CHAPTER 6

OPTIMISATION OF PHENOLIC EXTRACTION FROM CARAMBOLA POMACE BY RESPONSE SURFACE METHODOLOGY AND ITS MICROENCAPSULATION BY SPRAY AND FREEZE DRYING METHODS

6.1. Introduction

Fruits are rich sources of phytochemicals mainly polyphenols and carotenoids that have free radical scavenging capacity as well as antioxidant activities. Thus regular consumption of fruits could significantly prevent or reduce the risk of development of degenerative diseases like cancer, cardiovascular heart diseases, diabetes, etc. [1, 2] But despite the recommended amount of daily intake of fruits, the consumption remains very low. Thus, there is a need to deliver these phytochemicals to the consumer through different foods by developing newer functional food products fortified with phytochemicals derived from plant sources. The food industries mainly exploit the fruit and vegetable by-products as sources of phytochemicals.

Carambola or starfruit (*Averrhoa carambola)* is a tropical fruit belonging to the Oxilidaceae family and is found predominantly in Assam and other north eastern states of India along with the neighbouring countries like Myanmar, Bangladesh, Malaysia, and Indonesia. It is used traditionally for treatment of number of ailments. It contains major phytochemicals like alkaloids, flavonoids and tannins. [3] The major polyphenolic antioxidants in carambola consists of L-ascorbic acid, (-) epicatechin, gallic acid gallotannin forms and proanthocyanidins. [4] The by-product or pomace from carambola left after juice extraction contains much higher antioxidant activity than the extracted juice [5]. Thus, phytochemical extract of carambola pomace could be used for fortification purpose in functional products.

Generally, the phytochemicals are extracted using solvent extraction technique from different fruits and their by-products and incorporated to develop functional food products. The phytochemicals are added as such directly into the food to develop a product. But some kind of processing or delivering system is required in some cases to ensure the effectiveness of the phytochemical for their targeted functions. This is because phytochemicals as such are prone to destruction and oxidation from environmental factors like light and oxygen.

Moreover, phytochemicals mainly polyphenols have an inherent astringent taste which could hamper or mask the overall sensory desirability of the food product to which they are added. [6] Microencapsulation technique is widely studied to deliver the phytochemicals to the consumer without destruction. Microencapsulation protects the core material by encapsulating it with a wall or coating material that prevents exposure to adverse environmental conditions and also promotes controlled release of the encapsulate. [7] Apart from that, encapsulation also ensures masking of unwanted flavor of the core material. [8, 9]

Presently, spray drying, freeze drying, spray cooling, extrusion, coacervation, liposome entrapment, co-crystallisation, emulsion, etc. [9-11] are some of the widely used encapsulation techniques used in the food industries. The use of freeze drying is advantageous as no application of heat is involved but it adds to higher cost. [12] Basically, it has application in the encapsulation of heat sensitive volatile compounds and pharmaceuticals. [13] However, spray-drying for microencapsulation of polyphenols and other heat labile compounds is the commonly used technique in pharmaceutical and food industry. [14] Many researchers used the spray drying technique for the microencapsulation of fruit extract and studied the properties of the microcapsules.[15-17] Quispe-Condori et al. [18] used both spray and freeze drying method to encapsulate flax oil with zein.

Widely used coating or wall materials for encapsulation are maltodextrin, starch, modified starch and protein conjugates, gums, pectin, etc. The coating or wall material helps in reduction of the moisture/water content as well as acts as a physical barrier to oxygen and light to ensure protection from chemical and enzymatic destruction. [19] The use of maltodextrin is popular as it has number of functions including bulking and film formation properties, binding of flavour and fat in addition to playing a role in reduction of oxygen permeability of wall matrix. [20] Moreover, maltodextrin has high water solubility and hence, can contribute to the significant reduction of the apparent viscosity of feed favouring the atomization and drying of the liquid feeds. [21] Similarly, Silva et al. [22] studied the use of maltodextrin in microencapsulation of joboticaba peel extract.

A study was conducted to determine the optimum conditions for the extraction of polyphenols from pomace of ripe sour variety carambola or carambola using response

surface methodology and microencapsulate the extracted polyphenols with maltodextrin by spray and freeze drying methods for study of their properties.

6.2. Materials and methods

6.2.1. Chemicals and reagents

Chemicals used in the study were of analytical grade purchased from Sigma, Merck and Himedia. All the standards were purchased from Sigma.

6.2.2. Materials

Ripe sour variety of carambola was purchased from local market, Tezpur, India. The maturity stage of carambola was determined according to Narain et al. [23] The fruit was considered as ripe when the fruit skin appeared to be 100% and soft in texture. The juice from the fruit was extracted using a household juice processor and the pomace was collected. The obtained pomace was dried in a tray drier at 50°C for 12 h. The dried pomace was then powdered in a grinder and stored at -20°C until further analysis.

6.2.3. Extraction of polyphenols from the pomace

The pomace was extracted for polyphenols in acidified ethanol (1% of 1N hydrochloric acid, pH 3.0) in 1:10 ratio (solid: solvent) at different solvent concentrations and temperature conditions in a shaking incubator (Certomat 1S, Sartorius) for 3 h. After completion of the extraction time, the crude extract was centrifuged at 3000 rpm (Hettich-Zentrifugen, Germany) for 15 min. The supernatant was collected and analyzed for total phenolic content (TPC), ferric reducing antioxidant potential (FRAP) and DPPH radical scavenging activity.

6.2.4. Experimental design for optimisation using Central composite rotatable design (CCRD) by response surface methodology (RSM)

For the optimisation of the extraction process, CCRD model was applied. Two independent variables viz., temperature and solvent concentration were taken at 5 levels (Table 6.1). The CCRD consisted of 13 experiments including 5 centers, 4 axial and 4 factorial points. The design independent variables were temperature (X_1, °C) and solvent concentration (X_2, %) while, the dependent or response variables were total polyphenol content (TPC), ferric reducing antioxidant potential (FRAP) and DPPH radical scavenging capacity.

Experimental data obtained were fitted into a second order polynomial model. The generalized second order polynomial order equation used was:

$$Y_i = a_0 + a_1X_1 + a_2X_2 + a_{11}X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2 \qquad \textbf{Eq.6.1}$$

Where, Y_i (i= 1-3) is predicted response for TPC (total phenolic content), FRAP (ferric reducing antioxidant property) and DPPH radical scavenging activity. The a_o is the fitted response at the center point; $a_1$and a_2 are linear terms; a_{12} is the interaction effect, a_{11} and a_{22} are squared effects. X_1 and X_2 are the independent variables.

Design expert 6.0 software was used to generate response surfaces and the plot. The data were statistically analyzed by ANOVA. The p values $\leq$ 0.01 were considered to be statistically significant. Response variables viz. TPC, FRAP and DPPH were determined spectrophotometrically.

6.2.4.1. Total phenolic content (TPC)

Total phenolic content in the sample extracts was assessed using the Folin–Ciocalteau assay [24] with slight modification. For the analysis, 20 µL each of sample extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100 µL of Folin–Ciocalteau reagent, mixed well and within 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40°C. The absorbance was then measured at 765 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). The results were expressed in mg GAE/ 100g.

6.2.4.2. Ferric reducing antioxidant property (FRAP)

FRAP activity of the samples was measured by the method of Benzie and Strain. [25] Briefly, a 40 µL aliquot of properly diluted sample extract was mixed with 3 mL of FRAP solution. The reaction mixture was incubated at 37°C for 4 min and the absorbance was determined at 593 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) against a blank that was prepared using distilled water. FRAP solution was pre warmed at 37°C and prepared freshly by mixing 2.5 mL of a 10mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40 mM hydrochloric acid with 2.5 mL of 20 mM ferric chloride and 25 mL of 0.3M acetate buffer (pH 3.6). A calibration curve was prepared, using an aqueous

solution of ferrous sulfate (1-10 mM). FRAP values were expressed as μM of ferrous equivalent Fe (II) per 100 g of sample.

6.2.4.3. DPPH radical scavenging activity

Radical scavenging activity of the sample extracts was measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. [26] Precisely, 100 μL of extracts were added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). The absorbance at 517 nm was measured at 30 min against blank (100 μL methanol in 1.4 mL of DPPH radical solution) using a UV-Vis Spectrophotometer (Cecil Aquarius 7400). The results were expressed in terms of radical scavenging activity.

$$\text{Radical scavenging acitivity (\%)} = [(Ao-As)/ Ao] \times 100 \quad \textbf{Eq. 6.2}$$

Where, Ao is absorbance of control blank, and As is absorbance of sample extract.

6.2.5. RP-HPLC study of the polyphenol extract

Sample Preparation

For comparison of the difference of polyphenol content in the juice and pomace, both the fresh juice and extract from pomace were analysed by RP-HPLC. The carambola pomace extract was obtained by extracting in 65% ethanol at 40°C (as per the conditions obtained in the CCRD model). The ethanolic extract was then evaporated under vacuum in a rotary vapour evaporator, and then redissolved in 1mL HPLC grade methanol. The juice of carambola was squeezed and the obtained juice was centrifuged at 3000 rpm for 15 min. Both the juice supernatant and pomace extract was then filtered through a membrane filter (0.22 μm) before injection.

RP-HPLC (Waters) gradient elution method was used to identify the major phenolic acid composition of the polyphenol extract. Symmetry 300™ C_{18} (5 μm, 4.6 X 250 mm) column with a binary pump (Waters, 1525) and a UV-Vis detector (Waters, 2489) was used. The ethanolic extract was evaporated under vacuum and then redissolved in 1mL methanol. Mobile phases used were acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol (mobile phase B). The gradient method: 80 % A (0-8 min), 65 % A (9-12 min), 45 % A (13-16 min), 30 % A (17-20 min), 20 % A (21-30 min), 10 % of A (31-34 min) and then washing of the column with 65 % A (35-39 min) and lastly, 80 % A (40-45 min) was followed. Sample volume of 20 μL was used. The flow rate was maintained at

0.8mL/min and wavelengths used for UV-Vis detector were 254 nm and 325 nm. The standards used for comparison and identification were (±) catechin, caffeic acid, coumaric acid, gallic acid, syringic acid, chlorogenic acid, rutin hydrate, quercetin.

6.2.6. Encapsulation process

The crude extract obtained after optimisation process was concentrated under vacuum in an evaporator at 50°C to remove the ethanol and then freeze dried (Labtech Freeze drier) at -55°C. The freeze dried crude polyphenol powder was mixed with maltodextrin (≤20 DE) at three different core: coating material ratios (1:10, 1:15 and 1:20). The mixtures were homogenized at 12000 rpm in a homogenizer (UltraTurex 25, IKA) and divided into two lots. One lot was spray dried and the second lot was freeze dried.

Spray drying conditions

The three homogenized mixtures of polyphenol and maltodextrin were spray dried using a laboratory scale spray drier (Lab plant system, UK) at an inlet temperature of 185°C and an outlet temperate of 88°C. The feed rate was maintained at 6 mL/min and the nozzle size used for spraying was 0.1 mm in size. The obtained microencapsulate powder was kept in an airtight container under refrigeration.

Freeze drying conditions

The homogenized mixture was iced overnight at -40°C and then freeze dried at -55°C for 24 h. Microencapsulates thus obtained after drying were ground in a mortar and pestle and stored in an airtight container in a refrigerator.

6.2.6.1. *Moisture content*

Moisture content was determined by AOAC method. [27] Briefly, 5 g of sample was taken in previously dried and weighed covered dishes. The sample was allowed to dry in a hot air oven (Jiotech, South Korea) at 105°C for 8 h till a constant weight was attained. The final weight of the dish containing the sample was measured both before and after drying and moisture content was calculated.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_2} \times 100 \qquad \textbf{Eq. 6.3}$$

Where, W_1 is weight of the sample with the dish before drying; W_2 is final weight of the sample with dish after drying.

6.2.6.2. *Bulk density*

The bulk density was calculated by weighing 1g of sample powder into a graduated 10 mL cylinder and measuring the volume occupied by the sample. The results are expressed as g/mL. [16]

$$\text{Bulk density (g/mL)} = \frac{Weight\ of\ sample}{Volume\ \ occupied} \qquad \textbf{Eq. 6.4}$$

6.2.6.3. *Solubility*

The solubility was determined according to the method described by Chau et al. [28] Briefly, samples were mixed with distilled water (1:10 w/v), stirred for 1 h at room temperature and centrifuged at 1500 rpm for 10 min. The supernatant was collected, dried and weighed.

$$\text{Solubility (\%)} = \frac{W_f}{S} \times 100 \qquad \textbf{Eq. 6.5}$$

Where, W_f is the final weight (g) of supernatant after drying and S is the weight (g) of sample.

6.2.6.4. *Colour*

The colour variation among the obtained encapsulates was determined in a Hunter colour lab (Hunter ColourLab UltrascanVis). The '*L*' value indicates degree of lightness. '*L*' value in the range between 0-50 indicates dark and 51-100 indicates light. Similarly, '*a*' means measure of red (positive values) and green colour (negative values); '*b*' measures the yellow (positive value) or blue (negative values) colour. [29]

6.2.6.5. *Hygroscopicity*

The hygroscopicity property of the sample powders was determined according to Cai and Corke [30] with some modifications. Briefly, 2 g spray dried powder samples were placed in pre-weighed glass vials and placed in a desiccator containing saturated salt solution of sodium chloride (relative humidity of 75.09 %) maintained at 30°C and kept for 7 days. After the incubation period, sample vials were weighed and expressed as g moisture per 100 g solids.

6.2.7. Total phenolic content analysis (TPC), surface phenolic content (SPC) and encapsulating efficiency

TPC and SPC were determined by Folin–Ciocalteu method given by Slinkard and Singleton [24]. The sample extraction method given by Saenz et al. [17] was used. For the TPC, 100 mg of sample was dispersed in 1mL ethanol, acetic acid and water (50:8:42). The mixture was then vortexed (Tarsons, India) for 1 min and filtered through 0.45 μm filter. Similarly, in case of SPC, 100 mg of sample was dispersed in 1 mL of ethanol and methanol (1:1) mixture. The mixture was vortexed for 1 min and then filtered as mentioned above. The results were expressed as milligram of gallic acid equivalent per hundred grams (mgGAE/100g, dry weight). The encapsulating efficiency was determined.

$$\text{Encapsulating efficiency (\%)} = \frac{TPC-SPC}{TPC} X\, 100 \qquad \textbf{Eq.6.5}$$

6.2.8. *In vitro* simulated gastric and intestinal digestion release study of the encapsulates

In vitro digestion and release of TPC by microencapsulates was determined by simulation of gastric and intestinal fluid. The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared as given in U.S. Pharmacopea. [31] For SGF preparation, 2.0 g of sodium chloride was mixed with 3.2 g of pepsin (porcine stomach mucosa) and 7 mL of hydrochloric acid was added to the mixture and volume made up to 1000 mL with water maintaining pH at 1.2. For the study, 1.4 mL of SGF was added to 100 mg of sample in a 10 mL test tube and incubated at 37°C for 2 h at 80 rpm. After that, the solution was filtered and neutralized by adding 0.2M sodium hydroxide solution.

Similarly, SIF was prepared using monobasic potassium phosphate (6.8 g) dissolved in 250 mL water. To this solution, 77 mL of 0.2N sodium hydroxide was added. Finally, 10 g of pancreatin was added and the volume of the mixture was made up to 1000 mL and pH 6.8 was maintained. For the simulation study, 2.4 mL of SIF was taken in a 10 mL test tube and incubated with 100 mg of sample at 36.6°C for 2 h with no shaking. At the end of experiment, the solution was filtered and enzyme activity was inhibited by decreasing pH to 1.2 using 100 μL of 3M hydrochloric acid to 2 mL filtrate. After 15 min, solution was neutralized (pH 7.0) by adding 900 μL 0.2 N sodium hydroxide. Lastly, both the samples of SGF and SIF were analyzed for TPC by Folin-Ciocalteau method [24].

6.2.9. Surface morphology study by scanning electron microscope (SEM)

The powder samples, prior to SEM observation, were mounted on stubs with double-sided adhesive tape coated with a thin layer of gold. The SEM images were then obtained using a JSM-6390LV scanning electron microscope (SEM; JEOL, Japan) at 15 kV at 1000 X magnification.

6.2.10. Statistical analysis

All experiments were carried out at least in triplicates and reported as mean ± standard deviation of mean (S.E.M). The optimisation data were analyzed by ANOVA using Design Expert 6.0 software and the rest of the data were analyzed by Duncan's multiple range tests at $p \leq 0.05$ significance level using SPSS version 11.5. The HPLC data were subjected to paired-comparison t-test ($p \leq 0.05$).

6.3. Results and discussion

6.3.1. Optimisation and fitting of the model

RSM was applied to determine the effect of ethanol concentration and temperature on total phenolic content, FRAP and DPPH antioxidant activity of the carambola pomace extract. The results of the experiments performed for CCRD of the variables along with the responses are given in Table 6.1. The regression coefficients obtained for the different responses and ANOVA results for the model responses are presented in Table 6.2 and 6.3, respectively. For the good fit of a model, the R^2 value should be 0.80. [32] In the present study, R^2 values for the three responses were higher than 0.80 which implies the adequacy of the applied regression model. The lack of fit for all fitted models was found to be not significant ($p>0.05$). Myers and Montgomery [33] suggested that lack of fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. Therefore, it can be assumed that the selected model can be used for the simulation and optimisation of variables for the polyphenols extraction from carambola pomace.

6.3.1.1. *Effect of temperature and ethanol concentration on TPC*

From the response plot (Fig.6.1a), it was observed that initial rise in temperature up to 40°C resulted in increased TPC value but beyond 40°C slight drop occurred. However, ethanol concentration had imparted greater positive effect compared to temperature. The

interaction effect between the two variables showed a positive effect on the response. The regression equation obtained for TPC is given below

$$Y = +2255.80 + 4.17X_1 + 42.03X_2 - 53.21X_1^2 - 40.09X_2^2 + 55.63X_1X_2 \qquad \textbf{Eq. 6.6}$$

Table 6.1. CCRD variables (temperature & ethanol concentration) and responses

Run	Variables		Responses		
	Temperature (X_1, ° C)	Ethanol Conc. (X_2, %)	TPC (mgGAE/100g)	FRAP (µM/100g)	DPPH (%)
1	40 (0)	65 (0)	2222.50±0.34	14957.80±0.37	97.31±0.12
2	40 (0)	86.21(+1.141)	2227.50±0.24	14958.30±0.25	97.52±0.19
3	61.21(+1.41)	65 (0)	2152.50±0.19	13756.90±0.41	97.04±0.33
4	55 (+1)	50 (-1)	2057.50±0.21	13281.30±0.43	95.83±0.27
5	18.79 (-1.41)	65 (0)	2162.00±0.27	14970.40±0.28	95.16±0.21
6	40 (0)	65 (0)	2287.50±0.31	14998.40±0.32	97.07±0.36
7	40 (0)	43.79 (-1.41)	2140.00±0.38	14147.90±0.22	95.84±0.23
8	40 (0)	65 (0)	2252.00±0.15	14965.60±0.19	97.71±0.17
9	40 (0)	65 (0)	2267.00±0.18	15652.50±0.21	97.07±0.11
10	40 (0)	65 (0)	2250.00±0.22	14969.40±0.15	97.50±0.18
11	55 (+1)	80 (+1)	2275.00±0.36	14988.40±0.11	98.25±0.21
12	25 (-1)	80 (+1)	2140.00±0.33	14166.70±0.40	95.01±0.39
13	25 (-1)	50 (-1)	2145.00±0.27	14906.80±0.45	95.86±0.41

*results are mean of triplicate values

6.3.1.2. *Effect of temperature and ethanol concentration on FRAP*

In response plot for variables on FRAP (Fig.6.1b), it was seen that high temperature had a detrimental effect on the antioxidant activity of the extract. However, like TPC, increase in ethanol concentration showed a gradual rise and then slight drop in FRAP value.

A positive interaction effect between the temperature and ethanol concentration was observed. The regression equation for the response FRAP is given below

$$Y = +15108.73 - 314.99X_1 + 264.13X_2 - 403.19X_1^2 - 308.46_2^2 + 611.81X_1X_2 \quad \textbf{Eq. 6.7}$$

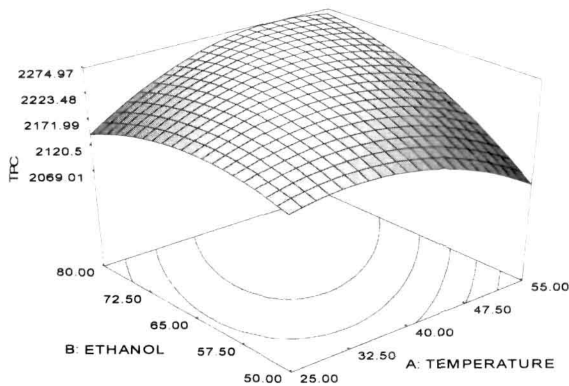
Table 6.2. Regression coefficients of the fitted second order polynomial for the three responses and their significance

Coefficient	TPC	FRAP	DPPH
a_0	2255.80[a]	15108.73[b]	97.33[a]
a_1	4.17	-314.99[b]	0.73[a]
a_2	42.03[b]	264.13[c]	0.49[a]
a_{11}	-53.21[a]	-403.19[b]	-0.65[a]
a_{22}	-40.09[b]	-308.46[c]	-0.36[b]
a_{12}	55.63[a]	611.81[b]	0.82[a]

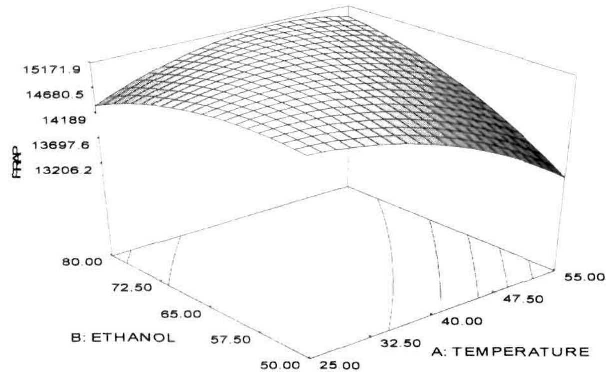
[a] significant at 0.1% ($p<0.001$); [b]significant at 1% ($p<0.01$); [c] significant at 5% ($p<0.05$)

Table 6.3. ANOVA values for the fitted models and lack of fit

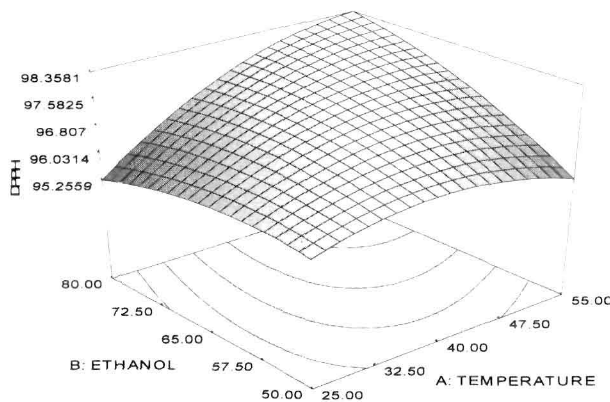
	df	Sum of squares		
		TPC	FRAP	DPPH
Model	9	54121.47	4.443E+006	12.45
Lack of fit	5	1987.80	1.381E+005	0.17
Pure error	5	2287.30	3.705E+005	0.31
R^2	---	0.9268	0.8973	0.9633



(a)



(b)



(c)

Fig.6.1. 3D graphic surface optimisation of three responses (a) TPC, (b) FRAP, and (c) DPPH

6.3.1.3. *Effect of temperature and ethanol concentration on DPPH radical scavenging activity*

The selected variable conditions had positive effect (Fig. 6.1c) on DPPH activity. With increase in temperature and ethanol concentration, increased DPPH activity was observed. In this case also, interaction effect was found to be positive on DPPH activity. The regression equation for the response DPPH is given below.

$$Y = +97.33 + 0.73X_1 + 0.49X_2 - 0.65X_1^2 - 0.36X_2^2 + 0.82X_1X_2$$ **Eq. 6.8**

The extraction of polyphenols from the plant sources is depended on number of factors like polarity of the solvent used, solubility of the phenolic acids present, the interaction of these phenolic acids with other plant compounds which consequently could lead to development of newer complexes that may be soluble or insoluble into a given solvent. Therefore, it is quite difficult to develop a standard extraction method that would be suitable for all the phenolic compounds. [34] The TPC and antioxidant activity of a particular sample may vary with the use of different solvent and extraction conditions.

6.3.1.4. *Verification of the predictive model*

The suitability of the model for the responses was determined under the optimum conditions of temperature (40°C) and ethanol concentration (65%). The experimental values for the responses were found to be quite comparable and with agreement with that of the predicted value (Table 6.4).

Table 6.4. Optimized solution obtained using the response optimizer

Optimal variables		Predicted value			Actual value		
Temp. (X_1, °C)	Ethanol Conc. (X_2, %)	TPC	FRAP	DPPH	TPC	FRAP	DPPH
40.00	65.00	2255.80	15108.70	97.33	2286.41± 0.48	15112.63±0.37	98.01± 0.41

*results are mean of triplicates. Units- TPC (mgGAE/100g); FRAP (μM/100g); DPPH (%)

6.3.2. RP-HPLC study of the crude polyphenols extract

The chromatograms of the polyphenol extract separation by RP-HPLC detected ascorbic acid and number of phenolic acids (Table 6.5 and Fig. 6.2 a & b). As the peak intensity of the sample at 325 nm was not that high and clear, only the peaks obtained at 254 nm were have been considered. The obtained peaks were compared with the retention time of their respective standards and identified. Compared to the pomace extract, phenolic acid content was much less in the juice samples. The phenolic acids obtained were, gallic acid (RT=3.23 min), catechin (RT=11.89 min), chlorogenic acid (RT=13.54 min), caffeic acid (RT=14.49 min), syringic acid (RT=14.73 min), ferulic acid (RT=16.55), coumaric acid

(RT=16.72 min), rutin (RT=17.31 min), kaemferol (RT=19.61 min), quercetin (RT=19.89 min) and ascorbic acid (RT=3.89 min). The ascorbic acid content was found to be 19.43±0.07 mg/100g while, among the phenolic acids, the concentration of gallic acid (41.90±0.05 mg/100g) and catechin (48.08±0.02 mg/100g) was highest followed by caffeic acid (38.09±0 mg/100g). The concentration of rutin (2.41±0.02 mg/100g) and quercetin (3.67±0.04 mg/100g) were relatively low in the extract.

Shui and Leong [4] reported the presence of ascorbic acid, gallic acid and catechin in the forms of epicatechin and epigallocatechin in the residue of carambola. Earlier studies reported the presence of rutin and quercetin in the carambola fruit. [35] The presence of caffeic acid, coumaric acid, chlorogenic acid and syringic acid has not been reported earlier in carambola pomace. The HPLC results showed absence of caffeic acid and coumaric acid in addition of rutin hydrate and quercetin in carambola juice when compared to the pomace extract. The gallic acid and syringic acid belong to the hydroxybenzoic acid group of phenolic acids whereas ferulic acid, chlorogenic acid, coumaric acid and caffeic acid were the hydoxycinnamic acid derivatives. Catechin, rutin and quercetin were the members of the flavonoid group. Therefore, the polyphenol extract from carambola pomace could be a good source of phenolic acids.

6.3.3. Microencapsulation of the polyphenols extract

6.3.3.1. *Physical properties of the encapsulates*

The encapsulation of the polyphenols extract with maltodextrin as the wall material at 3 different ratios by spray and freeze drying techniques showed significant variation in the properties of the encapsulate powder. Table 6.6 represents the physical properties of encapsulates. The moisture content in both the spray and freeze dried powders was between 2.79-4.98 %. The changes in moisture content of the samples are dependent on the type and concentration of carrier materials. [36] Solubility was greater in freeze dried encapsulates (60.98-64.22 %) than spray dried encapsulates. Bulk density was highest in both spray and freeze dried samples at 1:20 ratio concentration.

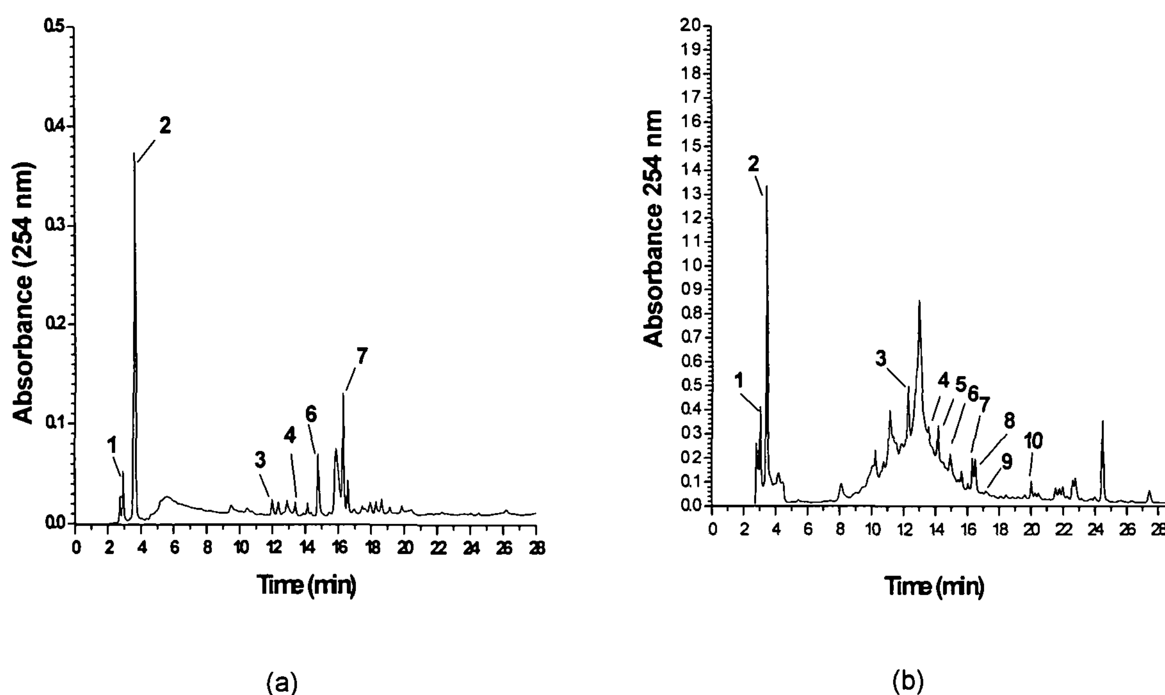


Fig. 6. 2. RP-HPLC chromatogram of (a) carambola juice and (b) polyphenol extract from carambola pomace at 254nm. **1=gallic acid; 2= ascorbic acid; 3= catechin; 4= chlorogenic acid; 5= caffeic acid; 6=syringic acid; 7= ferulic acid ; 8= coumaric acid; 9= rutin hydrate; 10= quercetin

Table 6.5. RP-HPLC results of the crude polyphenol extract from carambola pomace and carambola juice

Name	Polyphenol extract (mg/100g)	Juice (mg/100g)
Ascorbic acid	19.43±0.07*	10.11±0.09*
Gallic acid	41.90±0.05*	4.89±0.04*
Catechin	48.08±0.02*	2.90±0.01*
Caffeic acid	38.09±0.02	N.D.
Chlorogenic acid	22.01±0.05*	2.17±0.08*
Syringic acid	13.10±0.09*	3.51±0.04*
Ferulic acid	21.30±0.11*	4.21±0.03*
Coumaric acid	21.45±0.03	N.D.
Rutin hydrate	2.41±0.02	N.D.
Quercetin	3.67±0.04	N.D.

results were the mean of triplicate values. N.D.-not detected
* denotes statistically significant difference at $p \leq 0.05$ during paired t-test.

The colour parameter for lightness '*L*' revealed that spray dried powder was lighter in colour than freeze dried powder. The possible reason for lightness could be the destruction of some inherent pigments like carotenoids present in the sample on application of high heat during spray drying. Similarly, all the samples showed positive but low '*a*' values. The '*b*' value for the freeze dried samples was slightly more than spray dried encapsulates. The hygroscopicity values were higher in spray dried samples and also it was observed that with increase in maltodextrin concentration, encapsulates became less hygroscopic. The encapsulates of the spray drying technique were very fine in appearance, whereas the freeze dried samples appeared amorphous and glassy which on milling showed free flowing property. The appearance of the freeze dried samples was found to be in agreement with the results of 20% maltodextrin added freeze dried wine powder reported by Sanchez et al. [37]

Table 6.6. Physical properties of the encapsulated samples

Sample	Moisture (%)	Solubility (%)	Bulk density (g/mL)	Colour			Hygroscopicity (g/100g)
				L	*a*	*b*	
Spray dried							
1:10	4.98±0.17^{c}	43.80±0.23^{a}	0.212±0.08^{a}	87.80±0.12^{d}	1.05±0.12^{d}	7.60±0.10^{b}	9.45±0.21^{d}
1:15	2.85±0.11^{a}	42.23±0.20^{a}	0.257±0.05^{b}	86.62±0.15^{d}	0.77±0.07^{c}	7.19±0.22^{b}	9.36±0.18^{d}
1:20	3.73±0.21^{b}	45.56±0.34^{a}	0.330±0.09^{d}	86.29±0.19^{d}	1.41±0.10^{d}	6.82±0.17^{a}	8.32±0.14^{c}
Freeze dried							
1:10	2.79±0.10^{a}	60.98±0.14^{b}	0.296±0.01^{c}	59.68±0.23^{a}	0.55±0.10^{b}	8.89±0.34^{c}	6.83±0.11^{b}
1:15	3.46±0.12^{b}	64.22±0.23^{b}	0.288±0.05^{c}	68.49±0.19^{b}	0.80±0.08^{c}	8.95±0.21^{c}	6.28±0.18^{b}
1:20	3.67±0.19^{b}	61.87±0.37^{b}	0.344±0.04^{d}	74.16±0.20^{c}	0.28±0.12^{a}	7.88±0.19^{b}	5.55±0.16^{a}

* Means with the same letter between the rows are not significantly different at $p \leq 0.05$ by DMRT.

6.3.3.2. *Encapsulating efficiency, TPC and SPC*

The TPC, SPC along with the encapsulating efficiency are reported in Table 6.6. In both spray dried and freeze dried encapsulates, the TPC values were found to increase with increase in the maltodextrin concentration. [38] The reverse was noticed for SPC. The SPC

values of the spray dried encapsulate was higher than freeze dried encapsulates. Lastly, the encapsulating efficiency was much higher in the freeze dried samples (78-97 %) than the spray dried ones (63-79 %). Saenz et al. [17] reported encapsulation efficiency of 39.41-74.78 % in maltodextrin encapsulated cactus pear juice. Similarly, encapsulation by spray drying of anthocyanin extracted from black carrot coated with maltodextrin (20-23 DE) showed high retention of the pigment. [39] Microencapsulation with other coating materials like calcium alginate and calcium alginate-chitosan of polyphenols from *Illex paraguariensis* gave 48.5-87.1% efficiency. [40] The variation in efficiency could be due to destruction of some phenolic acids during the application of heat in the spray drying process. [41] The encapsulating agent type and core to coating ratio are the most important variable for the polyphenols encapsulation. [42]

During spray drying, atomization of the feed material results into very fine mist like droplets with increased surface area. More increase in surface area means more exposure to heat. Further, there may be instances when due to atomization some part of the coating material could get removed from the core material even after homogenization. Such partially covered encapsulates are easily affected by heat. In case of freeze dried sample after homogenization, the samples were dried without atomization and heat exposure. Also, it was observed that the surface phenolic content decreased with increase in maltodextrin content. This implies that higher core: coating material ratio leads to higher encapsulating efficiency. It was reported by Laine et al. [43] that freeze dried encapsulates were stable during storage for a longer period.

6.3.3.3. *In vitro gastric and intestinal release of the microencapsulate under simulated conditions*

The *in vitro* simulation of gastro-intestinal (GI) digestion was reported in Table 6.7. It determined the release of phenolic compounds from encapsulates and their role in prevention of oxidative damage of lipids and DNA by free radicals in the GI tract. [44, 45] In both spray and freeze dried encapsulates, the release of TPC was higher in gastric simulated medium with pH 1.2 than the simulated intestinal fluid having pH 6.8. Thus, release of TPC in the gut is influenced by pH. Again, the extent of resistance depends on the type and property of the coating material used for encapsulation. The behavior of encapsulate in a

simulated GI medium is always dependent on the surrounding matrix composition and their resistance or susceptibility to digestive enzymes as well as on the GI conditions like pH range. [46, 47]

Table 6.7. Surface phenolic content (SPC), total phenolic content (TPC), encapsulating efficiency and *in vitro* gastrointestinal release of TPC in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)

Sample	SPC (mgGAE/100g)	TPC (mgGAE/100g)	Encapsulating efficiency (%)	TPC (mgGAE/100g)	
				SGF	SIF
Spray dried					
1:10	198.00±0.23^{f}	535.00±0.51^{a}	62.99±0.48^{a}	257.50±0.19^{b}	155.50±0.26^{b}
1:15	165.00±0.27^{e}	637.00±0.32^{c}	74.10±0.26^{b}	202.00±0.23^{a}	167.00±0.27^{c}
1:20	112.00±0.11^{c}	825.00±0.17^{d}	79.07±0.11^{c}	267.50±0.41^{d}	189.00±0.22^{e}
Freeze dried					
1:10	129.00±0.17^{d}	588.00±0.11^{b}	78.06±0.27^{c}	267.00±0.32^{d}	205.50±0.43^{f}
1:15	39.00±0.21^{b}	585.00±0.19^{b}	93.33±0.11^{d}	264.00±0.21^{c}	182.00±0.38^{d}
1:20	23.00±0.09^{a}	827.00±0.23^{d}	97.22±0.28^{e}	282.50±0.25^{e}	132.00±0.32^{a}

* Means with the same letter between the rows are not significantly different at $p \leq 0.05$ by DMRT.

6.3.3.4. *Surface morphology study by Scanning electron microscope (SEM)*

Comparison of the SEM images showed marked difference in surface morphology among non-encapsulated phenolic powder, spray dried and freeze dried encapsulates (Fig. 6.2). The non-coated polyphenol powder showed irregular shapes of varying sizes. In freeze dried samples, variation in appearance among the three samples (1:10, 1:15 and 1:20) was observed. Compared to non-coated polyphenol powder, reduction in particle size was observed but irregularity in the shape persisted. With increase in maltodextrin concentration particle size became smaller with more sharp and prominent edges. Overall, coating with maltodextrin developed sharper edges and brittle texture in freeze dried encapsulates in addition to size reduction.

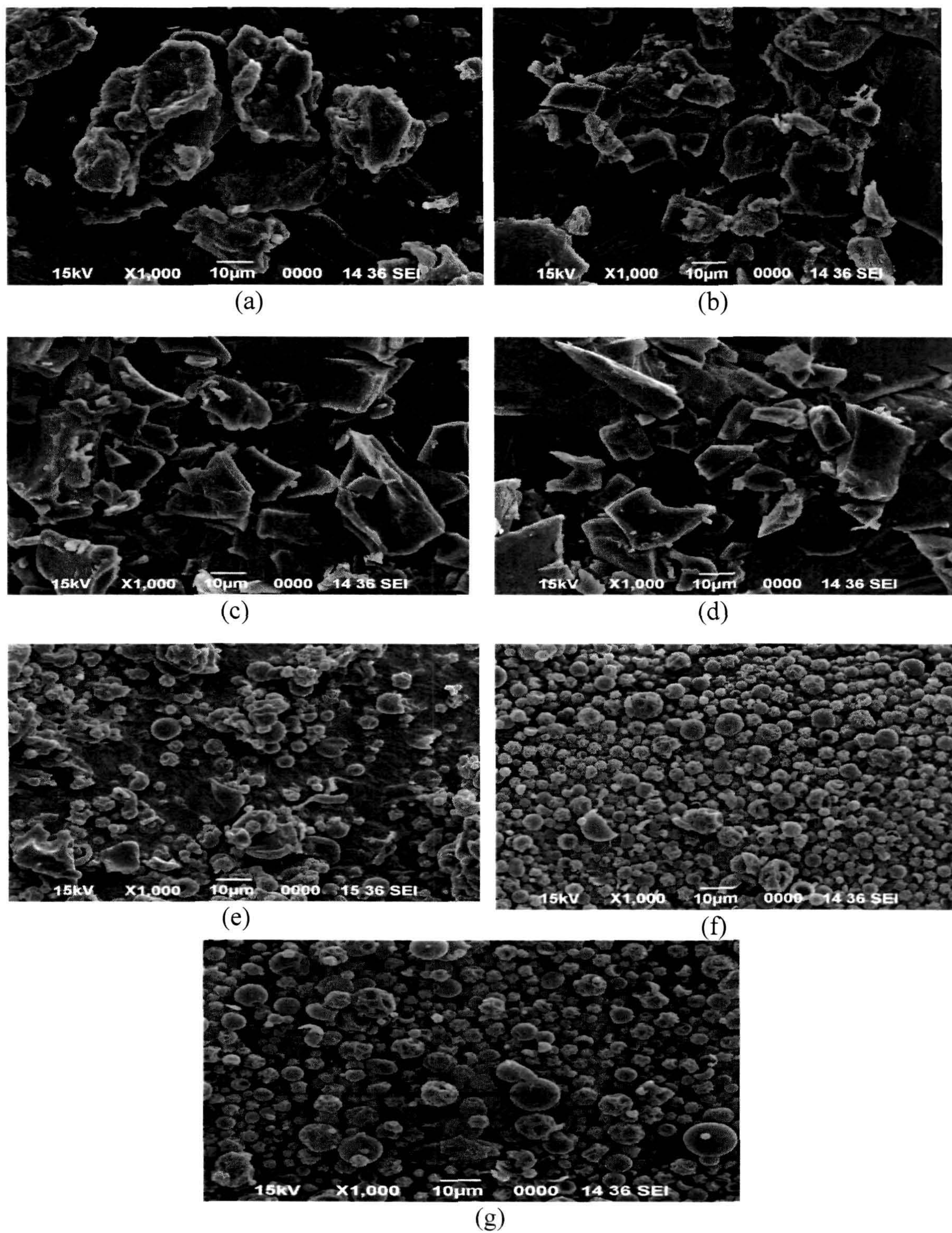


Fig. 6.3. SEM image of the surface morphology of the phenolic powder and microencapsulates.(a) Phenolic powder without maltodextrin, (b) FD 1:10, (c) FD 1:15, (d) FD 1:20, (e) SD 1:10, (f) SD 1:15, and (g) SD 1:20. SD=spray dried; FD= freeze dried

In the spray dried samples, unlike the other two samples, the major particles were of spherical shape while some were flat in appearance. The spheres contained some dent and holes on their surface and appeared to be hollow. These results are in agreement with that of Sahin-Nadeem et al.[48] This could be due to the instant removal of water during spray drying which might have caused collapse of the walls of the spheres.

6.4. Conclusion

The extraction of polyphenols from the fruits and vegetable sources depends on a number of factors. In the present study, it was observed that variation in ethanol concentration and temperature had both positive and adverse effects on TPC, FRAP and DPPH activity in the ethanol extract of carambola pomace. However, their combined interaction had a positive effect on the antioxidant activities. At ethanol concentration of 65% and temperature of 40°C used for extraction, TPC, FRAP and DPPH activity were optimum. RP-HPLC study of the extract showed presence of 9 different phenolic acids and ascorbic acid. Compared to the carambola juice, the polyphenol extract contained good amount of gallic acid, catechin and caffeic acid as well as moderate amount of ferulic acid, chlorogenic acid and coumaric acid. In microencapsulation study, variation in the physical properties between the two variants of microencapsulates was observed. Highest encapsulating efficiency was obtained in freeze dried samples. *In vitro* gastrointestinal simulation study showed greater release of phenolic compounds in the gastric fluid at pH 1.2. Lastly, it can be concluded that, the proposed extraction model of CCRD through RSM could be used for the polyphenols extraction from pomace of carambola and the extracted polyphenols can be used in different food models as such or in encapsulated form depending on the requirements of the end products.

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