

Chapter 5

*Encapsulation of natural antioxidant
from culinary banana pulp and peel*

5.1 Introduction

Bananas have been classified as one of the antioxidant foods by Kanazawa and Sakakibara.¹ These tropical fruits have strong ability to protect themselves from the oxidative stress caused by the intense sunshine and high temperature by increasing their antioxidant levels. They are known as a powerful secondary antioxidant source. The antioxidant compounds identified in bananas include ascorbic acid, tocopherol, β -carotene, phenolic groups, dopamine and gallic acid.² Astringent taste of unripe banana is due to phenolic compounds. Bananas are also rich in dopamine antioxidant. According to Ramcharan and George³ the role of banana and plantain is becoming more important with the increasing emphasis today on diets that are low in sodium but high in potassium and vitamins.

In today's world, much attention has been focused on the activity of natural antioxidants present in fruits and vegetables, because potentially these components may reduce the level of oxidative stress,⁴ i.e. preventing free radicals from damaging proteins, DNA and lipids.⁵ Besides, these compounds are also scientifically proven for their synergistic effects and protective properties against various degenerative disorders including cancer, stroke, cardiovascular, Alzheimer's and Parkinson's diseases.⁶⁻⁹ Natural antioxidants may be added to a wide range of processed food such as baked goods, biscuits, chewing gum, dry snacks, fruit drinks, mayonnaise, meat products etc.¹⁰ The aqueous extracts obtained from plants, exhibit low stability and some of it has an unpleasant flavour, therefore only a few extracts are currently employed in the food industry.^{11, 12} To protect and/or preserve the important bioactive ingredients from adverse environmental conditions like light, moisture, oxygen and to overcome undesirable interactions with the carrier food matrix encapsulation by cocrystallization technique has become an actual and cost effective choice.^{10, 13} Encapsulation by cocrystallization in sucrose matrix is a relatively new and simple method which offers an economical and flexible alternative for handling and preserving various active components used in the food industry.¹⁴ Cocrystallization with sucrose could improve the solubility, dispersibility, wettability, anticaking, antidusting, antiseperation, homogeneity, flowability and stability of food materials.¹⁰

Various studies have been reported on this technique dealing with flavours, natural extracts, essential oils, honey, glucose and fructose.¹⁵⁻¹⁹ However, no work has been reported on encapsulation of natural antioxidant from culinary banana. The present study was undertaken to

characterize the physical and chemical properties of the cocrystallized powder obtained by incorporating the natural antioxidant extract from both pulp and peel of culinary banana. This study will not just add value to culinary banana, but also provide a solution in utilization of its peel which is often considered and discarded as waste.

5.2 Materials and methods

5.2.1 Raw materials and preparation of the sample extract

The culinary banana at 20 DAE (days after emergence of inflorescence) matured stage were harvested from Tezpur University campus, Assam. The selection of fruit maturity was based on our previous study, at 20 DAE amounts of polyphenols and antioxidants was maximum in both pulp and peel. The samples were cleaned thoroughly with running tap water followed by soaking in deionised water for 1 h to remove any dust or dirt adhering to the peel surface and finally they were wiped with tissue papers and pulp and peel flour were separated using stainless knife, dried and ground. The extraction of natural antioxidant from both pulp and peel flour was done following the method described by Lopez-Cordoba et al.¹⁰ where three concentrations (3, 5 and 10 g) of samples were mixed with 100 ml of deionised water and placed in a sonication bath (Bandelin Sonorex, Germany) at 50°C for 30 min at 35 kHz. The extracts were then cooled, filtered and stored in dark glass bottles at 4°C.

5.2.2 Preparation of cocrystallized powder

Food grade commercial sucrose (50 g) (Merck, India) was heated at 132°C to obtain supersaturated solution. On attaining the supersaturated point the pan was removed from hot plate and 10 ml of aqueous extract of each pulp and peel extract (3, 5 and 10 g) were added to the sugar syrup with continuous stirring. When the slight turbidity was observed the samples were transferred to Petri plates and dried at 40°C for 15 h. The dried crystals were then ground, passed through 0.25 mm mesh screen, packed in polyethylene bags and stored inside desiccators at 25°C until further use. Six (6) batches of cocrystallized powders were prepared as described above and coded as CC-PU3, CC-PU5 and CC-PU10 for pulp and CC-PE3, CC-PE5 and CC-PE10 for peel.

Where, CC-PU and CC-PE are abbreviated for cocrystal from pulp and peel extracts respectively. A supersaturated sucrose solution without the active compound was also processed as a control and coded as CC-C.

5.2.3 Total polyphenols and radical scavenging activity

Total polyphenols content (TPC) and DPPH radical scavenging activity were determined following the methods of Malick and Singh²⁰ and Brand-Williams et al.²¹ (methods described in section 2.2.2.5)

5.2.4 Loading capacity and entrapment yield

The loading capacity (L_c) was calculated as the TPC of sample extract loaded in 1 g of cocrystallized material and measured following method given by Lopez-Cordoba et al.¹⁰ The entrapment yield (%EY) was calculated as:

$$\% EY = \frac{L_c}{L_o} \times 100 \quad \text{Eq. (5.1)}$$

Where, L_o is the initial TPC of the culinary banana extract per gram of raw mix.

5.2.5 HPLC analysis of phenolic compounds

High performance liquid chromatography analysis was carried out as described by Kumar et al.²² (detail method is described in section 2.2.2.10).

5.2.6 Characterization of the cocrystallized powders

5.2.6.1 Moisture content and water activity

Moisture content (%) was measured following the vacuum oven drying method of AOAC.²³ The water activity (a_w) of cocrystallized powder was determined using water activity meter (AquaLab-4TE, USA).

5.2.6.2 Solubility

The solubility of samples was determined by mixing 1 g of cocrystallized powder with 10 ml of distilled water at ambient temperature with continuous stirring. Aliquots were taken at every 15 s and the dissolved sucrose mass in the solution was determined using digital refractometer.

5.2.6.3 Flowability

The flowability of the cocrystallized powders was determined by dynamic angle of repose and Hausner ratio (H). The angle of repose was determined following method described by Geldart et al.²⁴ with a rotating measuring cylindrical which was tilted gradually until slipping occurred. The H value was calculated by the ratio of the tap bulk density to the loose bulk density.²⁵ The loose bulk density was determined by pouring a known mass of cocrystallized product delivered freely by gravity into a measuring cylinder and it was calculated by dividing the mass by the bulk volume. The tap bulk density was calculated from the weight of powder contained in the cylinder after being hand tapped for 100 times which was roughly 60 taps/min²⁶ and measurements were performed in triplicates.

5.2.6.4 Colour attributes

The colour measurement of samples was analyzed (L^* , a^* and b^* values) in a Hunter Lab Color Quest (Model Ultrascan Vis-Model, Virginia, USA). Where, L^* used to denote lightness, a^* to redness and greenness, and b^* to yellowness and blueness. Chroma and hue angle values were calculated using Eq. (5.2) and (5.3) respectively.

$$\text{Chroma} = [a^{*2} + b^{*2}]^{1/2} \quad \text{Eq. (5.2)}$$

$$\text{Hue angle} = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad \text{Eq. (5.3)}$$

5.2.6.5 Fourier transform infrared spectroscopy (FTIR)

IR spectra of encapsulated cocrystals were measured using Nicolet Instruments 410 FTIR equipped with KBr optics and a DTGS detector as described in section 2.6.2.2.

5.2.6.6 Differential scanning calorimetry (DSC)

Thermal behavior of encapsulated cocrystal samples was studied using differential scanning calorimetry (DSC) thermal analyzer (DSC60 SHIMADZU, North America). Samples (3 mg) were scanned in hermetically sealed aluminum pans under the nitrogen flow of 10 ml/min. Heat flow and temperature calibration was performed with indium. The melting temperature (T_m) and enthalpy (ΔH) of cocrystals were determined at scan rate of 10°C/min with heating rate from 50 to 250°C.

5.2.6.7 X-ray diffraction (XRD)

The powder X-ray diffraction patterns were obtained following the method described in section 2.6.2.4.

5.2.6.8 Microstructure evaluation

The microstructure of cocrystallized powders was studied in scanning electron microscope (JEOL JSM-6390LV, SEM, Oxford) at a magnification of 3000X at an accelerating voltage of 15 kV.

5.2.7 Water gain, hygroscopicity and chemical stability during storage

For storage study, 3 g of cocrystallized powders were weighed in Petri dishes and placed them in hermetically sealed glass desiccators containing supersaturated solution of NaCl (75% RH) and stored at 20°C. The weight of each Petri dish containing samples were measured in every 3 days interval where the total time taken for removal, weighing and keeping samples back

inside the desiccators was less than 20 s. This precaution helped minimization of atmospheric moisture during weighing. Hygroscopicity (HG%) is expressed as the final moisture content attained after exposing the samples under the conditions mentioned above. HG% values were calculated using modified Jaya and Das²⁷ equation as below:

$$HG (\%) = \left(\frac{b+H}{a-H} \right) \times 100 \quad \text{Eq. (5.4)}$$

Where b (g) is the weight increase, a (g) is the initial sample weight and H is the initial water content of the powder (g).

5.2.8 Statistical Analysis

Experiments were carried out in 3 replicates. The data analysis tool ‘Microsoft Excel’ was used for statistical analysis. Data were subjected to ANOVA and Fisher’s Least Significant Difference (LSD) was used to separate means.

5.3 Results and discussion

5.3.1 Total polyphenols, antioxidant activity, entrapment yield and loading capacity

Cocrystallized powder obtained by incorporating aqueous extract of natural antioxidant prepared with 3, 5 and 10 g from culinary banana pulp and peel, and they were coded as CC-PU3, CC-PU5, CC-PU10, CC-PE3, CC-PE5 and CC-PE10 respectively. The amount of total polyphenols content in the cocrystallized powder was found in the range of 0.86-1.35 mg chlorogenic acid/g powder in pulp sample and 1.03-1.46 mg chlorogenic acid/g powder in cocrystals obtained from peel extract (Table 5.1). The DPPH radical scavenging activity was 63.66-67.97% in pulp cocrystals and 75.24-83.78% in case of cocrystals obtained from peel extract (Table 5.1). The values of entrapment yield were obtained in the range of 75-80% in all cases. The results of entrapment yield reported by Lopez-Cordoba et al.¹⁰ for yerba mate extract processed at similar condition was around 80-85% which corroborates the present findings. Deladino et al.²⁸ working with cocrystallized freeze dried yerba mate extract obtained an average yield of 72%. Fig. 5.1 illustrates the relation between loading capacity (L_c) and measured DPPH

radical scavenging activity of cocrystals which showed the highest value of L_c was obtained for samples CC-PU10 and CC-PE10. Loading capacity parameter is important for calculating the mass of cocrystal powders which is essential for determining polyphenols levels in a food formulation.

Table 5.1 Total polyphenols and DPPH radical scavenging activity of cocrystal powders

Cocrystals	Total polyphenols (mg chlorogenic acid/g sample)	DPPH radical scavenging activity (%)
CC-C	0.05±0.01 ^a	9.34±0.07 ^a
CC-PU3	0.86±0.04 ^b	63.67±0.08 ^b
CC-PU5	1.02±0.08 ^c	65.43±0.07 ^c
CC-PU10	1.35±0.07 ^e	67.97±0.91 ^d
CC-PE3	1.03±0.11 ^d	75.24±0.77 ^e
CC-PE5	1.23±0.09 ^f	78.21±0.65 ^f
CC-PE10	1.46±0.05 ^g	83.78±0.97 ^g

^aMean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3.

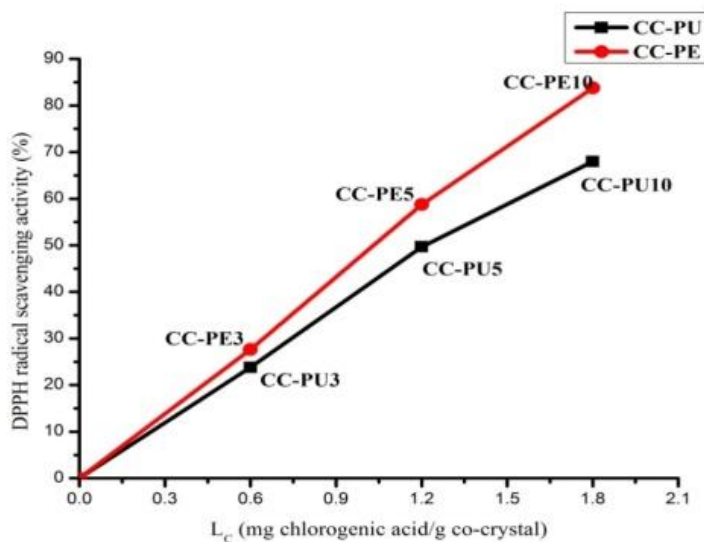


Fig. 5.1 Loading capacity vs. DPPH scavenging activity of cocrystallized powders

5.3.2 Polyphenols analysis by HPLC

The HPLC chromatograms of major polyphenols present in the cocrystals encapsulated with natural antioxidant extract from pulp and peel of culinary banana is presented in Table 5.2. Important polyphenols present in cocrystals encapsulated with aqueous extract of pulp were hydroquinone (42.33-60.32 ppm), chlorogenic acid (59.21-80.36 ppm), caffeic acid (21.54-45.67 ppm), p-coumaric acid (27.82-63.21 ppm), ferulic acid (21.64-35.32 ppm), quinic acid (109.57-187.08 ppm) and apigenin (67.98-97.26). The amount of entrapped polyphenols was significantly different to each other. Similarly, the important compounds of interest found in case of cocrystals incorporated with peel extract were hydroquinone (75.44-111.38 ppm), resorcinol (65.61-90.32 ppm), catechol (111.89-151.20 ppm), chlorogenic acid (17.47-46.87 ppm), p-coumaric acid (28.99-72.87 ppm), ferulic acid (104.28-151.09 ppm), quinic acid (236.26-275.54 ppm), salicylic acid (235.94-298.73 ppm), quercetin (81.14-116.87 ppm) and apigenin (143.30-208.37 ppm) varied significantly. From the results it has been observed that important polyphenols were appropriately entrapped in the sucrose cocrystals even working at high temperature, hence, it can be concluded that culinary banana is one of the preeminent sources of antioxidant and phenolic compounds. Results are in line with number of researchers who have also worked with different varieties of banana.^{1, 29,}

5.3.3 Characterization of the cocrystallized products

5.3.3.1 Moisture content and water activity

The cocrystallized powders revealed the final moisture content in the range of 5.34 -6.14 % (db) in CC-PU and 5.12-5.95 % (db) in CC-PE varied significantly in all the six samples studied (Table 5.3). The water activity (a_w) level was also recorded to be significantly varied and found considerably in a lower range (0.31-0.45 %) for CC-PU and (0.30-0.42 %) for CC-PE cocrystals and. The values obtained are in considerable range to prevent any microbial spoilage of encapsulated active compounds during storage. Various researchers have reported that microbial proliferation do not occur in sugar rich foods with a_w below 0.6, hence the product could be considered stable during storage.^{19, 31, 32}

Table 5.2 HPLC polyphenol profile of cocrystallized powders

Cocrystals	Major phenolic compounds (ppm)										
	Hydroquinone ($t_R = 8.42$)	Resorcinol ($t_R = 15.58$)	Catechol ($t_R=18.95$)	Chlorogenic acid ($t_R=19.25$)	Caffeic acid ($t_R=26.21$)	p-Coumaric acid ($t_R=32.72$)	Ferulic acid ($t_R=35.74$)	Quinic acid ($t_R=38.28$)	Salicylic acid ($t_R=38.51$)	Quercetin ($t_R=39.57$)	Apigenin ($t_R=40.14$)
CC-PU3	42.33±0.09 ^a	ND	ND	59.21±0.01 ^a	21.54±0.03 ^a	27.82±0.02 ^a	21.64±0.99 ^a	109.57±0.09 ^a	ND	ND	67.98±0.01 ^a
CC-PU5	51.64±0.07 ^b	ND	ND	72.44±0.02 ^b	33.97±0.11 ^b	48.69±0.04 ^b	29.87±0.75 ^b	135.04±0.01 ^b	ND	ND	83.54±0.09 ^b
CC-PU10	60.32±0.99 ^c	ND	ND	80.36±0.06 ^c	45.67±0.06 ^c	63.21±0.01 ^c	35.32±0.98 ^c	187.08±0.99 ^c	ND	ND	97.26±0.04 ^c
CC-PE3	75.44±0.32 ^d	65.61±0.45 ^a	111.89±0.15 ^a	17.47±0.13 ^d	ND	28.99±0.51 ^d	104.28±0.10 ^d	236.26±0.03 ^d	235.94±0.12 ^a	81.14±0.65 ^a	143.30±0.09 ^d
CC-PE5	83.80±0.97 ^e	78.32±0.36 ^b	126.75±0.09 ^b	29.07±0.07 ^e	ND	49.87±0.87 ^e	122.34±0.79 ^e	247.76±0.09 ^e	261.2±0.98 ^b	102.37±0.87 ^b	176.98±0.76 ^e
CC-PE10	111.38±0.65 ^f	90.32±0.97 ^c	151.20±0.03 ^c	46.87±0.15 ^f	ND	72.86±0.95 ^f	151.09±0.95 ^f	275.54±0.06 ^f	298.73±0.08 ^c	116.87±0.99 ^c	208.37±0.38 ^f

^aMean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3.

Table 5.3 Moisture content and water activity (a_w) of cocrystal powders

Cocrystals	Moisture content (% db)	Water activity
CC-C	5.23±0.09 ^b	0.37±0.01 ^b
CC-PU3	6.14±0.05 ^f	0.45±0.03 ^e
CC-PU5	5.56±0.07 ^d	0.36±0.01 ^{bc}
CC-PU10	5.34±0.09 ^c	0.31±0.02 ^a
CC-PE3	5.95±0.1 ^e	0.42±0.01 ^d
CC-PE5	5.12±0.06 ^a	0.30±0.04 ^a
CC-PE10	5.37±0.05 ^c	0.38±0.02 ^{bc}

^aMean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3.

5.3.3.2 Solubility and flowability of cocrystal powders

Dissolution of sucrose crystal is not instantaneous but depends on temperature, agitation rate, degree of under saturation and size of crystals.¹⁹ The kinetics of dissolution of control (CC-C) and all six culinary banana extract cocrystals are illustrated in Fig 5.2a and Fig. 5.2b. From the figures, it has been observed dissolution time was 40 to 45 s in all cocrystals compared to control (30 sec). This might be attributed to differences in shape and size of the control and cocrystal samples. Results are in agreement with Lopez-Cordoba et al.¹⁰ in case of cocrystals entrapped with yerba mate antioxidant extract According to Chen et al.³³ the cocrystallized active compounds are located primarily in the interstices between crystals. Due to the porosity of the agglomerates, aqueous solution can rapidly penetrate the agglomerate and free the active compound for dissolution.

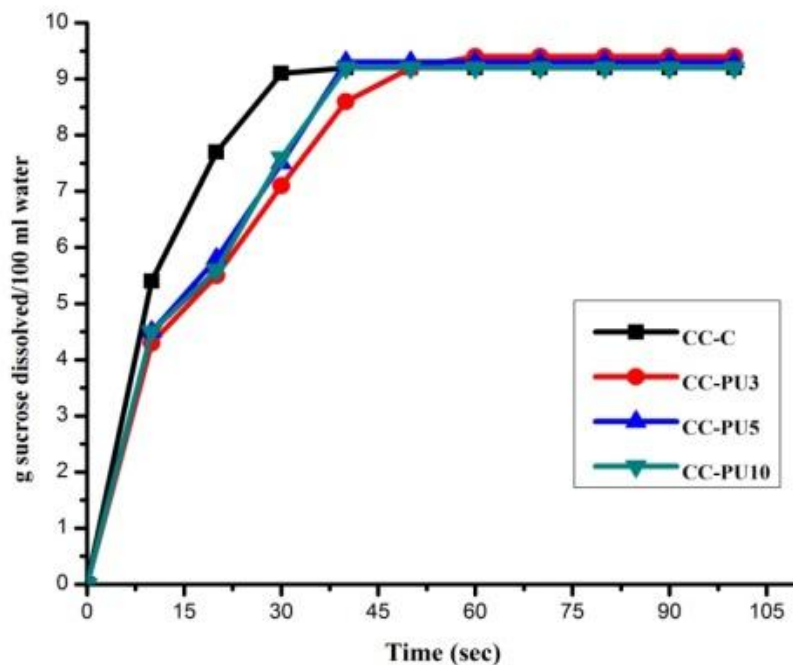


Fig. 5.2a Dissolution kinetics of sucrose cocrystals with and without pulp extracts

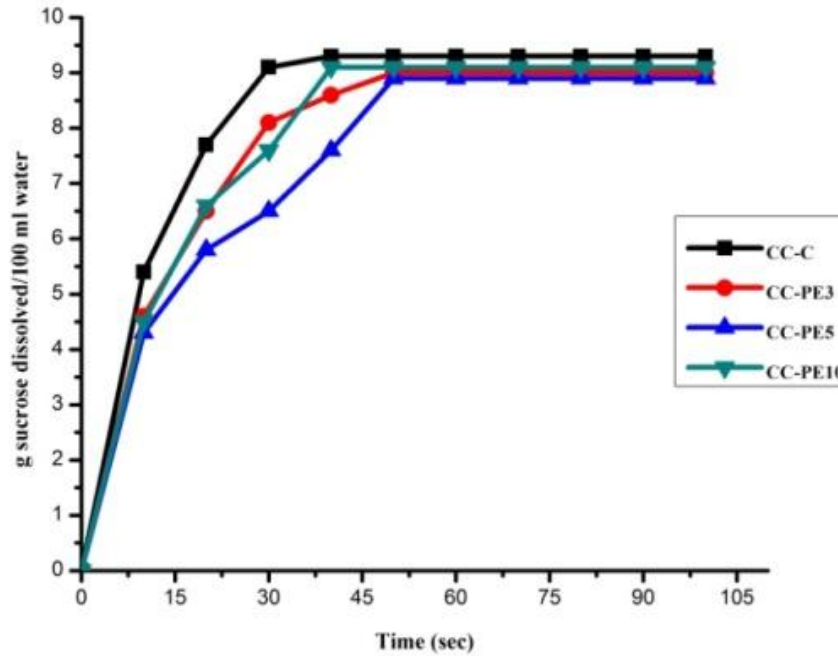


Fig. 5.2b Dissolution kinetics of sucrose cocrystals with and without peel extracts

Prediction of material handling during production and storage, material flow property is one the important aspects to be considered.²⁴ Table 5.4 represents the dynamic angle of repose and Hausner ratio which used in the present study to determine the flowability of the cocrystallized products. The dynamic angle of repose was recorded in the range of 40°-44° and Hausner ratio was found between 0.98-1.06 in all six sample and control sucrose crystal studied. It can be concluded that various concentrations used for extraction did not significantly affect the flowability properties of encapsulated samples studied, but they varied among pulp to peel cocrystals. To have a good flowability property for material handling, the angle of repose should be in the range of 40° to 45° and Hausner ratio values should be less than 1.2, whereas a value of 1.5 or higher Hausner ratio suggests a poor flow display by the material.^{24, 34-36}

Table 5.4 Flowability properties of cocrystal powders

Cocrystals	Angle of repose (°)	Hausner ratio (H)
CC-C	42.45±0.75 ^c	0.98±0.06 ^b
CC-PU3	41.64±0.97 ^b	1.01±0.05 ^c
CC-PU5	40.0±0.65 ^a	0.99±0.05 ^b
CC-PU10	41.56±0.96 ^b	1.03±0.07 ^d
CC-PE3	44.0±0.57 ^c	0.06±0.06 ^a
CC-PE5	43.68±0.67 ^d	0.99±0.02 ^b
CC-PE10	43.75±0.99 ^d	1.04±0.04 ^e

^aMean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3.

5.3.3.3 Colour attributes

Colour is one of the most important quality attributes and is significantly affected by the concentration of sample extract used. Table 5.5 showed decrease in lightness (L) value with the incorporation of extract. The chroma and hue angle values increased with the amount of sample extract loaded in the sucrose and were statistically significant to each other.

Table 5.5 Colour attributes of cocrystals

Cocrystals	Colour values		
	L*	Chroma	Hue angle (°)
CC-C	65.33±0.15 ^a	2.64±0.02 ^a	71.67±0.96 ^a
CC-PU3	67.75±0.86 ^b	2.72±0.01 ^b	74.03±0.86 ^b
CC-PU5	69.12±0.97 ^d	4.02±0.01 ^c	78.78±0.76 ^e
CC-PU10	70.36±0.76 ^f	8.43±0.04 ^d	76.25±0.75 ^c
CC-PE3	68.58±0.43 ^c	4.06±0.03 ^c	78.39±0.97 ^d
CC-PE5	70.12±0.47 ^e	11.36±0.04 ^e	80.56±0.51 ^f
CC-PE10	70.86±0.98 ^g	11.75±0.01 ^e	81.53±0.45 ^g

^aMean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3.

5.3.3.4 Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of cocrystals encapsulated with aqueous extract of pulp and peel are illustrated in Fig. 5.3a and Figs. 5.3b respectively. From the figures it is evident that the bands of functional groups present in the samples are typical bands of sucrose molecules. The bands observed at 523 and 795 cm^{-1} and attributed to the deformation mode of O-C-O group. The C-C stretching vibration modes were found at 871 and 1057 cm^{-1} . The characteristic peaks observed at 1050 and 1250 was C-O stretching. The bands observed at 1646, 1649, 1650, 1667 and 1691 cm^{-1} could be considered as deformation mode of hydroxyl group. The carboxyl group was found in the range of 1890 to 1950 cm^{-1} . At 2885 and 2928 cm^{-1} the C-H stretch were observed for $-\text{CH}_2$ group. Similarly at 2970 and 3395 cm^{-1} C-H stretching was observed. The peaks at 3360 and 3887 are assigned for $-\text{OH}$ stretching. The bands detected for specific functional groups are quite similar with the results of by Lopez-Cordoba et al.¹⁰ in case of yerba mate cocrystals.

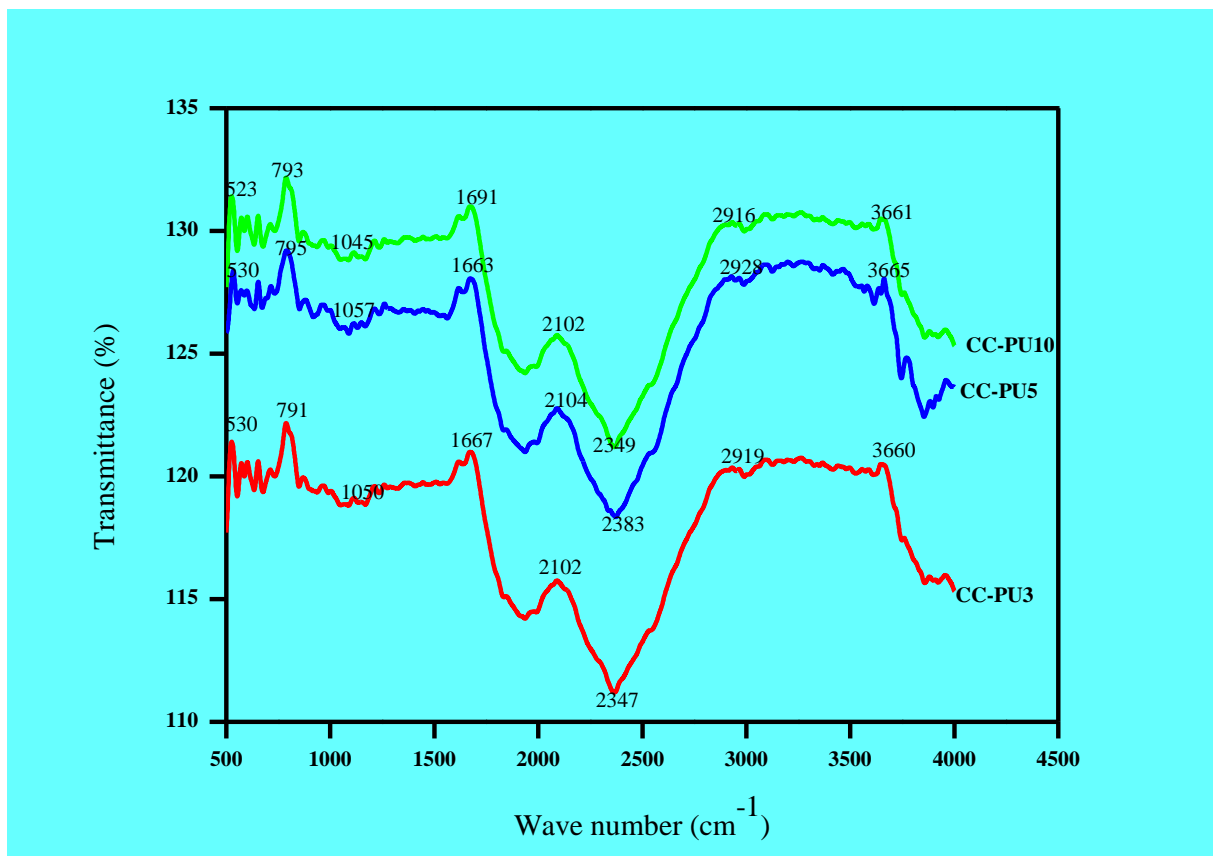


Fig. 5.3a FT-IR spectra of cocrystals encapsulated with pulp extract

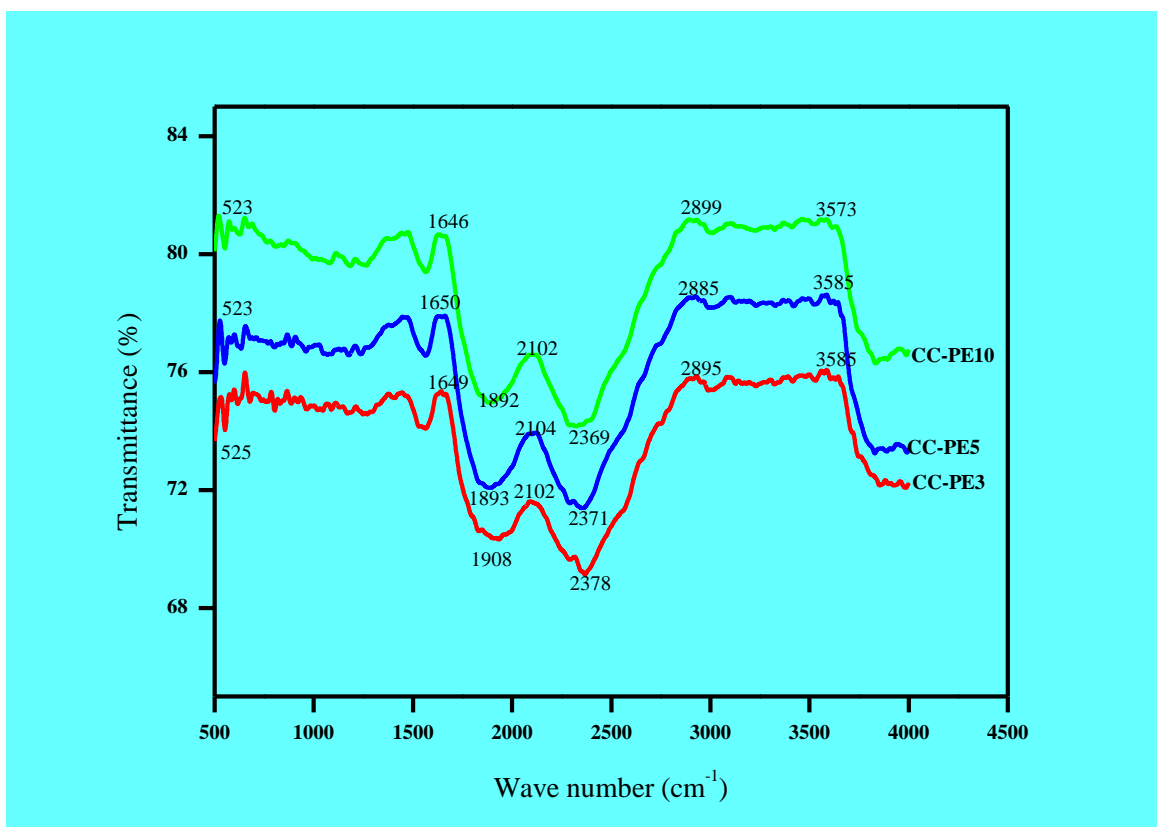


Fig. 5.3b FT-IR spectra of cococrystals encapsulated with peel extract

5.3.3.5 Differential scanning calorimetry (DSC)

The DSC thermographs studied for the cococrystals was carried out in hermetically sealed aluminum pans and the results are illustrated in Figs. 5.4a and Fig. 5.4b for pulp and peel cococrystals respectively. There was no base line deviation at the first scan which indicates the absence of residual amorphous material in the samples. The scanning of cococrystal (CC) at 300°C confirmed two major endothermic peaks at around 191.46°C and 225.26°C. The control sample (CC) confirmed an endothermic peak at around 191°C which is a typical melting point of sucrose. Bhandari and Hartel¹⁷ in their study reported that the pure crystalline sucrose had an endotherm peak at 190°C with a narrow melting range from 185 to 194°C which is in agreement with our results. The second peak at around 225°C could be attributed to the degradation of sucrose because caramelization of sugar might occur that have melting point above 150°C.³⁷ The thermographs obtained for all samples of CC-PU and CC-PE, the melting point slightly

decreased to lower temperatures and the endotherm was observed at 183.95-189.88°C for CC-PU and 186-190.46°C for CC-PE. Slight shifting of temperature in encapsulated cococrystals compared to the control one, might be due to the partial amorphous nature of cococrystal sucrose.¹⁹ Similar temperatures have also been reported by Lopez-Cordoba et al.¹⁰ in his study of yerba mate cococrystals. The diversity in endotherm transition shape might be due to presence of active compounds in the sample. In the DSC assay the total or partial disappearance of thermal events (melting point) corresponding to the active compound could be considered as proof of its incorporation into the matrix.³⁸

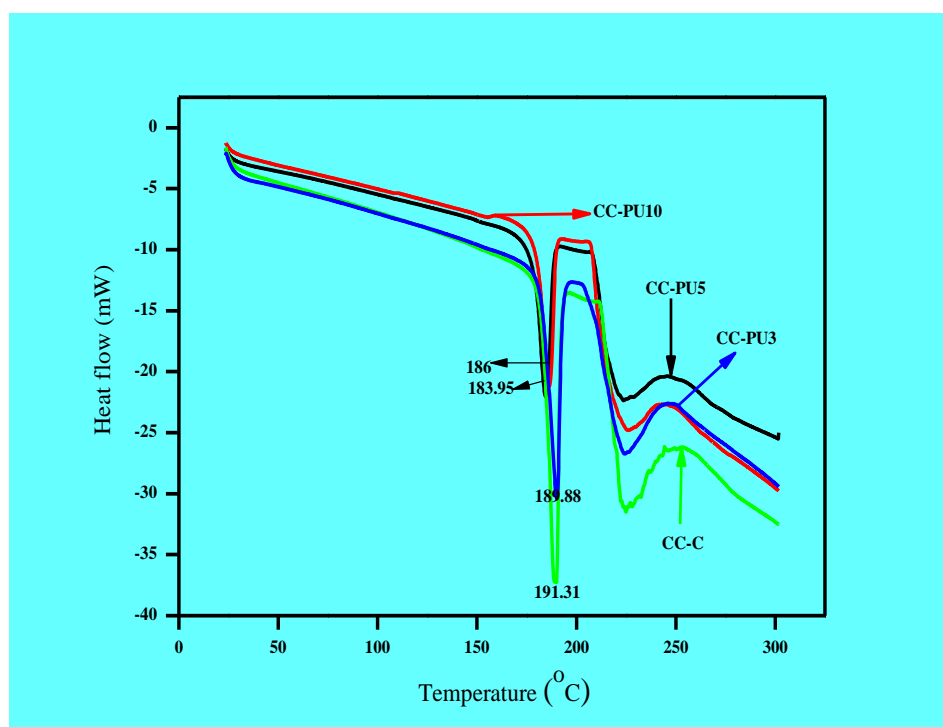


Fig. 5.4a DSC thermographs of cococrystals encapsulated with pulp extract

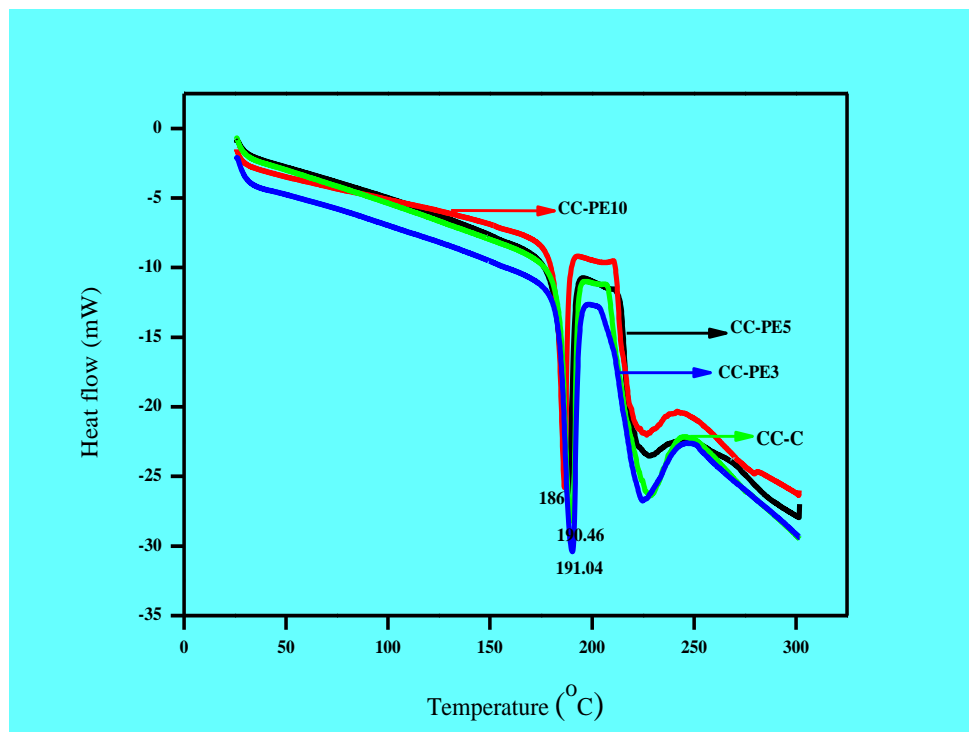


Fig. 5.4b DSC thermographs of cococrystals encapsulated with peel extract

5.3.3.6 X-ray diffraction (XRD)

The powder x-ray diffraction analysis of cococrystals with and without active compounds was observed to estimate the degree of crystallinity. The diffractograms of all the cococrystals presented in Figs.5.5a-d and 5.6a-d showed the similar type of x-ray crystalline pattern of sucrose. This might be due to the low content of active compound compared to the sucrose matrix.²⁸ For the cococrystal without active compound (CC-C) the peaks observed with the higher intensities emerged at 13.10, 18.87, 24.86, 31.02 and 38.55°C. The degree of crystallinity depends on the intensity of peak heights as well high intensity peaks. Therefore it could be concluded that higher the intensity of peak higher is the crystallinity. Cococrystal samples containing higher amount of extract concentration (CC-PU10 and CC-PE10) showed higher degree of crystallinity than the cococrystals with lower concentration extracts. As stated by Bhandari and Hartel¹⁷ a combination of techniques like DSC and XRD could give a complete analysis of the structural changes on cococrystallization. Our results are in line with Sardar and Singh¹⁹ in case of cococrystals entrapped with cardamom oleoresins.

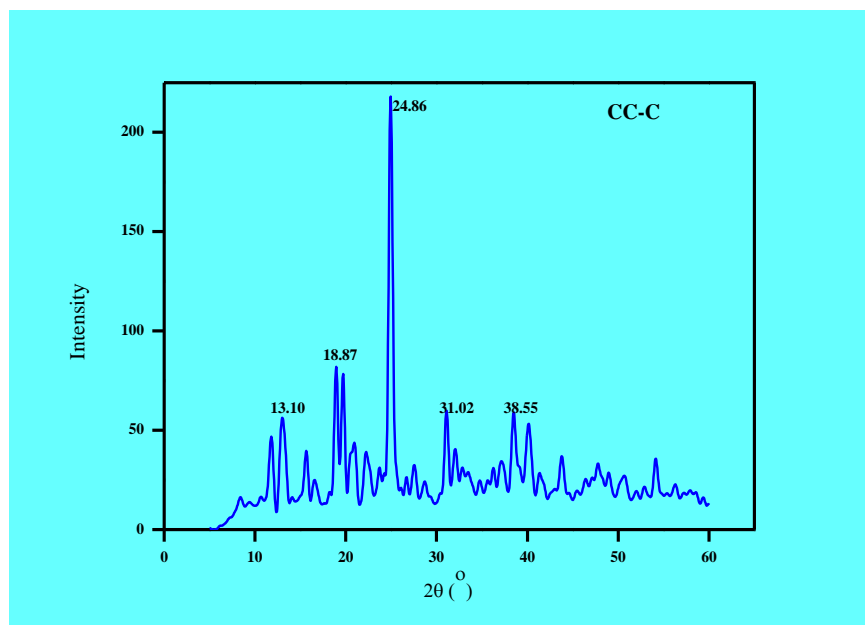


Fig. 5.5a X-rays diffraction patterns of sucrose cocrystals control (CC-C)

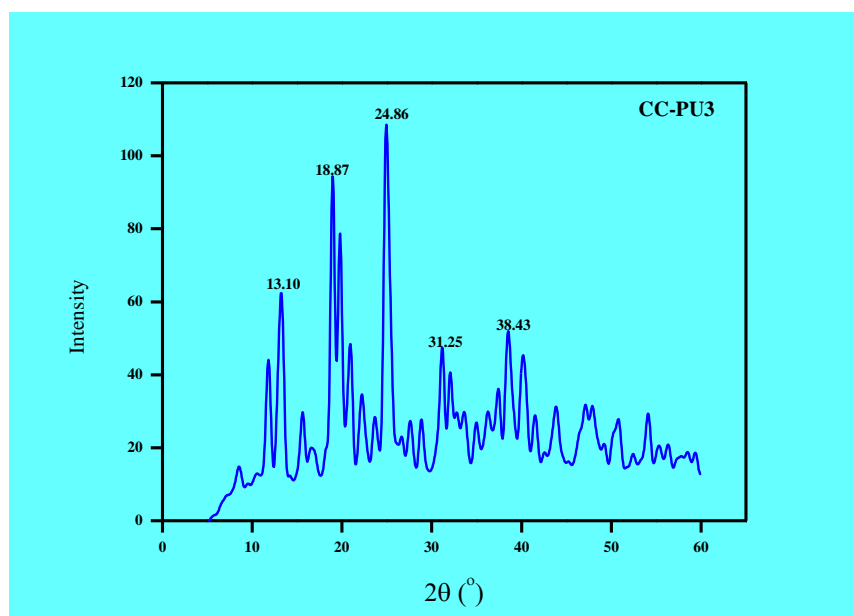


Fig. 5.5b X-rays diffraction patterns of cocrystals encapsulated with 3g pulp extract (CC-PU3)

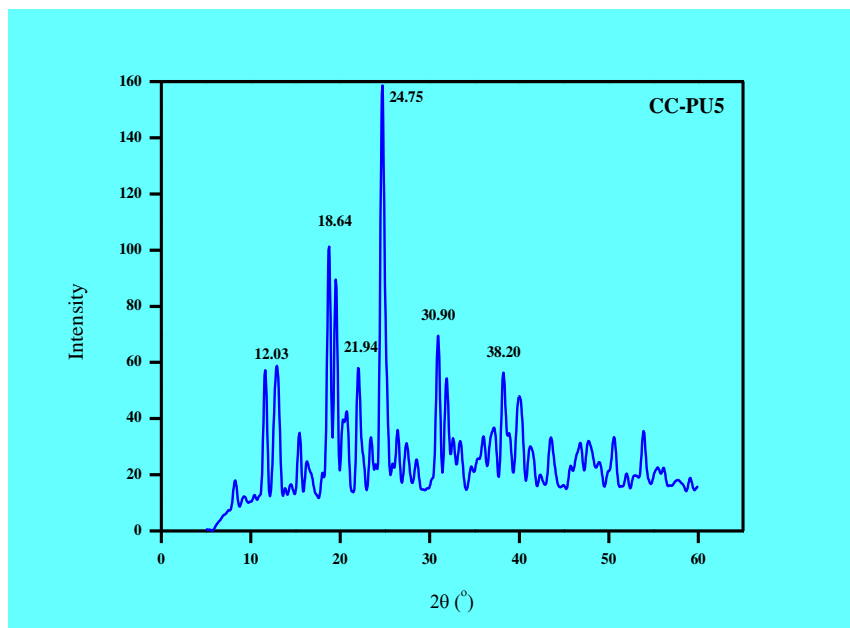


Fig. 5.5c X-rays diffraction patterns of cocrystals encapsulated with 5g pulp extract (CC-PU5)

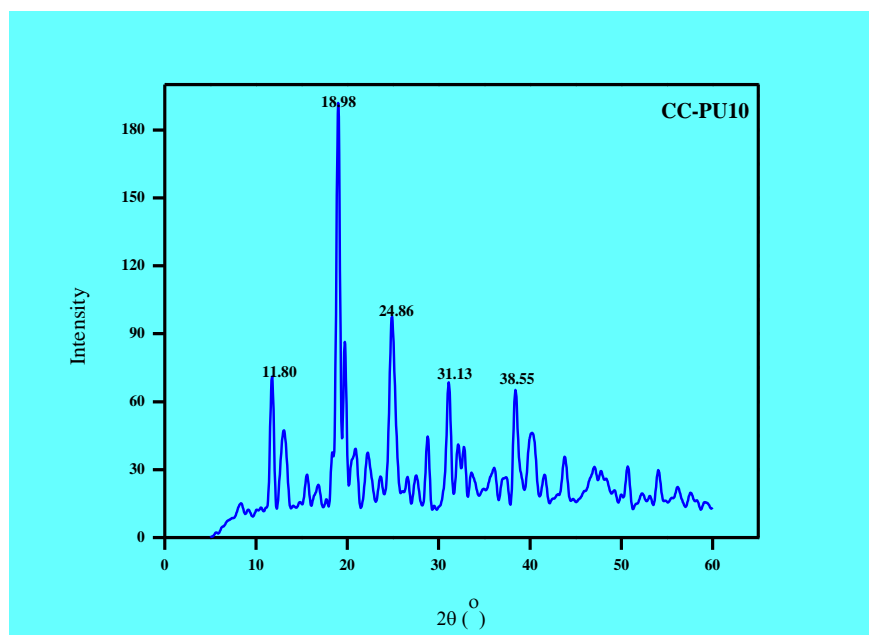


Fig. 5.5d X-rays diffraction patterns of cocrystals encapsulated with 10g pulp extract (CC-PU10)

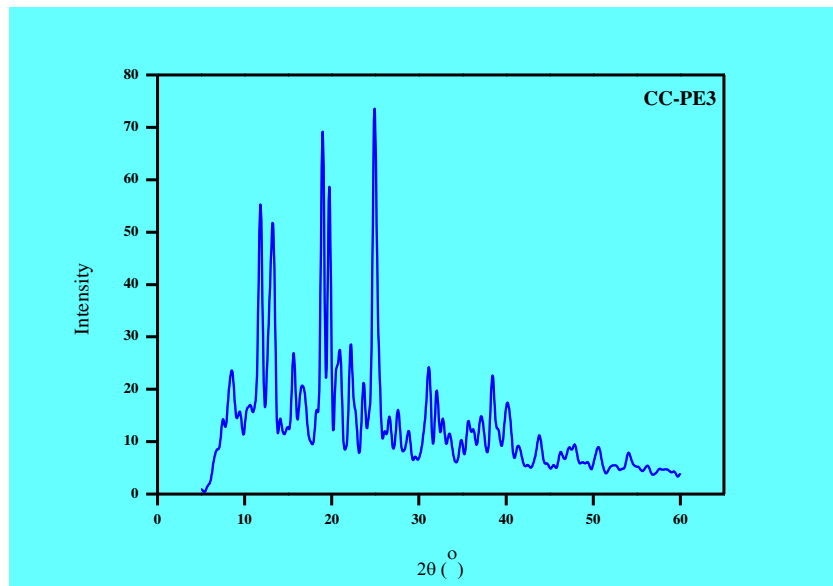


Fig. 5.6a X-rays diffraction patterns of cocrystals encapsulated with 3g peel extract (CC-PE3)

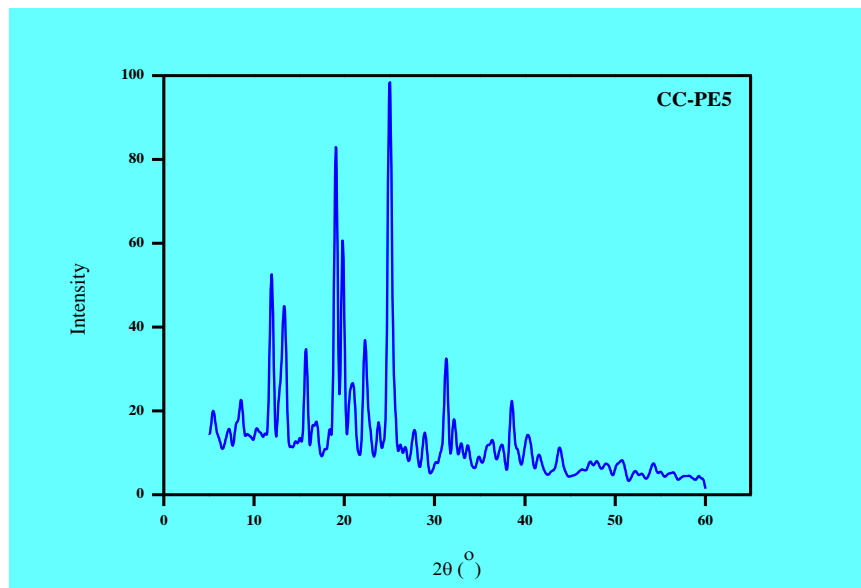


Fig. 5.6b X-rays diffraction patterns of cocrystals encapsulated with 5g peel extract (CC-PE5)

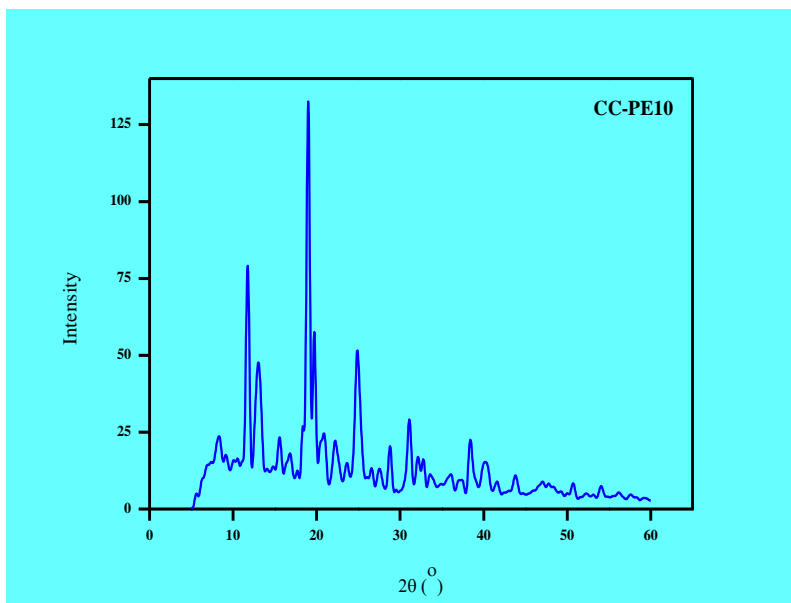


Fig. 5.6c X-rays diffraction patterns of cococrystals encapsulated with 10g peel extract (CC-PE10)

5.3.3.6 Microstructure evaluation

The results of scanning electron micrograms showed basic porous structure corresponding to typical cluster like agglomerates with void spaces and irregular cavities (Figs. 5.7 and 5.8). The cococrystallized sucrose without the active compound exhibit slightly modified crystalline structure with small cluster like agglomerates and void spaces with frequent neat edges. On the other hand, cococrystals entrapped with active compounds showed a surface structure containing rough cluster like agglomerates without neat edges but the presence of entrapped compounds in the sucrose matrix could be seen. Similar observations were also reported with other cococrystallized products like cococrystallized sucrose entrapped with cardamom oleoresin,¹⁹ cococrystals of yerba mate extract and mineral salts,²⁸ sucrose cococrystals.¹⁷

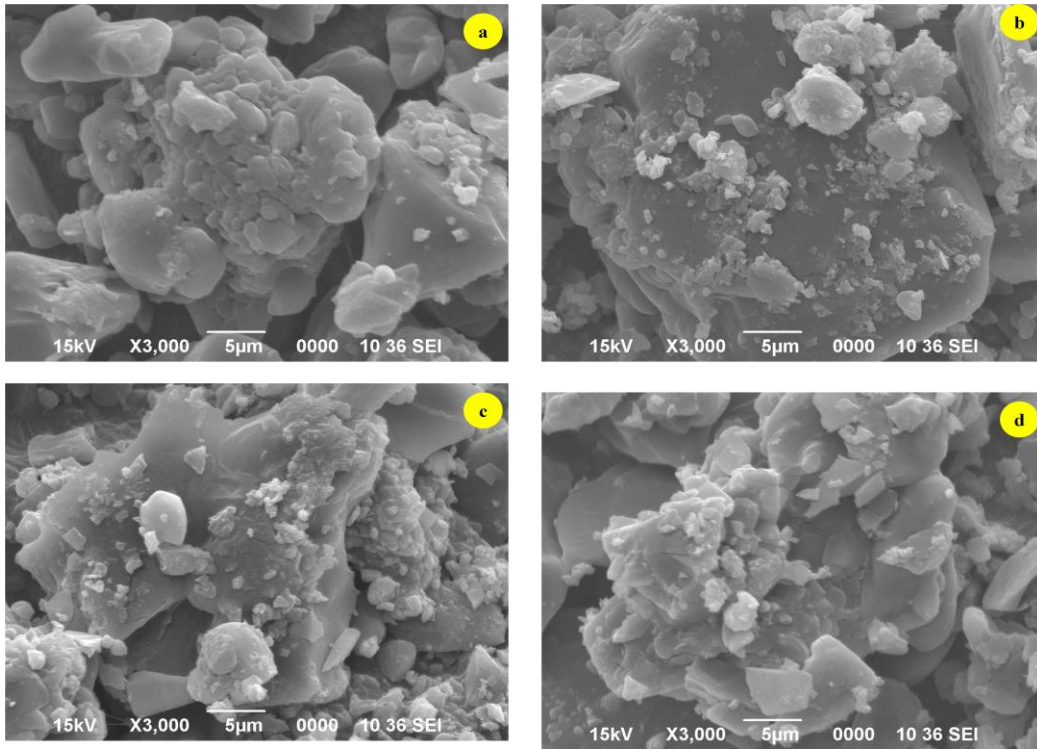


Fig. 5.7 SEM micrographs of control and cocystals entrapped with pulp extract a) CC-C, b) CC-PU3, c) CC-PU5 and d) CC-PU10

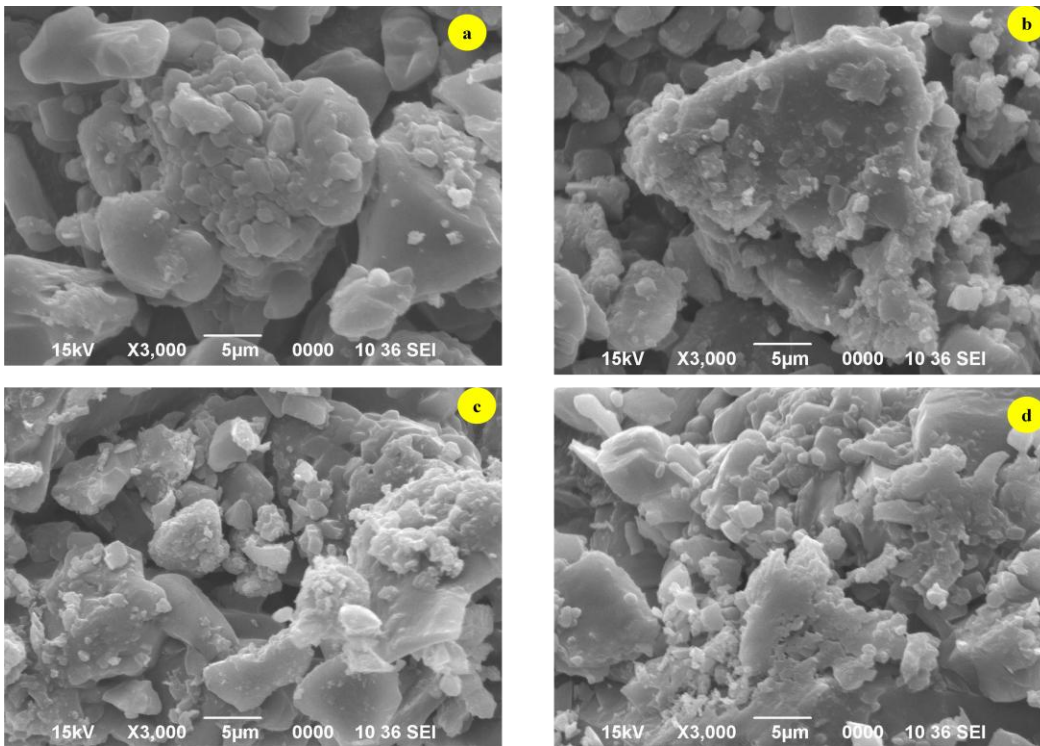


Fig. 5.8 SEM micrographs of control and cocystals entrapped with peel extract a) CC-C, b) CC-PE3, c) CC-PE5 and d) CC-PE10

5.3.3.7 Water gain, hygroscopicity during storage

The stability and physicochemical behaviour of pure crystal sucrose and various cocrystallized samples varied widely during storage. The hygroscopicity determination helps to predict handling and packaging behaviour. The water gain by samples during storage facilitates the occurrence of degradation reactions. Table 5.6 showed low hygroscopicity of cocrystals. It is known that at any given condition of temperature and relative humidity, the crystalline form of sucrose has significantly lower hygroscopicity compared to amorphous form.¹⁹ The hygroscopicity of the cocrystals is reported to depend on the fraction of amorphous crystals in a mixture of glucose and fructose.³⁹

Table 5.6 Hygroscopicity (HG %) of cocrystallized powders at 75 %RH and 20°C

Cocrystals	Hygroscopicity (%)
CC-C	0.16±0.01 ^a
CC-PU3	0.21±0.03 ^b
CC-PU5	0.24±0.01 ^c
CC-PU10	0.30±0.01 ^f
CC-PE3	0.26±0.02 ^d
CC-PE5	0.24±0.01 ^c
CC-PE10	0.27±0.01 ^e

^aMean in columns followed by the same letter are not significantly different at $p>0.05$; values represent mean±SD, n=3.

5.4 Conclusions

Natural antioxidant one of the most valuable compounds possessing scavenging properties was extracted using aqueous medium from both pulp and peel of culinary banana at early developmental stage (20 DAE) at different concentration of 3, 5 and 10 g. The aqueous extract obtained was further incorporated in supersaturated solution of sucrose. The cocrystals obtained with high entrapment yield showed desirable characteristics such as low water content and water activity, high solubility, low hygroscopicity and very good flowability. The presence

of major phenolic compounds in all sample studied was confirmed by HPLC chromatographs. The cocrystals studied demonstrated the typical bands of sucrose molecules in FTIR spectroscopy which was further confirmed by DSC endothermic peak at around 191°C showing a typical melting point of sucrose. The diffractograms obtained by XRD showed similar type of x-ray crystalline pattern of sucrose. The results of scanning electron micrograms illustrated basic porous structure corresponding to typical cluster like agglomerates with void spaces and irregular cavities. The cocrystallization process resulted in a good alternative to preserve and handle these materials for further application in food products. Hence, it can be concluded that the culinary banana extract powder constitutes a promising alternative as a natural antioxidant ingredient for the formulation of functional foods.

References

1. Kanazawa, K., & Sakakibara, H. High content of a dopamine, a strong antioxidant, in Cavendish banana. *J. Agric. Food Chem.* **48**(3), 844--848, 2000.
2. Qusti, S.Y., et al. Free radical scavenger enzymes of fruit plant species cited in Holy Quran. *World Appl. Sci. J.* **9**(3), 338--344, 2010
3. Ramcharan, C., & George, C. Growing banana and plantain in Virgin Islands. *Farmers Bulletins No.* **11**, 13--14, 1999.
4. Hassimotto, N.M., et al. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J. Agric. Food Chem.* **53**(8), 2928--2935, 2005.
5. Isabelle, M., et al. Antioxidant activity and profiles of common fruits in Singapore. *Food Chem.* **123**(1), 77--84, 2010.
6. Abdel-Hameed, E.S.S. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. *Food Chem.* **114**(4), 1271--1277, 2009.
7. Giasson, B.I., et al. The relationship between oxidative/nutritive stress and pathological inclusions in Alzheimer's and Parkinson's diseases. *Free Radic. Biol. Med.* **32**(12), 1264--1275, 2002.
8. Kawasaki, B.T., et al. Targeting cancer stem cells with phytochemicals. *Mol. Interv.* **8**(4), 174--184, 2008.
9. Ndhlala, A.R., et al. Antioxidant properties and degrees of polymerization of six wild fruits. *Sci. Res. Essays*, **1**(3), 87--92, 2006.
10. Lopez-Cordoba, A., et al. Yerba mate antioxidant powders obtained by cocrystallization: Stability during storage. *J. Food Eng.* **124**, 158--165, 2014.
11. Kosaraju, S.L., et al. Delivering polyphenols for healthy ageing. *Nutr. Diet.* **65**(3), S48--S52, 2008.
12. Makris, D.P., & Rossiter, J.T. Heat-induced, metal-catalyzed oxidative degradation of quercetin and rutin (quercetin 3-O-rhamnosylglucoside) in aqueous model systems. *J. Agric. Food Chem.* **48** (9), 3830--3838, 2000.
13. Onwulata, C.I. Encapsulation of new active ingredients. *Annu. Rev. Food Sci. Technol.* **3**(1), 183--202, 2011.

14. Jackson, L.S., & Lee, K. Microencapsulation and the food industry. *LWT--Food Sci. Technol.* **24**(4), 289--297, 1991.
15. Beristain, C.I., et al. Encapsulation of orange peel oil by cocrystallization. *LWT--Food Sci. Technol.* **29**(7), 645--647, 1996.
16. Bhandari, B.R., et al. Co-crystallization of honey with sucrose. *LWT--Food Sci. Technol.* **31**(2), 138--142, 1998.
17. Bhandari, B.R., & Hartel, R.W. Cocrystallization of sucrose at high concentration in the presence of glucose and fructose. *J. Food Sci.* **67**(5), 1797--1802, 2002.
18. Maulny, A.P.E., et al. Physical properties of cocrystalline sugar and honey. *J. Food Sci.* **70**(9), E567--E572, 2005.
19. Sardar, B.R., & Singhal, R.S. Characterization of cocrystallized sucrose entrapped with cardamom oleoresin. *J. Food Eng.* **117**(4), 521--529, 2013.
20. Malick, C.P., & Singh, M.B. *Plant Enzymology and Histo-enzymology*, 4th ed., Kalyani Publishers, New Delhi, 1980.
21. Brand-Williams, W., et al. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT--Food Sci. Technol.* **28**(1), 25--30, 1995.
22. Kumar, N., et al. Reversed phase-HPLC for rapid determination of polyphenols in flowers of rose species. *J. Sep. Sci.* **31**(2), 262--267, 2008.
23. A.O.A.C. *Association of Official Analytical Chemists Official methods of analysis of the Association of Official Analytical Chemists*. 18th ed., AOAC International, Washington, DC, 2010.
24. Geldart, D., et al. Characterization of powder flowability using measurement of angle of repose. *China Particuol.* **4**(3-4), 104--107, 2006.
25. Hausner, H.H. Friction conditions in a mass of metal powder. *Int. J. Powder Metall.* **3**(4), 7-13, 1967.
26. Pordesimo, L.O., et al. Food powder delivery through a feeder system: effect of physicochemical properties. *Int. J. Food Prop.* **12**(3), 556--570, 2009.
27. Jaya, S., & Das, H. Effect of maltodextrin, glycerol monostearate and tricalcium phosphate on vacuum dried mango powder properties. *J. Food Eng.* **63**(2), 125--134, 2004.
28. Deladino, L., et al. Cocrystallization of yerba mate extract (*Ilex paraguariensis*) and mineral salts within a sucrose matrix. *J. Food Eng.* **80**(2), 573--580, 2007.

29. Mohapatra, D., et al. Banana and its by-product utilization: an overview. *J. Sci. Ind. Res.* **69**(5), 323--329, 2010.
30. Alothman, M., et al. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chem.* **115** (3), 785--788, 2009.
31. Fennema, O.R., & Tannenbaum, S.R. Introduction to Food Chemistry, in *Food Science and Technology* Fennema, O.R. & Tannenbaum S.R. eds., New York: Marcel Dekker Inc., 1996, 1-16.
32. Fu, B., & Labuza, T.P. Shelf-life prediction: theory and application. *Food Control* **4**(3), 125--133, 1993.
33. Chen, A.C., et al. Cocrystallization: an encapsulation process. *Food Technol.* **42**(11), 87--90, 1988.
34. Santomaso, A., et al. Powder flowability and density ratios: the impact of granules packing. *Chem. Eng. Sci.* **58**(13), 2857--2874, 2003.
35. Antequera, M.V.V., et al. Evaluation of an adequate method of estimating flowability according to powder characteristics. *Int. J. Pharm.* **103**(2), 155--161, 1994.
36. Peleg, M. Flowability of food powders and methods for its evaluation: A review. *J. Food Process Eng.* **1**(4), 303--328, 1977.
37. Roos, Y.H. *Phase Transition of Foods*. 2nd ed., San Diego, CA, London: Academic press, Inc., 1995.
38. Cevallos, P.A.P., et al. Encapsulation of cinnamon and thyme essential oils components (cinnamaldehyde and thymol) in β -cyclodextrin: effect of interactions with water on complex stability. *J. Food Eng.* **99** (1), 70--75, 2010.
39. Saleki-Gerhardt, A., et al. Assessment of disorder in crystalline solids. *Int. J. Pharm.* **101**(3), 237--247, 1994.