Advance Journal of Food Science and Technology 6(5): 647-654, 2014
DOI:10.19026/ajfst.6.89
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: January 27, 2014 Accepted: February 10, 2014 Published: May 10, 2014

Research Article

Multi-response Optimization of Pectinase Processing Conditions on Blueberry Juice Extraction by Desirability Function Methodology

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Abstract: Response Surface Methodology (RSM) was employed to analyze the effect of pectinase processing on Juice Yield (JY), Total Anthocyanin content (TA), Total Phenol content (TP) and Total Flavonoid content (TF) of blueberry juice. The processing conditions included enzyme dosage, hydrolysis temperature and hydrolysis time. For resolving multi-response optimization, desirability function method was used to integrate JY, TA, TP and TF into a new target D (Desirability value). Predicted values of JY, TA, TP, TF and D were found to be in good agreement with experimental values as indicated by the high R^2 values of 0.9963, 0.9217, 0.9586, 0.9901 and 0.9933, respectively. The optimum conditions were: enzyme dosage of 0.6 mg/g, hydrolysis temperature of 51.6°C and hydrolysis time of 2.36 h. At this optimum point, JY, TA, TP, TF and D were 71.406%, 420.367 mg/L, 2.784 g/L, 3.121 g/L and 0.9206, respectively. The study showed that RSM was an effective technique to model the effect of pectinase processing on blueberry juice parameters and desirability function method could be used for multi-response optimization of blueberry juice extraction.

Keywords: Blueberry, blueberry juice extraction, desirability function, muti-response optimization, pectinase, response surface methodology

INTRODUCTION

The nutritional quality of processed foods is of great interest to the consumer and food processing industry because of its direct and indirect impact on consumer's health. Blueberries are a rich dietary source of different antioxidant phytonutrients, including anthocyanins, phenols, flavonoids, ascorbic acids and Superoxide Dismutases (SOD) (Nindo *et al*., 2005; Moyer *et al*., 2002). These antioxidant compounds from blueberry are believed to reduce the risk of heart and other chronic diseases (Mazza *et al*., 2002). Many researches on the antioxidation of blueberry (Srivastava *et al*., 2007; Wang and Chen, 2010), the effect of processing on anthocyanins and other antioxidant compounds (Kechinski *et al*., 2007; Rizzolo *et al*., 2003) have been reported.

Blueberries are highly perishable (Schotsmans *et al*., 2007) and therefore it is necessary to process them into juice or concentrate when their yields get large. Generally, three methods of juice extraction are employed, including cold, hot and enzymatic methods (Sreekantiah *et al*., 1971). As compared to cold and hot extraction methods, the use of enzyme in fruit juice extraction, especially pectinase, could significantly increase juice extraction yield (Kaur *et al*., 2009; Chauhan *et al*., 2001), extracted more antioxidative compounds from fruits (Buchert *et al*., 2005; Landbo and Meyer, 2001) and clarify fruit juice (Lee *et al*., 2006; Rai *et al*., 2004). Enzymatic treatment on fruit pulp leads to an extensive degradation of the middle-lamina and cell wall pectin by polygalacturonase, pectinmethylesterase and pectinlyase activities (Demir *et al*., 2000). That causes more juice and compounds would be extracted from fruits.

The enzymatic hydrolysis effect mainly depends on enzyme dosage, hydrolysis temperature and hydrolysis time. These three variables need to be optimized for the best juice quality in blueberry juice extraction. At the optimization, not only single variable effect, but also the interactive effect among variables should be considered. RSM is an efficient statistical methodology to optimize complex process and evaluate the interactive effect among variables. It has been proved that RSM could be used for optimizing processing variable conditions (Mangaraj and Singh, 2011; Rastogi *et al*., 2010).

In the former researches of pectinase processing optimization on juice extraction, the processing conditions were only optimized for the response of juice yield (Kaur *et al*., 2009). However, in this blueberry juice extraction work, the process conditions are expected to be optimized for both larger juice yield and higher contents of some antioxidant compounds,

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such as total anthocyanins, total phenols and total flavonoids. Therefore, this optimization work has 4 responses totally, including Juice Yield (JY), Total Anthocyanin content (TA), Total Phenol content (TP) and Total Flavonoid content (TF). Several approaches have been used to do the multi-response optimization and one of them is to combine all responses into one measurement by a desirability function (Eren and Kaymak-Ertekin, 2007). By recent product processing researches, desirability function methodology has been proved to be appropriate for multi-response optimization analysis (Karazhiyan *et al*., 2011; Erbay and Icier, 2009).

The aim of this study is to analyze the effect of pectinase processing on JY, TA, TP and TF of blueberry juice and optimize the conditions (enzyme dosage, hydrolysis temperature and hydrolysis time) for maximal JY, TA, TP and TF.

MATERIALS AND METHODS

Raw material and enzyme source: A blend of Individually Quick Frozen (IQF) rabbiteye blueberries, grown in Huaining Blueberry Planting Center (Anhui, China), was used. Lafase He Grand Cru, a kind of commercial pectinolytic enzymes, was obtained from LAFFORT Company (Sydney, Austrilia). This pectolytic enzyme preparation could make the production rich in coloring matter and with supple tannins.

Juice extraction processing: The blueberries were unfrozen at room temperature for 3 h in the dark, after which they were pulped by a commercial blender (Shanghai saikon electrical appliances co. LTD, China, Model SG280F). Then being treated with pectinase at a certain constant temperature to hydrolyze the pectin, the blueberry juice was separated from pulp by centrifugal filtration (3000 r/min, 10 min). The resulting juice samples were stored at 4.0±0.5°C and would be analyzed within 4 h.

Determination of JY: Juice yield was determined as the percentage of juice weight, as shown in Eq. (1):

$$\text{Juice yield} = \frac{\text{Juice weight}}{\text{Fruit weight}} \times 100\% \quad (1)$$

Determination of TA: The total anthocyanin content in juice was determined using pH differential method (Fuleki and Francis, 1968; Mónica and Ronald, 2001). The TA content was calculated by Eq. (2):

$$TA(\text{mg/L}) = \frac{A \times M \times DF}{\varepsilon \times l} \times 1000 \quad (2)$$

where, A (Total Absorbance) = $[(A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}]$, M is the molecular weight of anthocyanin Cyd-3-glu (449.2 g/mol), DF is the dilution factor, ε is the extinction coefficient of anthocyanin Cyd-3-glu (26,900 L/mol cm) and l is the path length (1.0 cm). The spectrophotometer was blanked with distilled water at all wavelengths used. A double beam spectrophotometer (Shanghai precision and scientific instrument co. LTD, China, Model UV-759S) was used.

Determination of TP: An adjusted method (Dragovic-Uzelac *et al*., 2001) with Folin-Ciocalteu reagent was used to determine total phenol content in juice. One milliliter of sample or standard solutions of gallic acid (50-500 mg/L) and 30 mL of deionized water were added to a 50-mL volumetric flask at the same time, then 2.5 mL of Folin-Ciocalteu reagent were added to the flask and shaken. After 5 min, 7.5 mL of 7% Na_2CO_3 were added to the flask and the flask was immediately filled up to 50 mL with deionized water. After equilibration at room temperature for 2 h, the absorbance of the solution in the flask was measured by the spectrophotometer UV-759S at 765 nm. The results were calculated according to the calibration curve for gallic acid (y = 0.0018 x + 0.0022, y is absorbance at 765 nm, x is concentration of gallic acid in mg/L, R^2 = 0.9996).

Determination of TF: The total flavonoid content in juice was determined by a method with Al $(NO_3)_3$ and $NaNO_2$ (Chinese Pharmacopoeia Commission, 2000). One milliliter of sample or standard solutions of rutin (50-500 mg/L) and 4 mL of deionized water were added to a 10 mL volumetric flask at the same time, then 0.3 mL of 5% $NaNO_2$ were added to the flask and shaken. After 5 min, 0.3 mL of 10% Al $(NO_3)_3$ were added to the flask and shaken. Six minutes later, 2 mL of 1 mol/L NaOH were added to the flask and the flask was immediately filled up to 10 mL with deionized water. After equilibration at room temperature for 15 min, the absorbance of the solution in the flask was measured by the spectrophotometer UV-759S at 510 nm. The results were calculated according to the calibration curve for rutin (y = 0.0013x - 0.0062, y is absorbance at 510 nm, x is concentration of rutin in mg/L, R^2 = 0.9996).

Experimental design: RSM was employed to analyze the effects of processing conditions on JY, TA, TP and TF. There key independent variables, namely enzyme dosage, hydrolysis temperature and hydrolysis time were chosen to generate the experimental design through the Box-Behnken Design method (Douglas, 2005). The levels of the selected parameters were listed in Table 1.

Statistic analysis: The experimental data were fitted to quadratic regression models by Design Expert version 7.1.4 (Stat-Ease, U.S.A). The generalized quadratic regression model was shown as Eq. (3). The

Table 1: Variables and their levels in response surface design

	Symbols		Levels		
Variables	Uncoded	Coded	-1	0	1
Enzyme dosage (mg/g)	X_1	x_1	0.1	0.5	0.9
Hydrolysis temperature (°C)	X_2	x_2	45	50	55
Hydrolysis time (h)	X_3	x_3	1	2	3

coefficients of the model were represented by a_0 (constant term), a_1, a_2 and a_3 (linear coefficient), a_{11}, a_{22} and a_{33} (quadratic term coefficient) and a_{12}, a_{13} and a_{23} (interactive term coefficient). Statistical significances of the terms in the regression equations were examined by Analysis of Variance (ANOVA). Model adequacies were checked by R^2, Adj-R^2, Pre-R^2, Adeq Precision (Douglas, 2005):

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_3 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 \quad (3)$$

Optimization and desirability function: In many cases, the production quality characteristics would be described by several responses, which should be considered during the industrial optimization processing. So far several feasible approaches have been used to investigate all the responses the optimization of industrial processing and one of them is to combine all responses into one measurement with desirability function. In this study, an adjusted kind of desirability functions (Derriger and Suich, 1980) was developed for the criteria that maximum JY, TA, TP and TF. The desirability value D was finally considered as juice quality. The form of desirability function was shown as Eq. (4). The parameters of desirability function were showed in Table 2 in detail. L_i of every response (JY, TA, TF and TP) was chosen to be a little smaller than the minimum of its experimental values in Box-Behnken experiments and H_i of every response was chosen to be a little larger than the maximum of its experimental values; t_i of every response was determined to be "1" because the shape of desirability function was expected to be linear; w_i of JY was determined to be "2" and other responses' were "1" because JY was expected to be more important than other three responses in this optimization:

$$D = \left[\prod_i d_i^{w_i}\right]^{\frac{1}{\sum w_i}}; d_i = \begin{cases} 0, y_i < L_i \\ \left[\dfrac{y_i - L_i}{H_i - L_i}\right]^{t_i}, L_i < y_i < H_i \\ 1, y_i > H_i \end{cases} \quad (4)$$

where,

d_i = The desirability value of the i^{th} response
y_i = The response value of i^{th} response
L_i, H_i = The lower and upper targets of the i^{th} response respectively

Table 2: Design of desirability function

Responses	Symbols	L_i	H_i	t_i	w_i
JY	y_1	62.5	72.5	1	2
TA	y_2	320	438	1	1
TP	y_3	2	2.8	1	1
TF	y_4	1.6	3.05	1	1

t_i = The parameter that determined the shape of desirability function
w_i = The relative weight of the i^{th} response

RESULTS AND DISCUSSION

Results and design of desirability function: The experimental results of Box-Behnken design were shown in Table 3. Then based on the parameters from Table 2, the Desirability values (D) of all experiments were calculated according to the Eq. (4) and were also shown in Table 3. The examination of every model adequacy was shown as the p-value of lack of fit, R^2, Adi-R^2 and Adeq Precision in Table 4. The model was not adequate unless its lack of fit >0.05, R^2>0.9 and Adeq Precision >4. The coefficients of the variables in the regression models and their significations were shown in Table 5.

JY: As shown in Table 4, the p-value of lack of fit, R^2, Adi-R^2 and Adeq Precision of the regression model for JY were 0.2049, 0.9963, 0.9896 and 39.791, respectively. So it was clear that the model for JY was adequate (Douglas, 2005). And it was observed that JY was significantly related to the linear effect of enzyme dosage ($p<0.01$) (Table 5). All of the quadratic terms had significant effects ($p<0.01$). The interactive effects of enzyme dosage and hydrolysis temperature ($p<0.01$), hydrolysis temperature and time ($p<0.05$) were also significant. According to Table 5, the quadratic regression model for JY was shown as Eq. (5):

$$JY = 71.83 + 2.065x_1 - 0.116x_2 - 0.122x_3 - 1.382x_1x_2 - 0.007x_1x_3 - 0.51x_2x_3 - 3.09x_1^2 - 2.458x_2^2 - 1.334x_3^2 \quad (5)$$

Figure 1 described the effects of all the interactive terms on JY. With the increase of enzyme dosage, JY increased sharply at the beginning and tend to be steady at a fixed hydrolysis temperature and time (50°C, 2 h). Moreover, with the increase of hydrolysis temperature JY also increased firstly and then decreased, but the effect of hydrolysis temperature was less significant than that of enzyme dosage. JY increased at first and then declined slowly with hydrolysis time at a fixed enzyme dosage and hydrolysis temperature (0.5 mg/g, 50°C). During the enzymatic treatment, pectinase

Table 3: Design and results of Box-Behnken experiments

Runs	x_1	x_2	x_3	JY (%)	TA (mg/L)	TP (g/L)	TF (g/L)	D
1	-1	-1	0	62.928	340.007	2.060	2.073	0.0940
2	1	-1	0	70.093	346.199	2.513	2.035	0.4758
3	-1	1	0	65.235	381.467	2.486	2.526	0.4295
4	1	1	0	66.874	400.587	2.712	2.842	0.6262
5	-1	0	-1	65.687	327.632	2.013	1.858	0.1132
6	1	0	-1	69.559	390.401	2.157	2.069	0.4490
7	-1	0	1	65.267	356.084	2.578	2.809	0.4227
8	1	0	1	69.112	392.199	2.675	3.015	0.7339
9	0	-1	-1	67.559	401.859	2.055	1.652	0.2115
10	0	1	-1	68.571	415.755	2.447	2.340	0.6150
11	0	-1	1	68.526	360.111	2.468	2.454	0.5282
12	0	1	1	67.498	434.552	2.787	2.982	0.7386
13	0	0	0	71.672	401.927	2.681	2.928	0.8481
14	0	0	0	71.997	411.014	2.683	2.935	0.8803
15	0	0	0	71.821	410.201	2.628	2.890	0.8518

Table 4: Analysis of variance for examination of every regression model adequacy

	Lack of fit				
Responses	S.S.	p-value	R^2	Adi-R^2	Adeq precision
JY (%)	0.320	0.2049	0.9963	0.9896	39.791
TA (mg/L)	1037.450	0.0689	0.9217	0.7808	9.165
TP (g/L)	0.039	0.0713	0.9586	0.8840	11.137
TF (g/L)	0.028	0.0591	0.9901	0.9723	23.195
D	0.006	0.1459	0.9933	0.9933	27.446

S.S.: Sum of square

Table 5: The coefficients of the variables in the regression models and their significations

Coefficients	JY	TA	TP	TF	D
a0	71.830	407.714	2.664	2.918	0.860
a1	2.065**	15.525*	0.115*	0.087*	0.153**
a2	-0.116	23.023**	0.167**	0.310**	0.137**
a3	-0.122	0.912	0.230**	0.418**	0.129*
a12	-1.382**	3.232	-0.057	0.089	-0.046
a13	-0.007	-6.664	-0.012	-0.001	-0.006*
a23	-0.510*	15.136	-0.018	-0.040	-0.048**
a11	-3.090**	-38.570**	-0.152*	-0.234**	-0.274**
a22	-2.458**	-2.079	-0.069	-0.315**	-0.180**
a33	-1.334**	-2.565	-0.156*	-0.246**	-0.157**

*: Significant at 5% level; **: Significant at 1% level

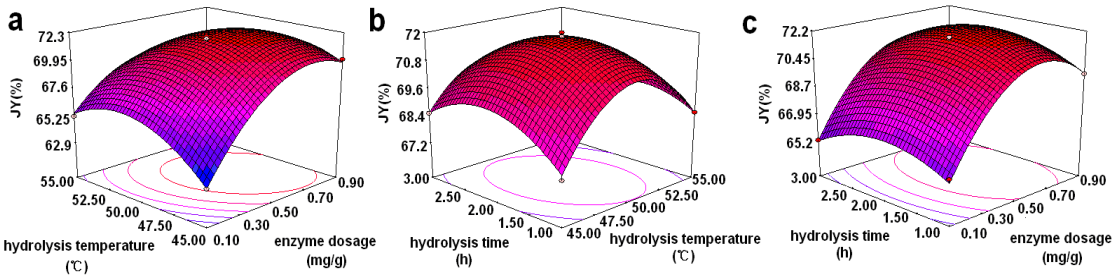

Fig. 1: Response surface and contour plots for Juice Yield (JY) as affected by all the interactive terms, (a) was enzyme dosage and hydrolysis temperature, (b) was hydrolysis temperature and time, (c) was enzyme dosage and hydrolysis time, (for each plot, the third machine parameter is fixed at "0" level)

breaks down the pectin molecules, degradation of pectin leads to a reduction of water holding capacity and consequently, free water is released to the system and JY was increased (Lee *et al*., 2006). The similar effect that enzymatic hydrolysis treatment resulted in extractive juice yield increasing was found in many other fruits (Kaur *et al*., 2009; Chauhan *et al*., 2001).

TA: From Table 4, the p-value of lack of fit, R^2, Adi-R^2 and Adeq Precision of the regression model for TA showed that the model for TA was adequate. As shown in Table 5, it was observed that TA was positive related to the linear effect of enzyme dosage ($p<0.05$) and hydrolysis temperature ($p<0.01$). The quadratic term of enzyme dosage had a negative effect on TA ($p<0.01$). All interactive terms had no effect on TA ($p>0.05$). According to Table 5, the quadratic regression model for TA was shown as Eq. (6):

$$TA = 407.714 + 15..525x_1 + 23.023x_2 + 0.912x_3 + 3.232x_1x_2 - 6.664x_1x_3 + 15.136x_2x_3 - 38.57x_1^2 - 2.079x_2^2 - 2.565x_3^2 \quad (6)$$

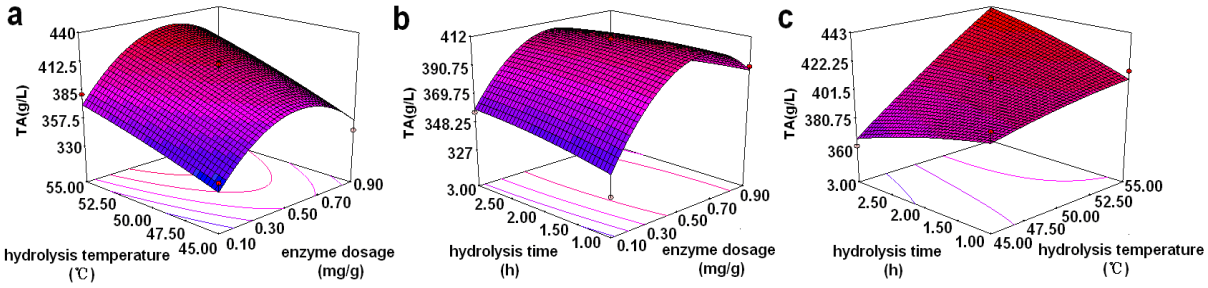

Fig. 2: Response surface and contour plots for Total Anthoyanin content (TA) as affected by all the interactive terms, (a) was enzyme dosage and hydrolysis temperature, (b) was enzyme dosage and hydrolysis time, (c) was hydrolysis temperature and time, (for each plot, the third machine parameter is fixed at "0" level)

The variation of TA with the interactive between variance is presented in Fig. 2. At a fixed hydrolysis temperature and time (50°C, 2 h), TA increased at the beginning and then declined with enzyme dosage. It is evident that TA was increased roughly linearly with hydrolysis temperature at a fixed enzyme dosage and hydrolysis time (0.5 mg/g, 50°C). This verified the linear effect ($p<0.01$) of hydrolysis temperature was dominant over its quadratic effect ($p>0.05$). The variation of TA with hydrolysis time was irregular as shown in Fig. 2. This coincided with the indistinctive effect ($p>0.05$) of hydrolysis time. The research of Lee and Wrolstad (2004) showed enzyme treatment had a little effect to increasing total anthoyanins recovery extracted from blueberry processing waste, but their anthoyanins recovery was not obtained at the optimal conditions. This present study showed at the optimal conditions much more anthoyanins could be extracted from blueberry fruits by pectinase treatment.

TP: According to Table 4, the p-value of lack of fit, R^2, Adi-R^2 and Adeq Precision of the regression model for TP showed the model was adequate. Table 5 clearly showed TP was affected linearly to enzyme dosage ($p<0.05$), hydrolysis temperature ($p<0.01$) and time ($p<0.01$). The quadratic terms of enzyme dosage and hydrolysis time had significantly negative effects ($p<0.05$). According to Table 5, the quadratic regression model for TP was shown as Eq. (7):

$$TP = 2.664 + 0.115x_1 + 0.167x_2 + 0.23x_3 - 0.057x_1x_2 - 0.012x_1x_3 - 0.018x_2x_3 - 0.152x_1^2 - 0.069x_2^2 - 0.156x_3^2 \quad (7)$$

The effect of the interactive terms on TP was described as Fig. 3. It was observed that at a fixed hydrolysis temperature and time (50°C, 2 h), TP increased with enzyme dosage up to a critical value beyond which it decreased. However, TP increased sharply with hydrolysis temperature and roughly linearly increased with hydrolysis time. This fact is corroborated by the value of the coefficients from Table 3.

TF: As shown in Table 4, the *p* value of lack of fit, R^2, Adi-R^2 and Adeq Precision of the regression model for TF showed the model was adequate. According to Table 5, it was observed that TF was positively related to the linear effect of enzyme dosage ($p<0.05$), hydrolysis temperature ($p<0.01$) and time ($p<0.01$). All of the quadratic terms had significantly negative effects ($p<0.05$). According to Table 5, the quadratic regression model for TF was shown as Eq. (8):

$$TF = 2.918 + 0.087x_1 + 0.31x_2 + 0.418x_3 + 0.089x_1x_2 - 0.001x_1x_3 - 0.04x_2x_3 - 0.234x_1^2 - 0.315x_2^2 - 0.246x_3^2 \quad (8)$$

Figure 4 presented the effect of the interactive terms on TF. At a fixed hydrolysis temperature and time (50°C, 2 h) TF with enzyme dosage increased up to a maximum value beyond which it decreased. This coincided with the fact that the quadratic effect ($p<0.01$) of enzyme dosage was dominant over its linear effect ($p<0.05$). However, TF increased roughly linearly both with hydrolysis temperature and time when other variables were fixed. This showed the linear effect ($p<0.01$) of both them was dominant over their quadratic effects ($p<0.01$).

The Desirability value (D): Based on Table 4, the p-value of lack of fit, R^2, Adi-R^2 and Adeq Precision of the regression model for *D* were 0.1459, 0.9933, 0.9933 and 27.446, respectively. So it was clear that the model for *D* was adequate. According to Table 5, it was observed that *D* the linear terms of enzyme dosage ($p<0.01$), hydrolysis temperature ($p<0.01$) and time ($p<0.05$) had significantly positive effects on *D*. All of the quadratic terms had significantly negative effects ($p<0.01$). The interactive effects of enzyme dosage and hydrolysis time ($p<0.05$), hydrolysis temperature and time ($p<0.01$) were also significant. According to Table 5, the quadratic regression model for *D* was shown as Eq. (9):

$$D = 0.86 + 0.15x_1 + 0.14x_2 + 0.13x_3 - 0.046x_1x_2 - 6.15\times10^{-3}x_1x_3 - 0.048x_2x_3 - 0.27x_1^2 - 0.18x_2^2 - 0.16x_3^2 \quad (9)$$

The effect of the interactive terms on *D* was described as Fig. 5. At a fixed hydrolysis temperature

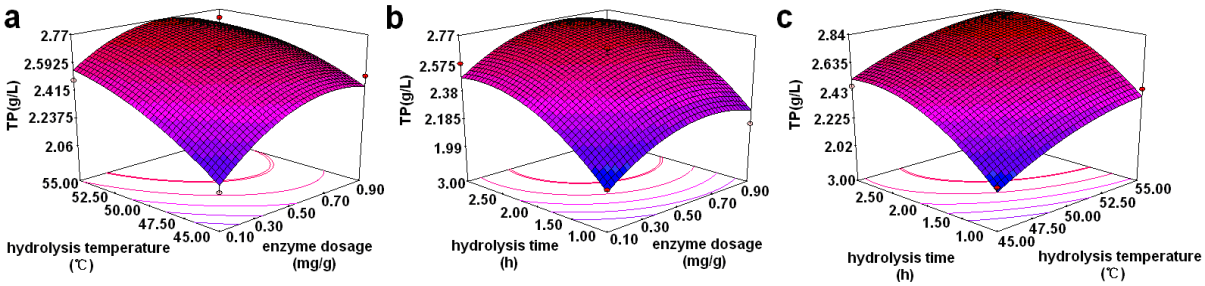

Fig. 3: Response surface and contour plots for Total Phenolic content (TP) as affected by all the interactive terms, (a) was enzyme dosage and hydrolysis temperature, (b) was enzyme dosage and hydrolysis time, (c) was hydrolysis temperature and time, (for each plot, the third machine parameter is fixed at "0" level)

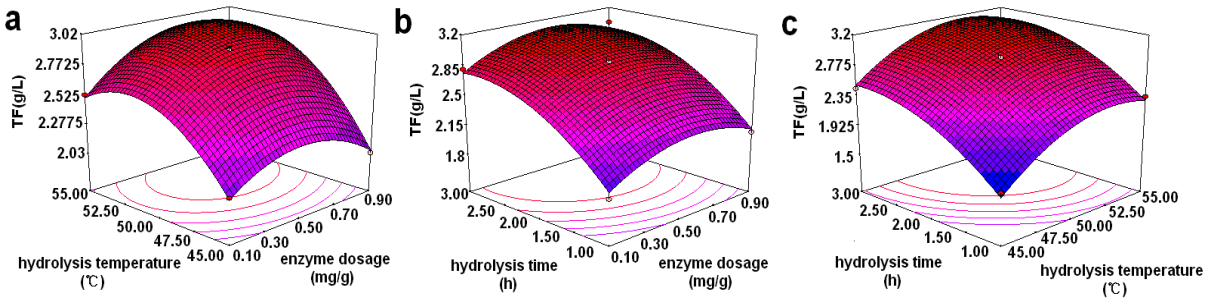

Fig. 4: Response surface and contour plots for Total Flavonoid content (TF) as affected by all the interactive terms, (a) was enzyme dosage and hydrolysis temperature, (b) was enzyme dosage and hydrolysis time, (c) was hydrolysis temperature and time, (for each plot, the third machine parameter is fixed at "0" level)

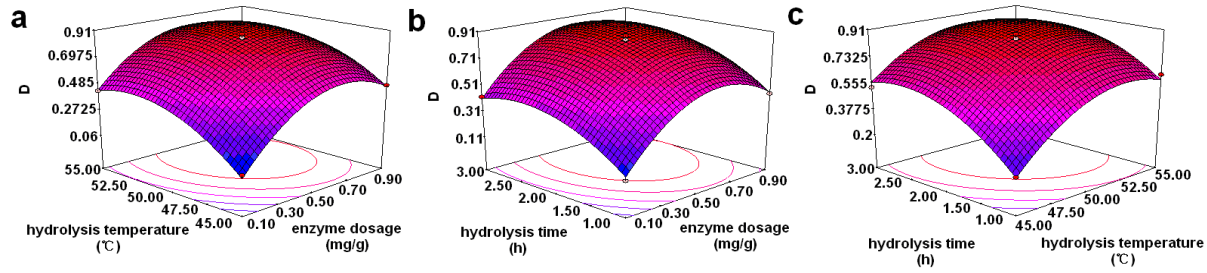

Fig. 5: Response surface and contour plots for Desirability value (D) as affected by all the interactive terms, (a) was enzyme dosage and hydrolysis temperature, (b) was enzyme dosage and hydrolysis time, (c) was hydrolysis temperature and time, (for each plot, the third machine parameter is fixed at "0" level)

and time (50°C, 2 h), *D* increased with enzyme dosage up to a critical value beyond which it decreased. But at different hydrolysis times, the variations of *D* with enzyme dosage were different. And the variances of *D* with temperature and time were similar when the other two variables were fixed at their 0 levels. All this showed that the quadratic effect of each variable was extremely significant and some interactive terms might have significant effects on *D*. And this coincided with the observation of the value of the coefficients in Table 5.

Optimization and verification: Pectinase processing conditions on blueberry juice extraction was optimized for four responses, including JY, TA, TP and TF, so that the optimization was based on the regression model for *D*:

$$\begin{cases} 0.15 - 0.54\,x_1 - 0.046\,x_2 - 06.15 \times 10^{-3}\,x_3 = 0 \\ 0.14 - 0.046\,x_1 - 0.36\,x_2 - 0.048\,x_3 = 0 \\ 0.13 - 6.15 \times 10^{-3}\,x_1 - 0.048\,x_2 - 0.32\,x_3 = 0 \end{cases} \quad (10)$$

To zero three partial derivatives of Eq. (9), an equation set of three liner equations of three variables was obtained as Eq. (10). The equation set was solved and its solutions were the optimal pectinase processing conditions. The solutions was x_1 = 0.247331, x_2 = 0.309952 and x_3 = 0.355004. According to the actual state, the optimal processing conditions were enzyme dosage of 0.6 mg/g, hydrolysis temperature of 51.6°C and hydrolysis time of 2.36 h. Calculated by the predicted models of all responses (Eq. 5~8), the estimated value of JY, TA, TP, TF, *D* was 71.501%, 418.622 mg/L, 2.778 g/L, 3.114 g/L and 0.9231,

respectively. The D value at optimal conditions was larger than the maximum of the Box-Behnken experiments and every response value of JY, TA, TP and TF was very close to the maximum of the Box-Behnken experiments. That indicated that the desirability function was an efficient tool to optimize the pectinase processing conditions of blueberry juice extraction for four responses.

To verify the reliability of the models and the optimal conditions, an experiment was performed at enzyme dosage of 0.6 mg/g, hydrolysis temperature of 51.6°C and hydrolysis time of 2.36 h. The results of the experiment were JY = 71.406%, TA = 420.367 mg/L, TP = 2.784 g/L, TF = 3.121 g/L and D = 0.9206. The errors between the predicted and experimental value were smaller than 1%. That demonstrated the regression models obtained by RSM could accurately predict JY, TA, TP, TF and D for any combination of enzyme dosage, hydrolysis temperature and hydrolysis time.

CONCLUSION

The response of JY, TA, TP, TF and D was found to be a function of the pectinase processing conditions, which obtained through RSM (R^2>0.92). Exploration of the response surfaces indicated a complex interaction between the variables. JY was found to be dependent on the interaction of enzyme dosage and hydrolysis temperature as well as hydrolysis temperature and hydrolysis time. The interaction of enzyme dosage and hydrolysis time as well as hydrolysis temperature and hydrolysis time had significant effect on D. For TA, TP and TF, the most significant effective variable was hydrolysis temperature, hydrolysis time and hydrolysis time, respectively. With desirability function, the optimal conditions were found to be: enzyme dosage of 0.6 mg/g, hydrolysis temperature of 51.6°C and hydrolysis time of 2.36 h. JY, TA JY, TA, TP, TF and D on the optimal conditions were 71.406%, 420.367 mg/L, 2.784 g/L, 3.121 g/L and 0.9206, respectively. D in the optimal conditions was obviously larger than D of all Box-Behnken experiments, which indicated that desirability function could be used for this optimization of four responses. The verification test showed the errors between the predicted and experimental value were smaller than 1%, which demonstrated the regression models obtained by RSM could predict JY, TA, TP, TF and D for any combination of enzyme dosage, hydrolysis temperature and hydrolysis time.

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