Pectinase enhances anthocyanin content in black carrot juice (*Daucus carota ssp.*)

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Abstract

The present study evaluates the effect of enzyme assisted processing (EAP), for enhanced recovery of total anthocyanin content (TAC) from black carrots, using pectinase.A Box-Behnken design with three-level, three-factor, under response surface methodology (RSM) was used to optimize the different concentrations of pectinase (0.1–0.3 %), incubation time (30–90 min) and extraction temperatures (50–70 °C). From a response surface analysis of the data, a three-degree polynomial equation was developed which provided the following optimal extraction conditions: enzyme concentration = 0.197%, temperature = 56.89 °C and extraction time = 51.46 min. Under the optimal conditions, black carrot juice extracted through EAP had TAC (670.28 mg/L). Overall there was 2 fold increase in TAC in juice processed with pectinase. Results demonstrate that pectinaseis a potential enzyme for enhancing anthocyanins and TPC from black carrots.

Keywords:Black carrots, Anthocyanins, Enzyme assisted processing, Pectinase, RSM

1. Introduction

Black carrot is considered as an excellent source ofanthocyaninswith significant antioxidant properties associated with alleviation of oxidative stress(Sun et al. 2012).Due to health promoting effect of anthocyanins, food and pharmaceutical industries are increasingly interested in these bioactive compounds. Acylated anthocyanins of black carrot imparts extraordinary thermal stability to the pigment in addition to strong antioxidant effects. Anthocyanins from black carrots have **G**enerally **R**ecognised **A**s **S**afe (GRAS) status and are promising alternatives to artificial colours in foods. Currently, black carrot concentrate is widely used to impart red shades to many acidic foods, such as soft drinks, Jams, marmalades and confectionary (Kirca et al. 2006 and Wrolstad and Culver 2012).

Extraction of anthocyanin is a challenging task and crucial to the recovery of high quality pigment for the industry. Traditional methods of extraction employ

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acidified water, sulphited water, organic or hydro alcoholic solvents, which have poor extraction yields, and take longer time and consume higher amounts of organic solvent.EAP is a rapid, inexpensive and green method for recovery of pigments and other bioactives. The EAP has been successfully used to enhance the recovery of various compounds such as polyphenols from blackcurrant; citrus peel and ginger, lycopene from tomato; protein from olive leaves; and essential oils from garlic (Boulila et al. 2015). Since the carrot roots display a wide distribution of polysaccharides with highly branched pectins to hemicellulose-enriched fractions, pectinase can be promosing effective enzyme for extraction of TAC.Till date, there is no report on the recovery of TAC from black carrots using pectinase by using statistical tool Response surface methodology (RSM).Keeping this in mind, the main objective of the present investigation was to optimize an enzyme assisted process for enhanced recovery of anthocyanin from black carrots employing pectinase enzyme.

2. Materials and methods

2.1. Extraction procedure

Freshly harvested, medium sized roots of black carrot variety 'PusaAsita' was generously supplied by Division of Vegetable Science, IARI, New Delhi, India. The crushed black carrot mash acidified with citric acid (pH 3.5) was poured in capped conical flasks. The mash was mixed thoroughly with pectinasefrom *Aspergillus sp.* (Sigma) and added at the w/v, from 0.1%-0.3% and placed in a thermostatically controlled incubator and incubated atdifferenttemperatures (40–60 ºC) for (30–90 min) as per designed RSM (Table 1).At the end of the incubation period, the extracted juice was filtered through double layered cheese cloth, heat processed at 90 ºC for 1 min to inactivate the enzyme and immediately analyzed for TAC. All treatments were carried out in triplicate.

2.2 Determination of TAC

TAC was determined using the pH differential method described by Wrolstadet al. 2005,expressed as cyanidin-3-glucoside equivalents/L.

2.3. Experimental design

A three-level, three-factor, Box–Behnken design was chosen to evaluate the combined effect of three independent variables viz. enzyme concentration, time and temperature on the response. The experimental design and statistical analysis were performed using Stat-Ease software (Design-Expert 8.0 Trial, Stat-Ease Inc., USA). The minimum and maximum values for enzyme (0.1-0.3 %), extraction time (30-90 min) and temperature (40-60°C) (Table 1). The response was evaluated in terms of TAC. The design consisted of 17 combinations, including five replicates of the center point used to determine the experimental error (Table 1).

3. Result and discussion

3.1. TAC

Anthocyanins are the most potential bioactive compounds present in black carrots, thus optimization of EAP is crucial. In the present paper, EAP of black carrots was optimized using, Pectinase enzyme preparation.Significant ($p<0.05$) variations in TAC were found in response to different enzymatic treatments and the results are presented inTable 1. The TAC ranged from 93.71 - 712.46 mg/L which is almost 2 folds more as comparison to untreated mash (50°C, 60 min). The TAC data, were consistent with

the interpretation that increased TAC is a result of enzyme catalyzed degradation of the cell wall in the plant matrix.

The experimental values of TAC in black carrot juice at various experimental conditions are presented in Table 1. As it was expected, the TAC increased with enzyme dosage, extraction temperature and incubation time. The maximum TAC (712.46 mg/L) was observed at 0.2% enzyme dose, incubation time of 60 minand temperature of 50°C. Both enzyme dosage and temperature are very crucial factors as excessive dosage can lead to breakdown of the anthocyanin structure, accompanied by color loss. Another critical factor in EAP is the length of incubation period. In the present study, 60 min was found optimum for maximum TAC.

3.2. Response surface modelling

In order to verify the predictive capability of the model, optimum conditions were established by RSM and comparisons between predicted results and the practical values were done by experimental rechecking using those presumed optimal conditions. The fitted model for total anthocyanin content (TAC) of the parameter estimates is as follows:

TAC = 666.032 + -0.57125 * A + -31.5512 * B + 35.76 * C + 46.82 * AB + -82.9325 * AC + -40.2575 * BC + -314.751 * A^2 + -188.306 * B^2 + -144.219 * C^2

Where A, B and C were the coded values of enzyme concentration, incubation time and temperature respectively.The regression coefficients and the significant values, represented by star mark, were also presented in Table 2. The importance of the independent variables to TAC value could be ranked in the following order: Temperature (C) >Time (B) > EC (A) can also seen in table 2. Interestingly, the linear coefficient of temperature (C) was positive, but its quadratic effect was negative.The response surface plots explained the relationships between TAC and the three extraction parameters involved (Fig. 1.1 – 1.3).

3.3 Responses at optimal extraction conditions

Optimal enzyme concentration, incubation time and temperature are critical for obtaining high TAC.The best combination of process variables for the best set of response properties included an enzyme concentration of 0.197%, incubation time of 56.89 min and incubation temperature of 51.46 °C.There is excellent agreement of the experiment values with the predicted values (670.28 mg/L) indicating the suitability of the models developed and the success of RSM in optimizing the extraction conditions.

4. Conclusion

Optimal enzyme concentration, incubation time and temperature are critical for obtaining high TAC. Under the optimized conditions, the experimental maximum TAC was 683.76 ± 35.54 mg/L. There is excellent agreement of the experiment values with the predicted values indicating the suitability of the models developed and the success of RSM in optimizing the extraction conditions.

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Table 1. Box-Behnken design and experimental data of TAC for Enzyme assisted processing (EAP) by using pectinase

		Factor 1	**Factor 2**	**Factor 3**	**Response 1**
Std	Run	A:EC	B:Time	C:Temp	TAC
Units		%	min	C	mg/L
7	1	0.1	60	60	319.12
4	2	0.3	90	50	198.05
16	3	0.2	60	50	668.68
10	4	0.2	90	40	278.87
3	5	0.1	90	50	93.71
12	6	0.2	90	60	296.13
17	7	0.2	60	50	678.45
15	8	0.2	60	50	628.61
11	9	0.2	30	60	468.66
2	10	0.3	30	50	138.6
14	11	0.2	60	50	712.46
5	12	0.1	60	40	107.99
9	13	0.2	30	40	290.37
1	14	0.1	30	50	221.54
8	15	0.3	60	60	140.27
13	16	0.2	60	50	641.96
6	17	0.3	60	40	260.87

Where TAC=Total anthocyanin content

Table 2. Model summary statistics-ANOVA (for significant values), Regression coefficient, coefficient of determination (R^2) and F- test value of the second order polynomial models for the TAC for Enzyme assisted processing (EAP) by using pectinase

		Pectinase
Coefficients	Degree of freedom	TAC

Intercept	9	666.03 (14.76)
Linear		
A	1	-0.57 (11.67)
B	1	-31.55** (11.67)
C	1	35.76** (11.67)
Interactive		
AB	1	46.82** (16.51)
AC	1	-82.93*** (16.51)
BC	1	-40.26** (16.51)
Quadratic		
A^2	1	-314.75*** (16.09)
B^2	1	-188.31*** (16.09)
C^2	1	-144.22*** (16.09)
Residual	7	
Lack of fit	3	0.4676
Pure error	4	
total	16	
R-Squared		0.9903
Adj R-Squared		0.9779
Pred R-Squared		0.9239
Adeq Precision		22.943
CV%		9.13

Where A=Enzyme concentration, B=Time, C=Temperature, AB= Enzyme concentration*Time, AC=Enzyme concentration* Temperature, BC= Time*Temperature and A^2, B^2 and C^2 are representing the quadratic terms for Enzyme concentration, Time, Temperature respectively and values shown are regression coefficient of respective term. Figures in parenthesis denotes standard error. * Significant at p<0.1, **Significant at p<0.05, ***significant at p<0.01 rest other values are non-significant. TAC=Total anthocyanin content, CV= Co-variance, Adj R-squared= Adjusted R- squared, Pred R-squared= Predicted R-squared, Adeq Precision= Adequate Precision.

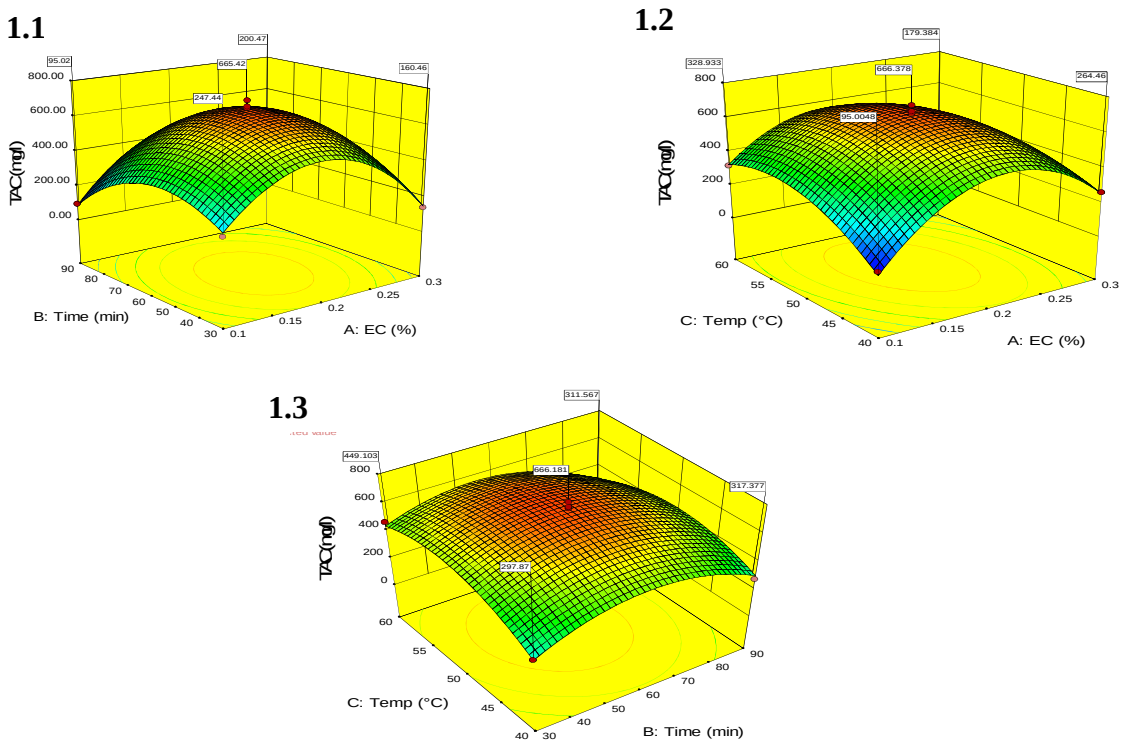

Fig. 1 Response surface analysis of black carrot for the effect of EC and time (1.1), EC and temperature (1.2), time and temperature (1.3) on TAC using pectinase. TAC= Total anthocyanin content in mg/L, EC= Enzyme Concentration in percent

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