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***Short communication***

# Cellulase enhances anthocyanin and phenolic content in black carrot juice

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## ABSTRACT

**The present study evaluates the effect of cellulase, for enhanced recovery of total anthocyanin and phenolic content from black carrot. A Box-Behnken design with three-level, three-factor, under response surface methodology (RSM) was used to optimize the different concentrations of cellulase (0.1–0.3 %), incubation time (30–90 min) and extraction temperatures (50–70 ºC). From analysis of the data, the following optimal extraction conditions were achieved: enzyme concentration = 0.215 %, temperature = 49.08 °C and extraction time = 63.3 min. Under the optimal conditions, black carrot juice extracted through EAP had TAC (1108.56 mg/kg) and TPC (305.88 mg GAE/100g).**

**Key words***: Daucus carota*, enzyme assisted processing, response surface methodology (RSM).

Black carrot is considered as an excellent source of polyphenols and anthocyanins (ACNs) with significant antioxidant properties associated with alleviation of oxidative stress (Kumar, 8; Kumar *et al.,* 7). However, extraction of ACN is a challenging task and crucial to the recovery of high quality pigment for the industry. Traditional methods of extraction employ 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. Enzyme assisted processing (EAP) is a rapid, inexpensive and green method for recovery of pigments and other bioactives. The process is based on hydrolytic activity of cellulase, pectinase, viscozyme and β-glucosidase to hydrolyze and degrade plant cell wall constituents to improve the release of intracellular compounds (Chen *et al*., 2). Previous work by Khandare *et al*., 4 and Kumar *et al*., 5, reported high recovery of ACN using pectinase from black carrot and viscozyme from black soybean respectively. Since the carrot roots display a wide distribution of polysaccharides with highly branched pectins to hemicellulose-enriched fractions, cellulase can be promising effective enzyme for extraction of TAC. Keeping this in mind, the main objective of the present investigation was to optimize an enzyme assisted process for enhanced recovery of ACN and phenolics from black carrot employing cellulase enzyme.

For carrying out extraction, freshly harvested, medium sized roots of black carrot variety 'Pusa Asita' was generously supplied by Division of Vegetable Science, IARI, New Delhi, India. The extraction procedure was followed as per Kumar *et al*., 2019b with minor modification. The crushed mash (≈50 g) acidified with citric acid (pH 3.5) was poured in capped conical flasks. The mash was mixed thoroughly with cellulase from *Aspergillus sp.* (Sigma) and added at the w/v, from 0.1%-0.3% denoted by 'A' and placed in a thermostatically controlled incubator and incubated at different temperatures (40–60 ºC) denoted by 'B' for (30–90 min) denoted by 'C' 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 and TPC. TAC was determined using the pH differential method described by Kumar *et al*., 6 and expressed as cyanidin-3-glucoside equivalents/L. The total phenolic content of juice was estimated spectrophotometrically using Folin–Ciocalteu reagent as described by Kumar *et al*., 5 and results expressed as gallic acid equivalents [mg gallic acid equivalents (GAE)/100g].

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 variables and their levels were chosen, based on the results of preliminary experiments and single response analysis. The minimum and maximum values for (A) enzyme (0.1-0.3 %), (B) temperature (40-60°C) and (C) extraction time (30-90 min) (Table 1). The response was evaluated in terms of TAC and TPC. The design consisted of 17 combinations, including

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five replicates of the center point used to determine the experimental error (Table 2).

In the present paper, EAP of black carrots was optimized using a multicomponent, cellulase enzyme preparation, produced by *Aspergillus* sp. (Table 1). TAC were found in response to different enzymatic treatments and the results are presented in Table 2. The TAC and TPC ranged from 628.43-1178.47 mg/kg and 182-329 mg GAE/100g which is almost 2.5 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. Further, the activities due to cellulase result in breakdown of insoluble matrix leading to complete disintegration and release of bound intracellular components. The results are in agreement with the reports of previous researchers. Boulila *et al*., 1 proved EAP resulted in enhanced release of phenolics from bay leaves (*Laurus nobilis* L.). The experimental values of TAC in black carrot juice at various experimental conditions are presented in Table 2. As it was expected, the TAC increased with enzyme dosage, extraction temperature and incubation time. The maximum TAC (1178.47 mg/kg) was observed at 0.2% enzyme dose, incubation time of 60 min and temperature of 50°C. Both enzyme dosage and temperature are very crucial factors as excessive dosage can lead to breakdown of the ACN structure, accompanied by color loss. Temperature plays a very vital role as it disrupts the cell wall and opens up the structure there by favoring the penetration of enzymes and making the release of phenolics easy (Costoya *et al*., 3). Similar improvements of ACNs in blueberry and strawberry juices have been confirmed by previous researchers (Puertolas *et al*., 9). The results obtained are in close agreement with the previous works. Enhanced ACNs from elderberry, purple corn, black currant and grape wines using pectinase enzyme have been reported (Puertolas *et al*., 9). Another critical factor in EAP is the length of incubation period. Excessive extraction period may prove detrimental for extraction of phenolic antioxidants, especially ACNs, which are sensitive and can polymerize and degrade (Silva *et al*., 10). However, optimal length of time is required to extract them efficiently from cellular matrix otherwise they remain entrapped and go waste into pomace. In the present study, 60 min was found optimum for maximum TAC and TPC. The optimum predicted values for 'A', 'B' and 'C' are obtained as A= 0.215%, B = 63 min, C = 49.09°C and under the optimized conditions, the experimental maximum TAC and TPC was 1112.15 ± 93.56 mg/kg and 287.5 ± 9.04 mg GAE/100, respectively.

**Table 1.** Independent variables with coded levels and actual values for fitting response surface model (RSM).

| Independent variables | Units | Code levels | | |
|---|---|---|---|---|
| | | -1 | 0 | +1 |
| Enzyme Concentration (A) | % | 0.1 | 0.2 | 0.3 |
| Temperature (B) | °C | 40 | 50 | 60 |
| Time (C) | minutes | 30 | 60 | 90 |

**Table 2.** Box-Behnken design and experimental data for TAC and TPC.

| Treatment Combinations | Independent variables | | | Dependent variables | |
|---|---|---|---|---|---|
| | A | B | C | TAC (mg/Kg) | TPC (mg GAE/ 100g) |
| 1 | -1 | +1 | 0 | 740.32 | 237.25 |
| 2 | +1 | +1 | 0 | 919.55 | 286.3 |
| 3 | 0 | 0 | 0 | 1120.50 | 291.35 |
| 4 | +1 | 0 | -1 | 731.41 | 237.45 |
| 5 | -1 | 0 | +1 | 696.34 | 214.1 |
| 6 | +1 | 0 | +1 | 696.90 | 213.65 |
| 7 | +1 | -1 | 0 | 768.71 | 248.3 |
| 8 | 0 | -1 | -1 | 870.01 | 275.7 |
| 9 | 0 | 0 | 0 | 1105.47 | 292.55 |
| 10 | 0 | +1 | +1 | 736.42 | 244.35 |
| 11 | 0 | 0 | 0 | 1049.25 | 296.95 |
| 12 | 0 | -1 | +1 | 700.24 | 201.25 |
| 13 | -1 | -1 | 0 | 628.43 | 182 |
| 14 | 0 | +1 | -1 | 733.08 | 233.15 |
| 15 | -1 | 0 | -1 | 628.43 | 251.1 |
| 16 | 0 | 0 | 0 | 1070.40 | 329 |
| 17 | 0 | 0 | 0 | 1178.47 | 305.6 |

Key to short form: 'A'= Enzyme Concentration, 'B'= Temperature, 'C'=Time, TAC=Total Anthocyanin Content, TPC=Total Phenolic Content

In conclusion, optimal enzyme concentration, incubation time and temperature are critical for obtaining high TAC and TPC. Under the optimized conditions, the experimental maximum TAC and TPC was 1112.15 ± 93.56 mg/kg and 287.5 ± 9.04 mg GAE/100 respectively. 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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