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The Encapsulation Efficiency and Physicochemical Characteristics of Anthocyanin from Black Carrot (*Daucus Carota ssp. Sativus*) as Affected by Encapsulating Materials

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Abstract

The anthocyanin pigment of black carrot juice was used as a core material for microencapsulation by spray dryer at 150°C using the mixed carrier material viz. whey protein isolate (WPI), jackfruit seed starch (JSS) and NBRE-15. The ratio of WPI: JSS ranged from 1:1 to 1:3 and NBRE-15 from 0.1-0.3% were taken to optimize the carrier material for encapsulation taking anthocyanin content, antioxidant activity and encapsulation efficiency as responses using Box-Behnken Design (BBD) by Response Surface Methodology (RSM). The anthocyanin (core material) was encapsulated at the optimized condition of the carrier material having five times the jackfruit seed starch as the whey protein isolate (5:1) and 0.3% NBRE-15 as an emulsifier. The optimized powder was found to have 2766.61 mg/100g (dry matter) of anthocyanin content, 290.56 µmolTrolox/g (dry matter) antioxidant activity with an encapsulation efficiency of 77.12% at 93.59% of desirability. Optimized powder retained the color value of 30.61, 29.39 and 0.03 for L*, a* and b*. Mean particle size distribution for the optimized encapsulated anthocyanin powder was 52.36 µm. Scanning Electron Microscope (SEM) images revealed the smooth surface characteristic of the optimized powder with slightly oval to globular in shape.

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Introduction

Anthocyanins (Greek *anthos*, flower and Greek *kyanose*, blue) are pigments of many flowers, fruits, and vegetables which are responsible for red, blue and purple colors.[1] Nowadays used as a natural colorant, only small doses of anthocyanins are required to display the color desired in several food matrixes (e.g. 30 to 40 ppm for soft drinks and 20–60 ppm for fruit preserves). Besides their use as a natural colorant, they play a major role in reducing the risk of coronary heart disease, cancer, and stroke,[2] improve visual acuity, anticancer and antioxidant activities[3] Anthocyanins are glycosides of anthocyanidins (also called aglycones). Till date, 539 anthocyanins have been reported from plants[4] but mostly six anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin) are found widespread in fruits and vegetables.[5,6]

Black carrot (*Daucus carota* ssp. *sativus*) anthocyanin attributes higher stability to hydration, light, *p*H and temperature changes due to the presence of acylated groups like *p*-coumaric, ferulic, *p*-hydroxybenzoic and sinapic acids.[7] In addition to their high anthocyanin stability, the black carrot is a rich source of anthocyanin having 1750 mg of anthocyanin per kilogram fresh weight.[8] Being a rich source of natural colorant, black carrot in India is underutilized and could not gain much consumer acceptance as a vegetable. Therefore, some processing techniques implied to renovate the raw food materials by means of either chemical or physical changes into stable, consumable, marketable and valorized form.[3,6,9-11]

Increase awareness about health and healthy food development of functional ingredients food products with nutritional and medicinal values are under consideration.[12-18] Microencapsulation is a process technique of incorporating natural ingredients, polyphenols, volatile additives, colors, enzymes, and bacteria in small capsules in order to stabilize and protect them against nutritional as well as health losses. It does package materials in the form of micro- and nanoparticles.[19] The active agent inside the microcapsule is referred to as the core, encapsulants, internal phase, payload phase or fill, whereas the wall is called the shell, coating, wall material, encapsulating agents or membrane shell, carrier material, external phase, or matrix.[20] Microencapsulation by spray dryer is an economical method for preservation of natural colorants by entrapping the active ingredient in a wall material.[21] The most significant parameters of spray dryer, which affects the physicochemical properties of core materials as well as wall materials, are inlet temperature, outlet temperature and feed speed.[22-25] Microencapsulation of black carrot extract using spray dryer at inlet and outlet temperature of 150°C and 76°C with 2 mL/min feed rate were studied the best for efficient quality anthocyanin powder.[26] Several studies have been conducted for the protection of core material from adverse environmental conditions *viz*., high acidity, oxygen stress, and temperature many researchers using different carrier materials.[27-30] The choice of carrier material should be low solubility so that it is protected while passing through *in vitro* and *in vivo* environmental conditions.[31,32] Jackfruit seed starch (JSS) has been chosen as a carrier material because jackfruit seed is a source of protein (13.50%), carbohydrates (79.34%), crude fiber (3.19%) and total mineral matter (3.1%)[33,34] and it has excellent gelling, thickening capacity, stability to mechanical & thermal shear, binding capacity, acid resistivity and non-toxicity.[35] Whey protein isolate (WPI) with polysaccharides has been also studied by several researchers in microencapsulation of different bioactive compounds using spray drying.[30,36,37] Similarly gum arabic also plays a key role in the encapsulation of different bioactive compounds.[38,39] However, availability and caste of production have been limited the utilization of gum arabic in food industries.[39-43] Hence, NBRE-15 has similar chemistry to gum arabic which acts as an emulsifying agent was tried to optimize the effective concentration in microencapsulation of anthocyanin.

In this study the feed mixture containing Jackfruit Seed Starch, Whey protein and NBRE-15 as carrier materials for the encapsulation of anthocyanin pigment of black carrot juice was evaluated statistically by the use of RSM; the process was optimized in terms of the modeling equations for the anthocyanin content, antioxidant activity, and the encapsulation efficiency and the particle size and its structure have been characterized using Particle Size Analyser and Scanning Electron Microscope (SEM) respectively.

Materials and Methods

Materials

Black carrot (Pusa asita - black carrot variety) sample was procured from the archer of Indian Agricultural Research Institute, New Delhi, India. The carrot was properly cleaned, washed and kept at (4-10) °C in cold storage before juice extraction. WPI (>99.0%) was purchased from the local market of New Delhi, India. NBRE-15 (an emulsifier) was obtained National Botanical Research Institute (NBRI), Lucknow, India. Jackfruit seed starch was isolated from jackfruit seeds purchased from the local market. Other chemicals were purchased from Sigma Aldrich, India and Central Drug House (CDH) Private Ltd. Pvt, New Delhi, India.

Juice Extraction

Juice extraction was done as per the method optimized by Kirca *et al.*[10] with slight modification. The carrot was manually peeled and crushed by a hydraulic press to extract the juice. Solid fibers were separated from the juice by filtering through Whatman No.1 filter followed by addition of 1% citric acid as a preservative. It was given a pectinase enzyme treatment at 50°C for 2 hours. The total solids were measured to be 6°Brix of the extracted juice and stored in cold storage at (4-10) °C for further use.

Carrier Agents for Spray Drying

Dry Jackfruit seeds were procured from the local market, New Delhi, India. The seeds were decorticated and powdered using hammer mill and further used for starch extraction. The starch was extracted using the distilled water method as described by Murali *et al.*[22,24] with slight modification. Jackfruit seed powder was sieved using 150-170µ pore-size and slurry made with distilled water. The slurry was centrifuged for 2 min at 9000 rpm and the pellet collected was washed with 50% ethanol followed by vacuum drying for 48 hours. The fat was removed by adding petroleum ether which was evaporated at room temperature. The starch obtained was fineally ground with a mortar and pestle and packed in plastic bag and kept at room temperature for further use. Whey protein isolate (WPI) was obtained from the local market. NBRE-15 a type of emulsifier was excessed from National Botanical Research Institute (NBRI), Lucknow, India.

Preparation of Feed Mixtures

Jackfruit seed starch (JSS), whey protein extract and NBRE-15 were combined with the black carrot juice (6% solid content) in different proportions as per the BBD arrangements of design-expert 8.0.7.1 (Table 1) and stirred to homogeneity with IKA®T25 digital Ultra-Turrax (USA) homogenizer for 30 min.

Spray Drying Conditions

The feed mixtures were spray dried using SonoDry 1000 (Sono-Tek Corporation, USA) with the inlet temperature of (150 ± 2) °C and the outlet temperature of (76 ± 2)°C being optimized for the encapsulation of black carrot juice using spray drying.[26] The aspirator speed was set at 79 rpm with a constant feed flow of 2 ml/min with D-block on time of 1 second.

Determination of Anthocyanin Content

The total monomeric anthocyanin pigment was determined using pH differential method.[26] The samples were diluted in two buffer systems- *p*H1.0 buffer (potassium chloride, 0.025M) and pH 4.5 buffer (sodium acetate, 0.4M) and the absorbance measured at 520 nm and 700 nm for each using UV-Visible spectrophotometer (GENESYS 10 VIS, USA). The results were expressed as cyaniding-3-glucoside equivalents as:

Monomeric anthocyanin pigment (*mg/L*) =
$A \times MW \times DF \times 10^3 / sx1$ ...(1)

Where
A = Absorbance = $(A_{520nm} - A_{700nm})pH\ 1.0 - (A_{520nm} - A_{700nm})pH\ 4.5$

MW = Molecular weight = 449.2; DF = Dilution factor; 1 = path length in cm and ε = 26,000 $L.mol^{-1}.cm^{-1}$.

Determination of Antioxidant Activity

The Antioxidant activity was done by Cupric Reducing Antioxidant Capacity (CUPRAC) method developed by Apak *et al.*[41] 100 µl of sample solution was added to 1 ml of distilled water which was further added to 1mL each of copper (II) chloride solution (10^{-2}M), neocuproine solution (7.5×10^{-3}M), and ammonium acetate buffer solution (pH 7) solution in a test-tube to make the final volume of 4.1 ml. The tubes were capped properly and left for 1 hour. The absorbance

was taken at 450 nm against a reagent blank using UV-Visible spectrophotometer (GENESYS 10 VIS, USA). The results were expressed in μmol TE/g.

Encapsulation Efficiency

The encapsulation efficiency is the content of anthocyanin being encapsulated with respect to the content in the juice. It was calculated using the following method.[26]

Encapsulation efficiency (%) = Total anthocyanin content in the feed / Total anthocyanin content in the powder x 100 ...(2)

Experimental Design for Optimization Using Box-Behnken Design (BBD) by Response Surface Methodology (RSM)

Box-Benhken Design (BBD) was used for the optimization of the encapsulation process. Three independent were taken as TSS, the ratio of whey and JSS and NBRE-15 each at 3 levels (Table 1) with anthocyanin content (mg/100g of dry matter), antioxidant activity (μmolTrolox/100g dry matter) and encapsulation efficiency (%) taken as the dependent variables. The BBD consisted of 17 experiments with 1 block containing 5 center points per block. Experimental data obtained were fitted into the second order polynomial equation.

$$R_i = \alpha_o + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{33} C^2 + \alpha_{12} AB + \alpha_{13} AC + \alpha_{23} BC \quad ...(3)$$

Where R_i is predicted response; α_o is fitted response at the centre point; α_1, α_2 and α_3 are linear terms; α_{12}, α_{13} and α_{23} are interaction effects; α_{11}, α_{22} and α_{33} are squared effects. A, B and C are the independent variables.

Color Measurement

The color of the optimized powder was measured using a Hunter-Lab Colorimeter (Miniscan® XE Plus 4500L). The color was expressed in hunter lab units,

Table 1: The arrangement of the BBD for the three independent variables along with the experimental responses.

Experimental order	Actual and coded value for design variable			Responses		
	TSS (°Brix, X1)	WPI:JSS (X2)	NBRE-15 (%,X3)	Anthocyanin content (mg/100g of dry matter)	Antioxidant activity (μmol Trolox/g dry matter)	Encapsulation efficiency (%)
1	20 (-1)	1:1 (-1)	0.2 (0)	2409.24	204.86	67.12
2	30 (1)	1:1 (-1)	0.2 (0)	2524.84	253.86	70.34
3	20 (-1)	1:5 (1)	0.2 (0)	2445.10	232.52	68.72
4	30 (1)	1:5 (1)	0.2 (0)	2651.78	283.54	73.88
5	20 (-1)	1:3 (0)	0.1 (-1)	2253.24	160.28	62.76
6	30 (1)	1:3 (0)	0.1 (-1)	2522.54	206.72	70.28
7	20 (-1)	1:3 (0)	0.3 (1)	2486.23	179.03	66.48
8	30 (1)	1:3 (0)	0.3 (1)	2551.42	220.54	71.14
9	25 (0)	1:1 (-1)	0.1 (-1)	2560.36	242.62	71.33
10	25 (0)	1:5 (1)	0.1 (-1)	2734.25	260.56	76.18
11	25 (0)	1:1 (-1)	0.3 (1)	2680.54	253.53	74.67
12	25 (0)	1:5 (1)	0.3 (1)	2807.59	289.32	78.22
13	25 (0)	1:3 (0)	0.2 (0)	2630.57	245.59	74.93
14	25 (0)	1:3 (0)	0.2 (0)	2622.77	246.45	74.72
15	25 (0)	1:3 (0)	0.2 (0)	2696.06	234.34	73.97
16	25 (0)	1:3 (0)	0.2 (0)	2684.89	245.24	76.45
17	25 (0)	1:3 (0)	0.2 (0)	2664.54	248.68	73.09

L* (varying from 0 black to 100 white), a* (varying + value, red to – value, green), b* (varying from +value, yellow to – value, blue). The test was performed in triplicates and average data was recorded.

Moisture Content

5g of powder was weighed in a clean and tarred aluminum dish and dried in hot air oven at 105°C with the open lid for 3 hrs. The dish was cooled in a desiccator and weighed. All samples were analyzed in triplicate and the result was expressed in (w.b.) percentage using the formula given below:

Moisture (%) = Initial weight - Final weight / Initial weight x 100 ...(4)

Analysis of Particle Size

The optimized product contained five times more Jackfruit starch (JSS) than the whey protein. So, the particle size distribution in the optimized encapsulated powder was for JSS using laser scattering particle size distribution analyzer LA-950 (Horiba Scientific Instruments, Japan).

Surface Morphology by Scanning Electron Microscope (SEM)

The morphological analysis of the optimized encapsulated powder was done by coating the powder with gold/palladium to a thickness of 27 nm using an SC7620 model mini sputter coater (Quorum Technologies Ltd, West Sussex, UK) followed by SEM analysis using EVO/MA10 model (CarlZeiss, Germany). The SEM was operated at 5000X magnification.

Statistical Analysis

All the experiments were carried out in triplicates and the mean values were used for the optimization

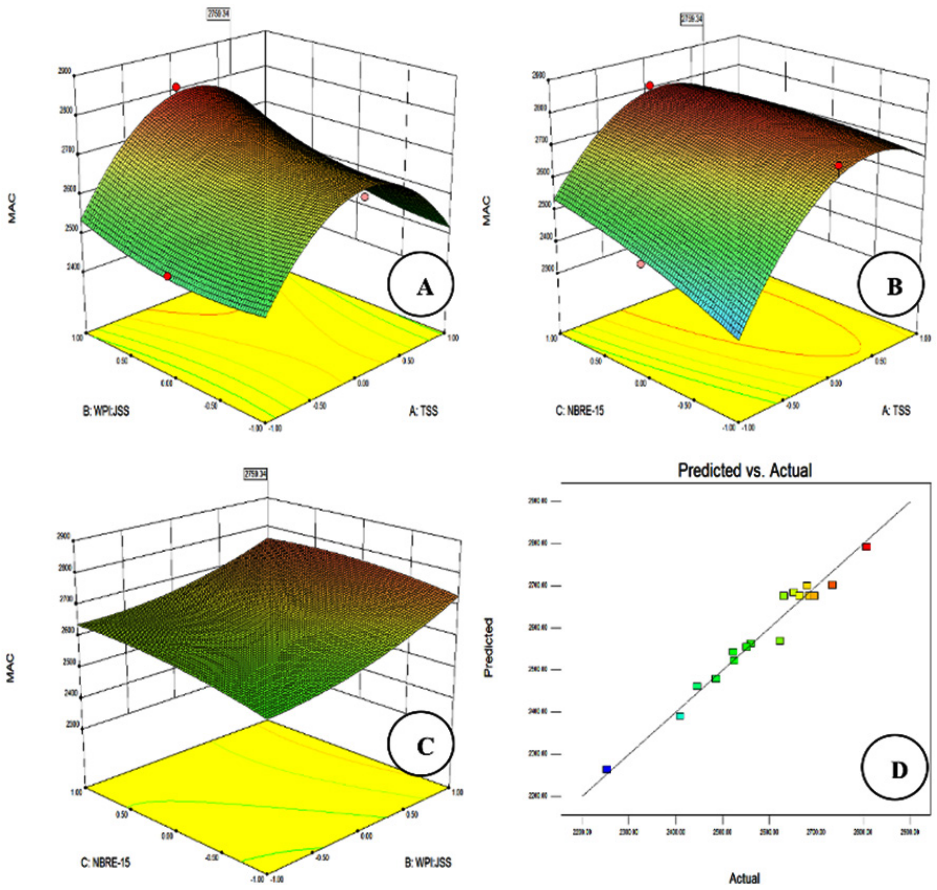

Fig. 1: 3D surface plot for effect of three variables on the MAC. TSS & WPI: JSS (A), TSS & NBRE-15 (B) and WPI: JSS & NBRE-15(C).

using Design-Expert 8.0.7.1 software. The data were analyzed statistically by ANOVA considering p values ≤ 0.05 as statistically significant.

Results and Discussion

Model Fitting for the Optimization

Process optimization for the encapsulation of black carrot anthocyanin was done by RSM taking total anthocyanin content, antioxidant activity and encapsulation efficiency as the responses. The results of the experiments performed as per BBD design are given in Table 1. The regression coefficients, $R,^2$ P and F values for the quadratic model of the dependent variables are shown in Table 2. The model for all the responses was found to be significant ($p<0.05$) and the R^2 value was more than 0.90 as for the good fit of the model it should be more than 0.80 respectively.[44] The lack of fit was not significant ($p > 0.05$) for all the models being analyzed by ANOVA.

Effect of TSS, the Ratio of Whey Protein and JSS and NBRE-15 on the Anthocyanin Content of the Encapsulated Powder

The effect of WPI: JSS, TSS, and NBRE-15 the variables on the anthocyanin content has been depicted through the 3D surface plot (Fig. 1). It was observed that with the increase of total soluble solids (TSS), the anthocyanin content increased but beyond 28 slight drops was observed. The ratio of whey protein and JSS has a positive effect and the anthocyanin content increased considerably. NBRE-15 also imparted positive effect and the increase in the anthocyanin content was observed. With regard to the interaction effect, the interaction of TSS and NBRE-15 has a negative effect with regression

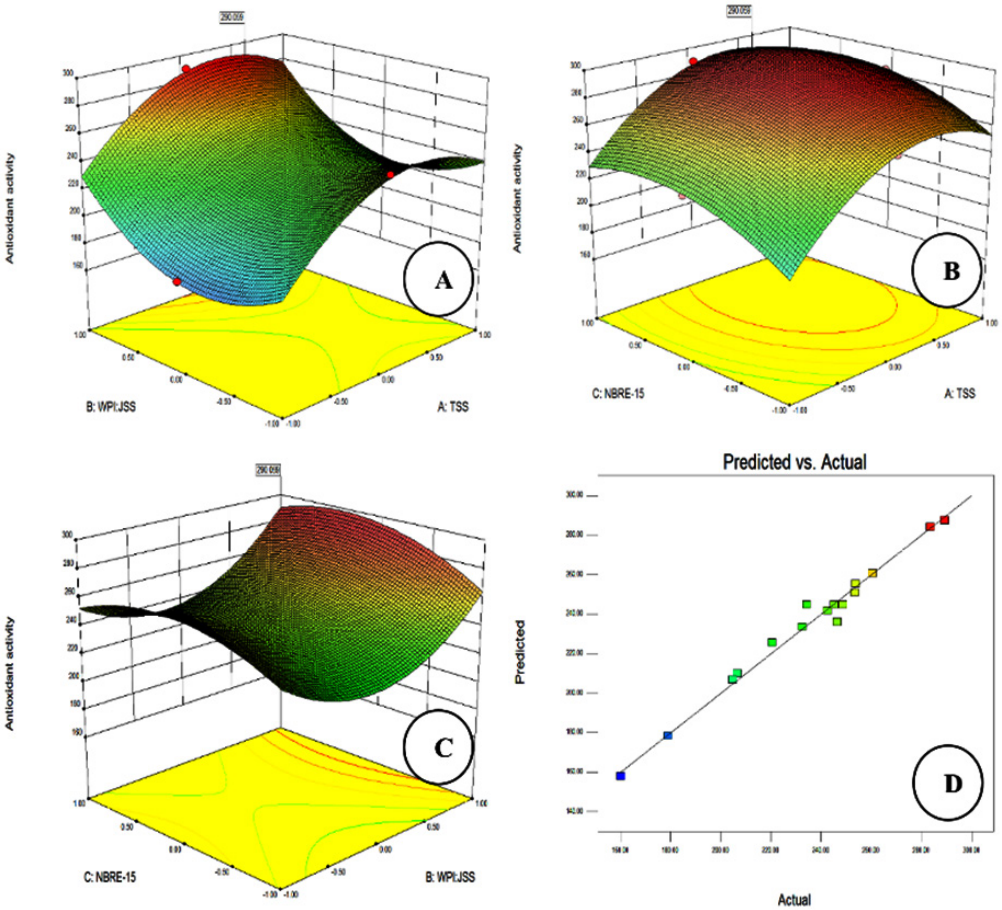

Fig. 2: 3D surface plot for effect of three variables on the antioxidant activity. TSS & WPI: JSS (A), TSS & NBRE-15 (B) and WPI: JSS & NBRE-15 (C).

coefficient -51.027 while the rest interactions have a positive effect (Table 2).

Effect of TSS, the Ratio of Whey Protein and JSS and NBRE-15 on the Antioxidant Activity of the Encapsulated Powder

The selected variables had a positive effect on the antioxidant activity (Fig. 2). The effects were similar to that on the anthocyanin content. With the increase in TSS, antioxidant activity increased and after then dropped slightly. Unlike TSS, with the increase of the ratio of whey and JSS, the antioxidant activity increased. Similarly, there was a proportionally increase in antioxidant with the increase in NBRE-15. The interaction effects of AB and BC were observed positive while AC had a negative effect.

Effect of TSS, the Ratio of Whey Protein and JSS and NBRE-15 on the Encapsulation Efficiency of Encapsulated Powder

The response surfaces for the effect of the variables on the encapsulation efficiency are shown in Fig. 3. In this case, also, the three variables effect positively. Encapsulation efficiency increases with the increase in TSS to a certain level and then decreases while with the ratio of whey and JSS there is a logarithmic increase. With NBRE-15 a similar trend to that of the ratio was observed. Regarding the interaction effect, in this case, only AB has a positive effect while the rest AC and BC negatively affects the encapsulation efficiency.

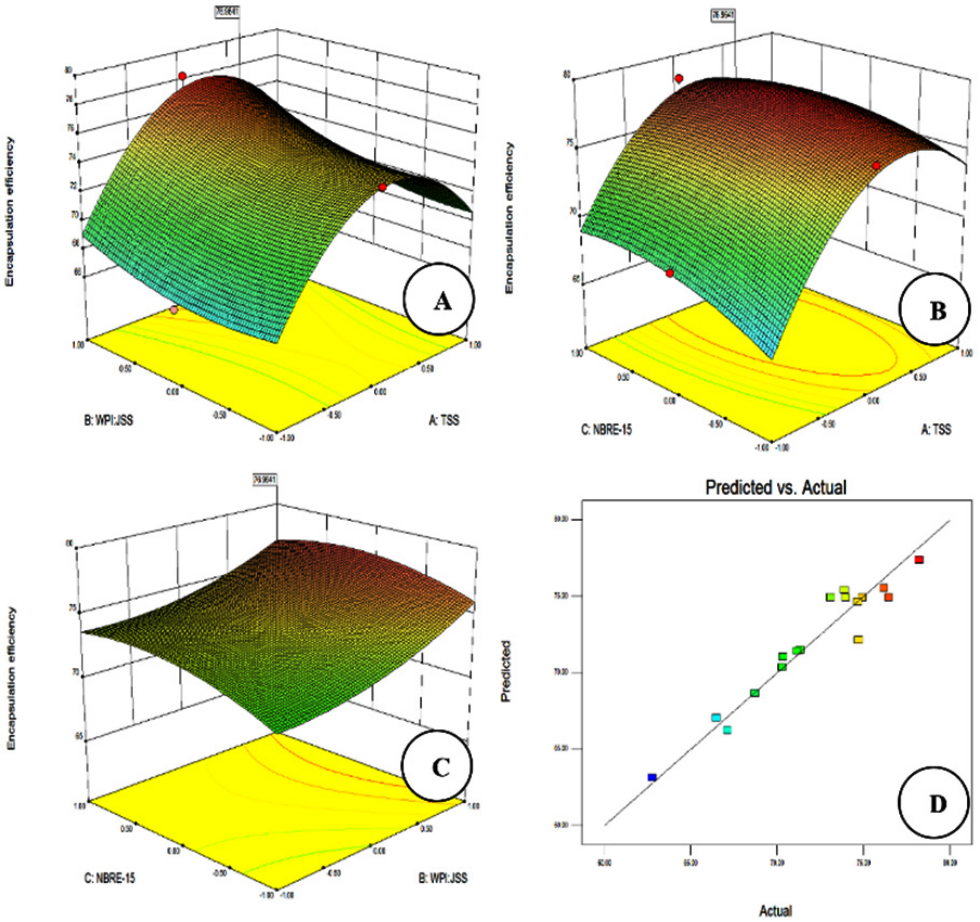

Fig. 3: 3D surface plot for effect of three variables on the encapsulation efficiency. TSS & WPI: JSS (A), TSS & NBRE-15 (B) and WPI: JSS & NBRE-15 (C).

Optimization Constraints and Solution

The optimization of different constraints was done targeting the variables and the responses both to the maximum with high importance. The optimized condition selected for variable was 27.8°Brix (TSS), 1:5 (WPI:JSS) and 0.3% (NBRE-15) having 2766.61 mg/100g dry matter of anthocyanin content, 290.56 µmolTrolox/g dry matter antioxidant activity with encapsulation efficiency of 77.12% at 93.59% desirability. The color was reported to have L*, a* and b* values as 30.61, 29.39 and 0.03 respectively. The moisture content in the optimized encapsulated powder was 4.04%, respectively.

Predicted Model Verification

The predicted model was verified by confirming the actual values against the predicted under the optimum conditions of TSS, the ratio of WPI: JSS and NBRE-15 respectively at 27.8 °Brix, 1:5 and 0.3%. The actual values were found to be significant with the predicted values at the 5% level of significance.

Size Distribution of the Encapsulated Powder

The particle size distribution for the optimized powder containing whey protein isolate, jackfruit starch, and NBRE-15 is shown in Fig. 4. The size distribution for the encapsulated powder is as follows:

D10 = 8.71 µm; D50 = 21.57 µm; D90 = 132.12 µm; Dmean = 52.36 µm ...(5)

Where, D10 implies that 10% of the particles are below this size 8.71 µm; D50 denotes the median particle size distribution of 21.57 µm; D90 denotes the 90% of the particles are above 21.57 µm, however, below 132.12 µm and Dmean is the average particle size distribution 52.36 µm of the optimized encapsulated anthocyanin powder.

Surface Morphology Study by Scanning Electron Microscope (SEM)

The SEM image of the encapsulated powder (Fig. 5) shows a complex mixture of encapsulant materials.

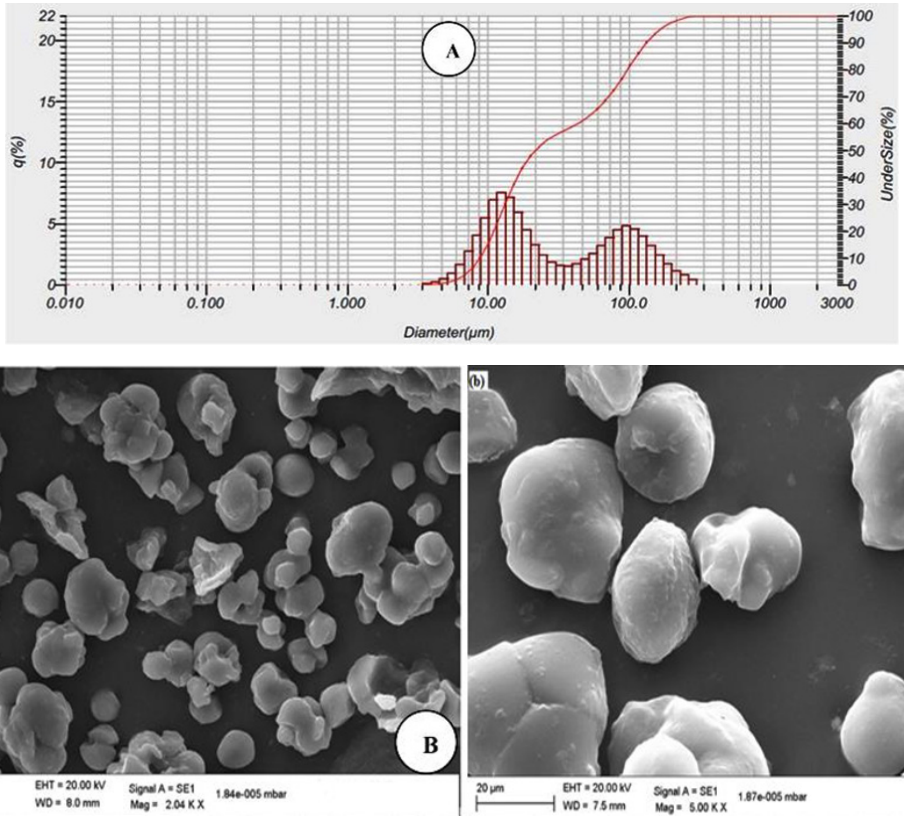

Fig. 4: Particle size distribution of the optimized microcapsule (A) and SEM image for surface characterization of the encapsulated powder (B) 2000X magnification (C) 5000X magnification

The powder molecules are slightly oval to globular in shape without any cracks ensuring the safety of core material inside which are similar to reports confirmed by Ersus and Yurdagel.[9] The particle size ranged approximately from 2µm to 20µm. The molecules are attached to each other which could be due to the emulsifying property of NBRE-15 in presence of whey protein isolate and jackfruit starch.

Conclusion

The anthocyanin pigment of black carrot was encapsulated using the carrier materials *viz*., whey protein isolate, jackfruit starch, and NBRE-15. NBRE-15 in presence of whey protein isolate formed the best carrier with the jackfruit seed starch which ultimately gave best encapsulation efficiency with optimum anthocyanin content and antioxidant activity. This was also revealed through the particle size distribution and SEM images for the optimized powder. The proportion of jackfruit starch five times the whey protein isolate with 0.3% NBRE-15 was optimized to be the best carrier material for the encapsulation of anthocyanin pigment.

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Ethical Approval

This article does not contain any studies with either animals or human participants performed by any of the authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors Contributions

Mr. Avinash Singh Patel design the experiments and conducted the entire research work and write the manuscript under supervision of Dr. Abhijit Kar. Mr. Dipendra Kumar Mahato helped in the qualitative analysis of sample. Dr. Lalit M Bal did the statistical analysis and interpretation of the data.

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