 0.9924$) and the adjusted determination coefficient ($R^2_{adj} = 0.9827$) further reflected a high degree of correlation between experimental and predicted values. The model was hence found to be very reliable and adequate for predicting the real relationships among the selected factors. The linear coefficients without B were found to be significant ($P < 0.01$). The smaller *the P value* was, the more significant the effect of the factor on the desugar rate was [25]. The variable with the greatest influence was the initial sugar content (C), followed by the initial pH (A) and temperature (B). Besides, the quadratic term coefficients (A^2, B^2, C^2) were significant ($P < 0.05$) and the interaction coefficients (AB, AC, BC) were not significant ($P > 0.05$). The analysis of the model coefficients proved that the model indeed represented the actual relationships among these selected factors.

Two-dimensional contour plots and three-dimensional response surface plots were shown in Figure 4, depicting the interactions between two variables and the relationship between the sugar removal rate and the experiment parameters [26]. The color shifting from blue to red in the response surface plots indicates the change in response value from less to more, in which a faster one is demonstrated by a steeper surface with a better interaction between the experimental factors. The sugar-removing rate increased at first and then decreased with increase of initial pH and temperature, while a decline trend was found with increasing initial sugar content, which were consistent with the results of the above single-factor experiments (Figure 4A-4C). However, there was a slight hill running along the concentration axis on the response surface plots, suggesting that the combined effect of various variables on the desugar rate were insignificant. Besides, the contour plots (Figures 4D to 4F) exhibited a circular characteristic, further indicating the negligible interactions between the corresponding variables [27].

The optimal conditions for maximizing the sugar-removing rate were determined by setting the partial derivatives of the model equation (Equation 1) to zero with respect to the independent variables [28], *i.e*., the initial pH, fermentation temperature, and the initial glucose content were 3.23, 29.69°C, and 160 g/L, respectively, and the predicted desugar rate was estimated as 94.75%. In consideration of practical convenience, the above conditions were adjusted as initial pH 3.2, temperature 30°C, initial sugar content of 160 g/L, yielding the corresponding response of 94.54%. To confirm the accuracy of the model predictions, the adjusted optimal conditions were employed to three independent replicates for sugar removal from the concentrated juice of purple carrot. The average sugar-removing rate was 91.03%, which was close to the predicted value of the model equation, demonstrating the validation of model for the optimization of desugar process by yeast fermentation. Overall, sugar removal by ethanol fermentation could effectively reduce the sugar content of the purple carrot concentrated juice with the resulting alcohol as a by-product. The alcohol could reduce the possibility of bacterial contamination of the concentrated juice and be prone to be separated from the pigment product, improving the suitability for industrial production. More interestingly, alcohol as a substrate could be oxidized to acetic acid by acetobacter for the development of purple carrot vinegar.

The preparation of purple carrot vinegar *via* acetic acid fermentation

Purple carrot vinegar is a novel product with high nutritional value and bright color, forming by acetic acid fermentation based on the purple carrot concentrated juice (about alcohol content 10% Vol) treated with alcohol fermentation as raw materials. Purple carrot vinegar not only retains the antioxidant and other physiological activities of anthocyanidin, but also contains some active substances including amino acids produced by fermentation, which will meet the needs of different people for nutrition and health care.

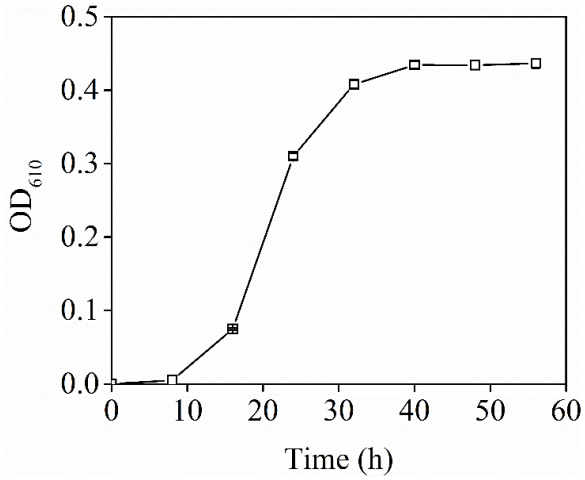

Figure 5. Growth curve of *Acetobacter Pasteurianus* AS1.41.

(1) The growth trend of acetic acid bacteria

To evaluate the growth of *Acetobacter Pasteurianus* AS1.41, the liquid medium was inoculated with AS1.41, incubated at 30℃, and 180 rpm. The samples were taken every 8 h for measuring the absorbance at 610 nm. The OD value of the bacterial suspension increased with increasing time and tended to the maximum at 40 h (Figure 5). The biomass quantity began slowly at initial period with a 16 h around lag time due to the adaptation of acetobacter to the new growth environment. After the lag period, the biomass quantity increased rapidly within about 40 h and remained almost unchanged thereafter, because AS1.41 cells reached the stable growth phase. Thus, the seed solution of AS1.41 inoculated for 32 h was selected as the optimal for the acetic acid fermentation of the purple carrot concentrated juices. At this stage, AS1.41 cells were in a logarithmic growth phase, with rapid growth and high metabolic activity, allowing the rapid conversion of alcohol to acetic acid in the fermentation broth and to form purple carrot vinegar. Subsequently, 10% of the above seed solution was added to the purple carrot concentrated juice treated with alcohol fermentation for yielding acetic acid. The acetic acid content in purple carrot juice as a function of time was depicted in Figure 6. The acetic acid content increased rapidly at the first 5 days, followed by a slight increase over time, owing to the accumulation of metabolic products along with the fermentation. Meantime, alcohol as a substrate was rapidly degraded by *Acetobacter pasteurianus* during fermentation and became nearly depleted within 6 days. Negligible alteration was observed in the acetic acid yield of the purple carrot juice after the fermentation of 6 days. Overall, the fermentation time of 6 days was optimal for acetic acid fermentation.

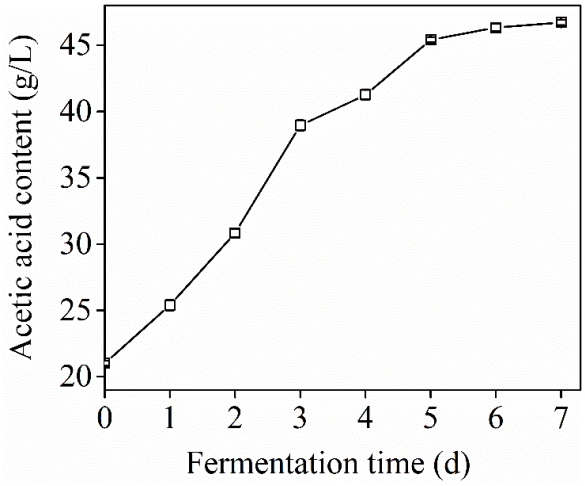

Figure 6. The acetic acid content in purple carrot juice as a function of time.

(2) Single factor optimization for acetic acid fermentation

The effect of different temperature on the acetic acid content in the purple carrot juice was shown in Figure 7A. The acetic acid content increased

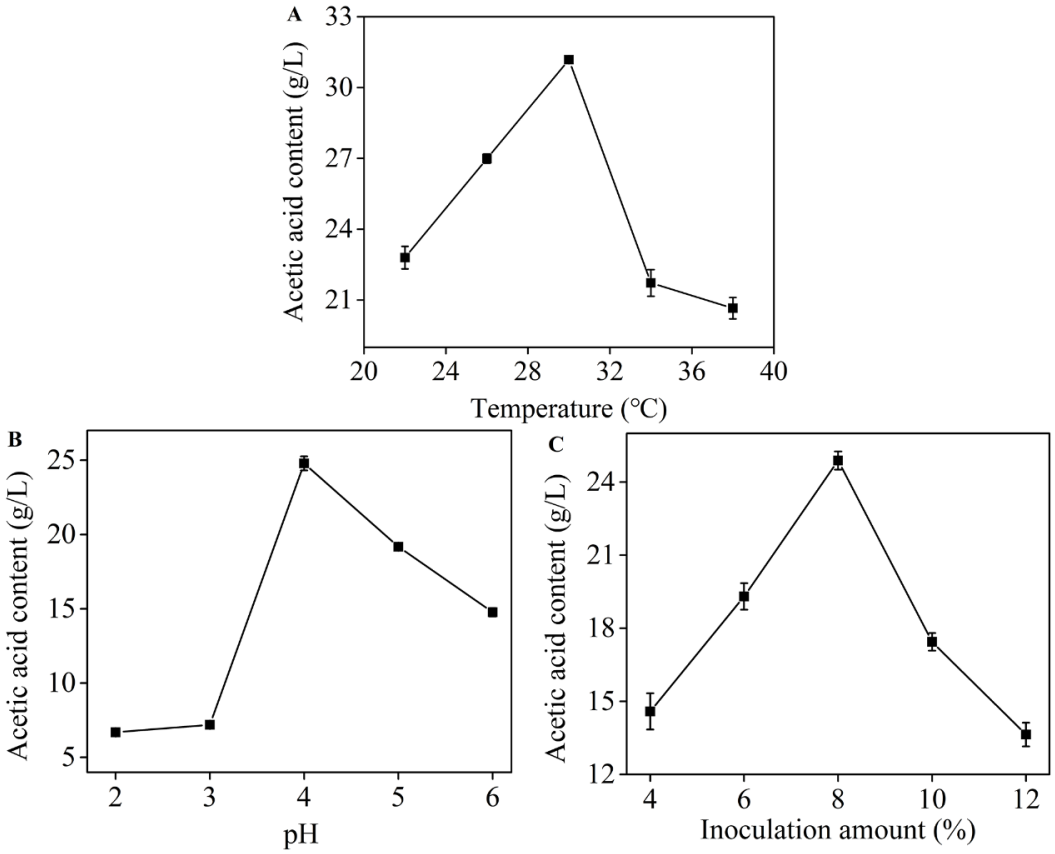

Figure 7. The single factor effect of fermentation temperature (**A**), pH (**B**), and inoculation amount (**C**) on the yield of acetic acid.

with fermentation temperature increasing from 22 to 30℃, reached a maximum of 31.2 g/L at 30℃, and followed by a decrease with higher temperature, which might be attributed to the influence of temperature on the growth and survival of microorganisms. Low or high temperature was infaust for the growth of *Acetobacter pasteurianus*, leading to a slow metabolic activity of cell, inhibiting its growth and reproduction. With temperature inching closer to the optimal one, *Acetobacter pasteurianus* cell proliferated faster, improving the yield rate of acetic acid. pH was another important factor that would influence the acetic acid yield. The acetic acid content in the juice increased from 6.69 g/L to 24.79 g/L with pH shifting from 2.0 to 4.0 and began to reduce above pH 4.0 (Figure 7B), which indicated that the optimal pH value for acetic acid fermentation was 4.0, with the maximum growth and metabolic activity for *Acetobacter pasteurianus* cell. Higher or lower pH would represent a negative impact on the enzyme activity in metabolic processes. The effect of inoculation amount on the yield of acetic acid was shown in Figure 7C. The acetic acid content in the purple carrot first increased and then decreased with the inoculation amount of *Acetobacter pasteurianus* increasing from 4% to 12%. The maximum yield of 24.89 g/L was obtained with the inoculation volume of 8%. A higher inoculation amount decreased the acetic acid yield. Therefore, inoculation amount 8% was favorable for acetic acid fermentation.

(3) Response surface analysis

The design matrix and the corresponding responses of RSM experiments to evaluate relationship of the acetic acid content (Y_2) with three variables including fermentation temperature (D), pH (E), and inoculation amount (F) were given in Table S4. The experiment data

Table 2. ANOVA results of response surface quadratic model for the acetic acid yield.

Source [a]	Sum of squares	Degree of freedom	Mean square	*F* value	*P* value
Model	169.55	9	18.84	22.02	0.0002
D	5.54	1	5.54	6.48	0.0383
E	70.09	1	70.09	81.94	< 0.0001
F	0.1682	1	0.1682	0.1966	0.6708
DE	0.0012	1	0.0012	0.0014	0.9709
DF	0.0002	1	0.0002	0.0003	0.9875
EF	0.0030	1	0.0030	0.0035	0.9542
D^2	32.22	1	32.22	37.67	0.0005
E^2	48.71	1	48.71	56.94	0.0001
F^2	4.79	1	4.79	5.60	0.0499
Residual	5.99	7	0.8554		
Lack of fit	1.33	3	0.4421	0.3794	0.7740
Pure error	4.66	4	1.17		
Total	175.54	16			
R^2		0.9659			
R^2_{adj}		0.9220			

Notes: D: temperature (°C). E: pH. F: inoculation amount (%). D^2, E^2, and F^2 were the quadratic terms of the corresponding variables (D, E, F).

were analyzed *via* multiple regression, resulting in the following second-order polynomial equation. $Y_2 = 36.41 - 0.8325D + 2.96E - 0.1450F + 0.0175DE + 0.0075DF + 0.0275EF - 2.77D^2 - 3.40E^2 - 1.07F^2$ (Equation 2). ANOVA was performed to estimate the significance of the fit of the quadratic model for the experiment data (Table 2). The *P* values showed that the linear coefficients (D, E) and three quadratic coefficients (D^2, E^2, F^2) were statistically significant, while the other term coefficients (F, DE, DF, EF) were insignificant ($P > 0.05$). However, to minimize error, all the coefficients were considered in the design. The model *P* value of 0.0002 indicated that the quadratic model was significant at 1% level, while the lack of fit ($P = 0.7740$) was statistically non-significant at 5% level. Moreover, R^2 and R^2_{adj} were 0.9659 and 0.9220, respectively, suggesting a close correspondence between observed and predicted values. The regression model was thus adequate for predicting future responses for acetic acid fermentation.

The corresponding two- and three-dimensional response surfaces of the quadratic model were shown in Figure 8. The acetic acid content in the purple carrot juice first increased and then decreased with the increase of various factors (Figure 8A-8C), indicating the optimal conditions just occurred within the design boundary. The elliptic contour plots were observed in Figures 8E and 8F, while the other was circular (Figure 8D), indicating the weakness mutual interactions between temperature and pH comparing with other variables. By analyzing the plots, the optimum conditions for acetic acid fermentation were determined as temperature of 29.7℃, initial pH 4.22, and inoculation amount of 7.94%, with a predicted maximum acetic acid of 37.12 g/L. For ease of operation, the optimal values of the variables were adjusted to temperature of 30℃, initial pH 4.2, and inoculation amount of 8%. The corresponding response was estimated as 37.05%. Three independent replicates for acetic acid fermentation were conducted under the above conditions, and the mean value of the experimental yield of acetic acid was 35.62 g/L. The excellent correlation between predicted and actual values confirmed that the employed model could effectively predict the optimal conditions for the fermentation process of purple carrot vinegar. The fermentation product had a strong sour taste and good color.

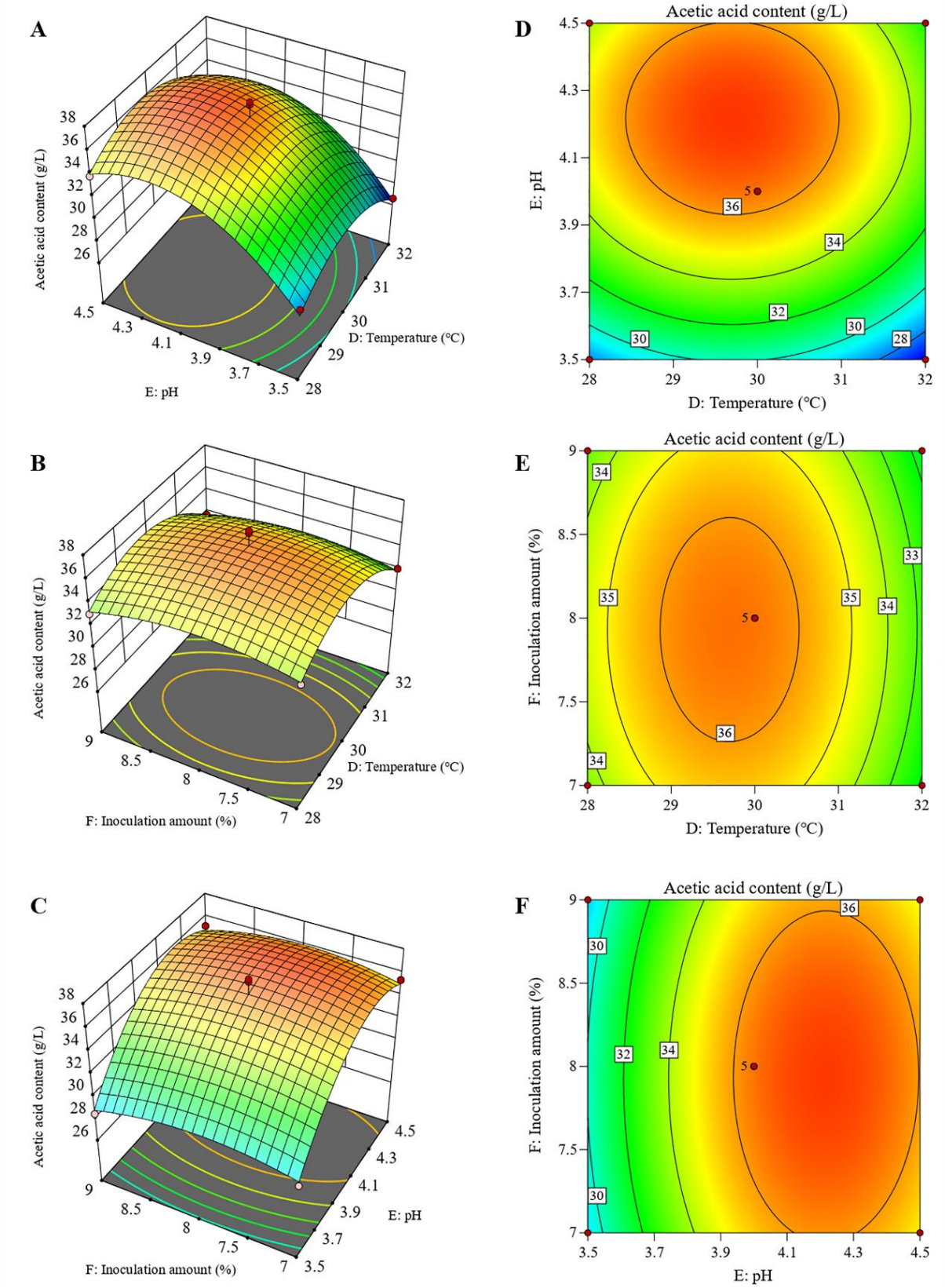

Figure 8. Response surface plots and the corresponding contour plot for acetic acid yield with respect to (A, D) temperature and pH, (B, E) temperature and inoculation amount, and (C, F) pH and inoculation amount.

Conclusion

In this study, the sugar-removal process by yeast fermentation was developed for the concentrated juice from purple carrot. 0.15‰ of RA was firstly determined to be the optimal by evaluating the fermentation ability in six wine dry yeasts for sugar-removing. Then, the single-factor experiments and Box-Behnken design along with RSM were employed for optimizing desugar parameters in the alcohol fermentation. The optimal conditions for the sugar removal were determined as initial pH 3.2, temperature 30℃, initial sugar content of 160 g/L, with an actual desugar rate of 91.03%. Meantime, the resulting fermented juices with low sugar reached alcohol content of about 10% (v/v), which not only had antibacterial effects, but more importantly, could serve as a fermentation substrate for preparing purple carrot vinegar. Therefore, the acetic acid fermentation was further conducted, and the maximum acetic acid yield of 35.62 g/L was obtained by using *Acetobacter Pasteurianus* AS1.41 with 8% of inoculation amount at pH 4.2 and 30℃. The development of purple carrot vinegar provided more possibilities for the application of purple carrot. The research on fermentation on sugar removal and fruit vinegar production was undergoing pilot tests, which was of great significance for promoting the development of natural food additives.

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