

Storage stability of microencapsulated anthocyanin extracted from kokum (*Garcinia indica*) rind

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Abstract

The present research aimed to study the storage stability of microencapsulated anthocyanin. The anthocyanin was extracted from kokum processing industry waste and microencapsulated by spray drying. The effect of different process variables like core (i.e., anthocyanin) to wall (i.e., maltodextrin) ratio, inlet temperature and feed rate on encapsulation efficacy (EE) and Encapsulation yield (EY) was observed. The storage stability of anthocyanin was carried out by subjecting it to different storage conditions like the presence and absence of air, sunlight, room temperature, and refrigeration condition. The maximum EE ($82.2 \pm 0.95\%$), and EY ($92.6 \pm 0.86\%$) were recorded at the core to wall ratio (1:3), inlet temperature (140°C), and feed rate (2 mL/mins). The highest retention was recorded in encapsulated anthocyanin stored in refrigerated conditions ($R^2 = 0.982$), with a half-life of 245.77 weeks, and a D value of 354.57 weeks. This concludes that the stability of anthocyanin was increased due to encapsulation.

1. Introduction

Kokum (*Garcinia indica* Choisy) is an indigenous and valuable fruit crop. It is also known as wild Mangosteen or Red mango (Padhye *et al.*, 2009). The ripe kokum fruits are red or dark purple in colour, and the presence of anthocyanin i.e., cyanidin-3-glucoside and cyanidin-3-sambubioside are responsible for red colour (Krishnamurthy *et al.*, 1982; Du and Francis, 1977). At present, India produces 10,200 metric tons of Kokum out of which 9000 metric tons/ha is being processed into different products (Braganza *et al.*, 2012). Kokum is generally used for the manufacturing of different products like dried ripe Kokum rind (Amsul), Kokum syrup, Kokum agal, Kokum butter, Kokum juice etc. The dried rind is extracted with water to make a syrup which is sweetened and carbonated to make a cold drink. In some parts of India, rinds are spiced and sweetened with Jaggery for feasts (Patil *et al.*, 2005). Aqueous Kokum extract also has 4% sugar which can be fermented to make excellent quality wine. Dried kokum rind pieces are powdered, sieved and stored in airtight containers. The powder is used in coconut and fish curries as an acidulating agent (Nayak *et al.*, 2010). While preparation of these products waste is generated and leads to serious environmental problems.

It is observed that anthocyanin is an unstable pigment and undergoes discolouration during storage (Seeram *et al.*, 2001). The stability of anthocyanin is affected by various factors such as pH, temperature, structure and concentration, light, presence of co-pigment enzymes, oxygen, ascorbic acid, metallic ions, sugar, sulphur dioxide and proteins (Mazza and Miniati, 1993; Rodriguez-Saona *et al.*, 1999).

Microencapsulation is considered the best method to protect the bioactive components. There are various methods for microencapsulation such as spray drying, Freeze drying, Fluidized bed drying, centrifugation and Extrusion (Ranveer *et al.*, 2021). Microencapsulation by using a spray drier is an economical method for the preservation of natural colourants by entrapping the ingredient in a coating material (Cai and Corke, 2000). There are various encapsulation materials such as Carbohydrates (Cellulose and derivatives, Chitin and Chitosan, Starch, Agar, Alginate, Carrageenan's, Gums and Pectin's), Proteins (Gelatine, Corn zein, Wheat gluten, Soy protein and Whey protein) and Lipids and waxes (Waxes and paraffin's, Acetoglycerides and Shellac resins) are used for encapsulation (Khanvilkar *et al.*, 2016). Microencapsulation of pigments was studied by different researchers; those include anthocyanin from

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black carrot (Ersus and Yurdagel, 2007), anthocyanins from *Berberis vulgaris* (Homayoonfal *et al.*, 2021) and betalains from Amaranthus (Cai and Corke, 2000). Encapsulating materials protects active food ingredient against oxidation (Shahidi and Han, 1993; Wang *et al.*, 2021), these materials have low viscosity at high solids ratio and are available in different molecular weights which provides different wall thickness around the encapsulated materials (Desorby *et al.*, 1997; Cai and Corke, 2000).

The stability of encapsulated anthocyanin extracted from blue barriers (Xu *et al.*, 2019) and Roselle (Idham *et al.*, 2012) is well documented. The anthocyanin stability inclined by the temperature of storage was well fitted to a first-order rate law (Ochoa *et al.*, 2001; Gradinaru *et al.*, 2003; Kirca and Cemeroglu, 2003). However, there are no kinetic data for the degradation of encapsulated kokum anthocyanin. Also, there is no evidence found for stability studies at different conditions such as the presence and absence of oxygen, sunlight and different storage temperatures.

Therefore, the present research work focused on the encapsulation of anthocyanin extracted from kokum processing industry waste and studies the stability of encapsulated anthocyanin stored at different storage conditions.

2. Materials and methods

2.1 Kokum rind

Kokum rinds of a variety kokum Amruta were procured from Kokum processors of the Ratnagiri district of Maharashtra. The kokum processing waste should be free from any blemish part and spillage. The waste samples should be dark red in colour. The seeds and rinds were separated manually. The rinds were cleaned and dried in a cabinet dryer at 45–50°C up to 10% moisture content stored in polyethylene bags at refrigerated temperature.

2.2 Extraction and purification of anthocyanin

The anthocyanin was extracted as per the procedure suggested by Nayak and Rastogi, (2011) with slight modification. The dried rinds were ground and mixed with acidic water (0.1% Hydrochloric acid) in a proportion of 1: 2 and continuously stirred on an orbital shaker at 100 rpm for 30 mins. Further, colour was extracted using a hydraulic press. The filtered extract was stored in brown bottles at 4 to 5°C. The anthocyanin extract was purified by adopting a procedure suggested by Nayak *et al.* (2010). The aqueous extract of kokum was passed through a 500 mg sorbent C-18 Sep-Pak cartridge, which was previously activated with methanol

followed by 0.01% aqueous HCl. Anthocyanins were adsorbed onto the cartridge, whereas sugars, organic acids and other water-soluble compounds were washed off the cartridge with 0.01% aqueous hydrochloric acid. Anthocyanins were eluted using acidified methanol (0.01%, v/v HCl). Methanol was evaporated using a rotary evaporator at 35°C and pigments were dissolved in double distilled water containing 0.01% HCl. These partially purified concentrated anthocyanins were loaded (0.8 mL) onto a Sephadex LH20 column (1.0 × 60 cm) and eluted with a mixture of methanol/water/trifluoroacetic acid at a ratio of 20:79.5:0.5 to obtained purified anthocyanins.

2.3 Microencapsulation of anthocyanin

500 mL of anthocyanin extract (6°Bx) was mixed with Maltodextrin (DE 21) and stirred using a high-speed homogenizer for 1 hr. The mixture was spray-dried in a spray dryer (LU-222, Labultima, Mumbai). The emulsion was fed at a different feed rate into the drying chamber, entrance air temperatures of 140±2°C, air pressure of 2.5 kg f/cm² in co-current flow mode. After spray drying, microcapsules were collected in the cyclone separator driven by an exhaust blower.

2.4 Experimental design for response surface methodology and statistical analysis

The effect of core to wall Ratio (A), inlet temperature (B) and feed rate (C) on EY and EE was investigated by using a central composite rotatable design (CCRD) (Montgomery, 2005). The design of the experiment was carried out by statistical software Design –Expert 8 (Table 1).

Table 1. Values of independent variables at three levels

Independent Variables	Code	Level in code form		
		-1	1	+1
Core: Wall	A	1:1	1:2	1:3
Inlet Temperature (°C)	B	140	150	160
Feed rate (mL/mins)	C	2.0	2.5	3

Each factor was investigated at three levels i.e., -1, 0, +1 (Table 1). A total of 27 runs were designed based on CCRD with three factors. A quadratic model was then fitted to the data upon regression using Design expert version 8. The relationship of the independent variables and the response was calculated by the second-order polynomial (Equation 1).

$$Y = \beta_0 + \sum_{i=1}^K \beta_i X_i + \sum_{i=1}^K \beta_{ii} X_i^2 + \sum_{i=1}^K \sum_{j=1}^K \beta_{ij} X_i X_j \quad (1)$$

Y is the predicted response, β_0 a constant, β_i the linear coefficient, β_{ii} the squared coefficient and β_{ij} the cross-product coefficient; k is a number of factors. The second-order polynomial coefficients and surface plots were obtained using the software.

2.5 Encapsulation yield

The proportion of the mass of capsules obtained at the end of the process and the mass of initial substances added including anthocyanin and carrier materials is known as encapsulation yield (EY) (Xu *et al.*, 2019). The EY was calculated by the following formula

$$EY(\%) = \frac{MSA \times 100}{MSB}$$

Where MSA = total mass of microcapsules obtained after encapsulation and MSB = total mass of solids before encapsulation.

$$EE(\%) = \frac{(TL - SL) \times 100}{TL}$$

2.6 Encapsulation efficiency

The Encapsulation efficiency (EE) was calculated according to (Xu *et al.*, 2019), and it was calculated as follows:

Where TL = total anthocyanin and SL = surface anthocyanin.

2.7 Structural study

The microstructure of the encapsulated anthocyanin sample was obtained by using a scanning electron microscope (JSM – 6360, JEOL, Germany). The sample was mounted horizontally and vertically using carbon tape on an aluminium disc of diameter 3.0 cm and thickness 0.5 cm. The disc containing specimens was placed in a vacuum sublimator sputter coater (JFC – 1600, JEOL, Germany) and sprayed with platinum. The stub was placed on a pre-cryogenic electron microscope specimen holder that was pre-frozen by liquid nitrogen and observed at acceleration voltages 10kV and 20kV (Ranveer *et al.*, 2015).

2.8 Pigment retention

A pH differential method was employed for measuring the anthocyanin content in the extract. Briefly, 200 g of encapsulated powder was diluted in 1000 mL 0.1N HCl, further diluted 25 mL of stock to 50 mL using water. The sample is rested in dark for 1 hr and analyzed at 510 nm (Nayak, and Rastogi, 2011). The anthocyanin content was calculated using the following equation.

$$\text{Anthocyanin Content (mg/L)} = \frac{A \times M \times DF \times 10^3}{\epsilon \times l}$$

Where A is total absorbance = $[(A_{\lambda_{\max}} - A_{700}) \text{ at pH 1.0} - (A_{\lambda_{\max}} - A_{700}) \text{ at pH 4.5}]$, M is the molecular weight of anthocyanin (449 g mol^{-1}), DF is the dilution factor, ϵ is the extinction coefficient ($29,600 \text{ L cm}^{-1} \text{ mol}^{-1}$), and l is the path length (1.0 cm).

The ratio of the percentage of total anthocyanin present in the encapsulated powder after spray drying to that of total anthocyanin present in the extract was used to determine the anthocyanin retention efficiency.

2.9 Stability studies of microencapsulated anthocyanin

The encapsulated non-samples (lyophilized) were packed in pre-sterilized glass bottles. The bottles were stored under different storage conditions in the presence and absence of air, and sunlight, at room temperature ($25 \pm 2^\circ\text{C}$) and under refrigerated conditions ($5 \pm 2^\circ\text{C}$). Retention of anthocyanin content was analysed periodically at regular intervals of 7 days.

2.10 Kinetics of anthocyanin stability

The stability data of anthocyanin content were subjected to regression analysis using the following first-order model: $\ln(C_t/C_0) = kt$

Where, C_t and C_0 are the anthocyanin content at time t and t_0 , respectively, k is the reaction rate constant, and t is the storage time (days). Moreover, the half-life value $t_{1/2}$ of anthocyanin was calculated as $t_{1/2} = \ln 2/k$, D-value was also calculated as a kinetics parameter of anthocyanin degradation. D-value meant the time that the degradation of 90% anthocyanin would take and was calculated as $D = -1/k$ (Hernández-Herrero and Frutos, 2011).

3. Results and discussion

3.1 Effect of processing parameters on EE and EY for encapsulation anthocyanin

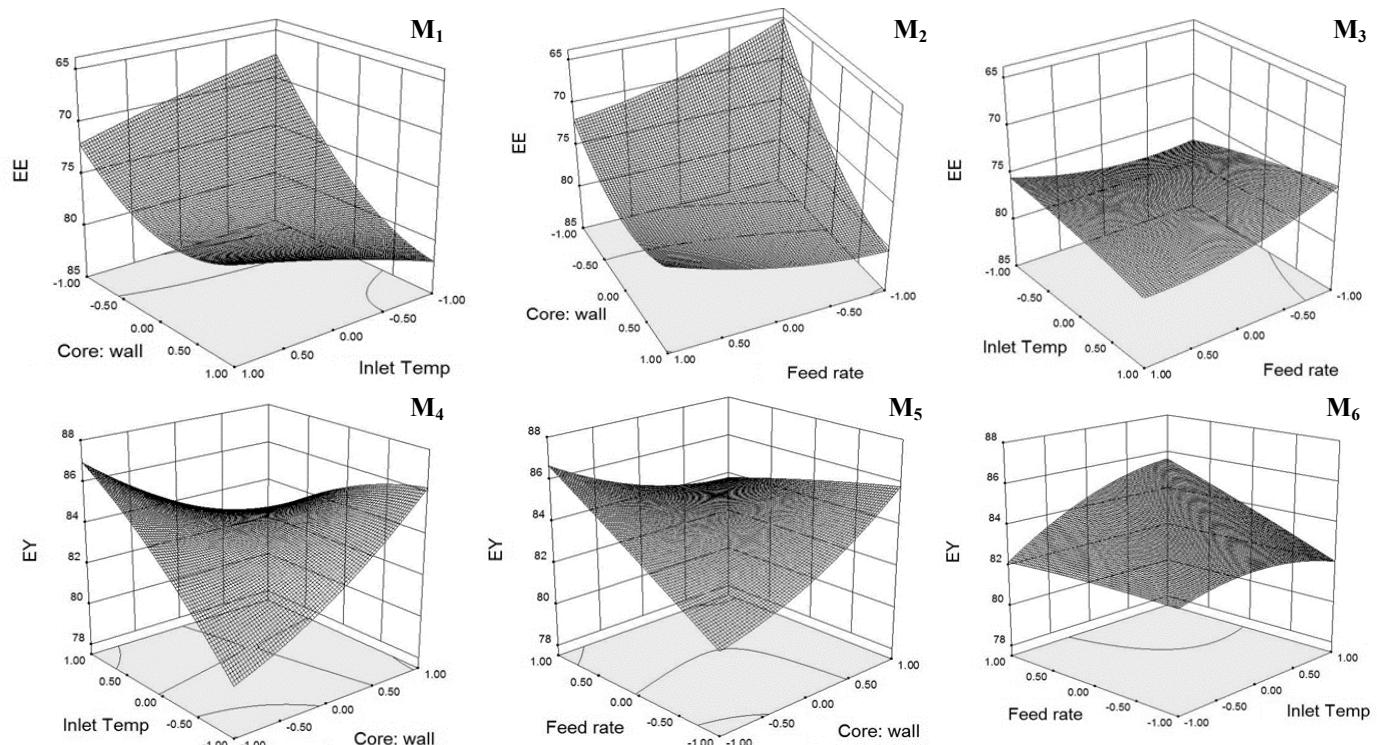
The optimization study for the encapsulation of anthocyanin was carried out by response surface methodology (RSM). The effect of the core to wall ratio, inlet temperature and feed rate on Encapsulation efficiency (EE) and Encapsulation yield was studied and obtained results are given in Table 2 whereas its surface plots are shown in Figure 1.

The ANOVA, i.e., analysis of variance was used to analyse the obtained results. The model F value of 8.21 and 13.16 for EE and EY means that the model is significant. Model P-value (Prob >F) is very low (0.0500) which suggests the model is significant. The coefficient approximation and the corresponding P values of EE and EY, among the test variables used in the study, A × B (Core to wall ratio × Inlet Temperature), A × C (Core to wall ratio × Feed rate) are the significant model terms. The corresponding second-order response model (Equations 2 and 3) of free and microencapsulated anthocyanins that was found after regression analysis was

Table 2. Influence of process parameters on EE and EY during encapsulation of anthocyanin

Run	M _{core} /M _{wall}	Inlet Temperature	Feed rate (mL/Mins)	EE (%)	EY (%)
1	1:1	140	2.0	65.9±0.18	75.4±0.07
2	1:1	140	2.5	66.1±0.99	77.5±0.99
3	1:1	140	3.0	68.9±0.32	78.2±0.57
4	1:1	150	2.0	66.4±0.58	86.8±0.28
5	1:1	150	2.5	68.7±0.14	88.1±0.78
6	1:1	150	3.0	69.3±0.73	90.2±0.99
7	1:1	160	2.0	65.8±0.28	79.2±0.71
8	1:1	160	2.5	68.9±0.20	82.5±1.06
9	1:1	160	3.0	79.2±0.78	90.3±0.49
10	1:2	140	2.0	67.9±0.21	76.8±0.42
11	1:2	140	2.5	69.8±0.42	78.9±0.28
12	1:2	140	3.0	71.5±0.35	80.2±0.92
13	1:2	150	2.0	81.0±0.42	81.3±0.28
14	1:2	150	2.5	81.9±0.49	84.7±0.64
15	1:2	150	3.0	82.4±0.53	86.1±0.57
16	1:2	160	2.0	75.8±0.21	85.2±0.92
17	1:2	160	2.5	78.9±0.14	87.5±0.85
18	1:2	160	3.0	79.2±0.99	90.3±0.64
19	1:3	140	2.0	83.6±0.71	90.6±0.78
20	1:3	140	2.5	82.4±0.85	90.1±0.37
21	1:3	140	3.0	83.2±0.42	89.8±0.92
22	1:3	150	2.0	83.4±1.06	86.1±0.71
23	1:3	150	2.5	82.9±0.49	84.7±0.26
24	1:3	150	3.0	70.2±0.99	79.9±0.21
25	1:3	160	2.0	72.3±0.71	80.6±0.35
26	1:3	160	2.5	77.9±0.57	81.3±0.92
27	1:3	160	3.0	67.9±0.71	77.8±0.15

Results are mean±SD of 3 determinations

Figure 1. Effect of different parameters on EE (M₁-M₃) and EY (M₄-M₆) for anthocyanin encapsulation using maltodextrin as a carrier material

$$\text{EE}(\%) = 4.444 + 0.011 \times A - 0.056 \times B + 0.024 \times C + 0.014 \times AB - 0.011 \times AC + 0.004 \times BC + 0.007 \times A^2 - 0.031 \times B^2 - 0.001 \times C^2 \quad (2)$$

$$R^2 = 0.81$$

$$\text{EY}(\%) = 4.369 + 0.064 \times A + 0.057 \times B + 0.027 \times C + 0.028 \times AB - 0.014 \times AC + 0.004 \times BC - 0.041 \times A^2 - 0.051 \times B^2 - 0.003 \times C^2 \quad (3)$$

$$R^2 = 0.87$$

The result of the EE ranges from $65.8 \pm 0.28\%$ to $83.6 \pm 0.71\%$ and EY ranges from $75.4 \pm 0.07\%$ to $90.6 \pm 0.78\%$ with different process parameters. After optimization of a mathematical model of the three independent variables for microencapsulation, it can be found that core to wall ratio (1:3), inlet temperature (140°C), and feed rate (2 mL/mins) gave the maximum EE (82.2 ± 0.95) and EY (92.6 ± 0.86).

The maximum EY recorded for the core to wall ratio was 1:3 ($90.6 \pm 0.78\%$). EE was lowest in the case of the core to wall ratio was 1:1 ratio (i.e. $65.8 \pm 0.28\%$) than ratio was 1:2 and 1:3. The reason may be associated with the instability of the emulsion when the core to wall ratio was low.

Both EY and EE increased with an increase in temperature from 140°C to 160°C . The high inlet temperature could have broken the balance between the rate of water evaporation and film-formation; this resulted in the breaking of the wall system of microcapsules, and thus a low EE. It is known that anthocyanin could easily decompose when exposed directly to heat. The low EY could be due to the breaking of the microcapsules. Ersus and Yurdagel (2007) reported more anthocyanin losses in spray drying of black carrot anthocyanin when inlet temperatures are higher ($>160\text{--}180^\circ\text{C}$). Cai and Corke (2000) found that a higher drying temperature (>180) is unsuitable for spray drying of betacyanin.

EE and EY were significantly influenced by the feed rate. When the feed rate was low (2mL/mins) higher EE and EY were observed at 1:3 core to wall ratio and 160°C Inlet temperature, whereas increased feed rate decreases the EE and EY. It is a well-known fact that lowers the feed rate is higher was the moisture of encapsulated materials. This may lead to higher EE and EY.

3.2 Morphological properties of microcapsules

Encapsulated microcapsules are characterized by their spherical shape and smooth surface. Particles of microcapsules prepared using maltodextrin as carrier material was observed as a spherical shape with a smooth surface without any groove (Figure 2B) as compared to anthocyanin without encapsulation (Figure 2A). Similar findings related to the shape and surface of microencapsulated were reported by Varavinit *et al.*

(2001). The maltodextrin was used as wall material in the encapsulation of anthocyanin. These ruptures seem to be due to the low glass transition temperature of maltodextrin as encapsulating material and the lack of emulsification ability of this encapsulating material, which results in a considerable reduction in encapsulation efficiency and so significant release of core material throughout drying (Varavinit *et al.*, 2001).

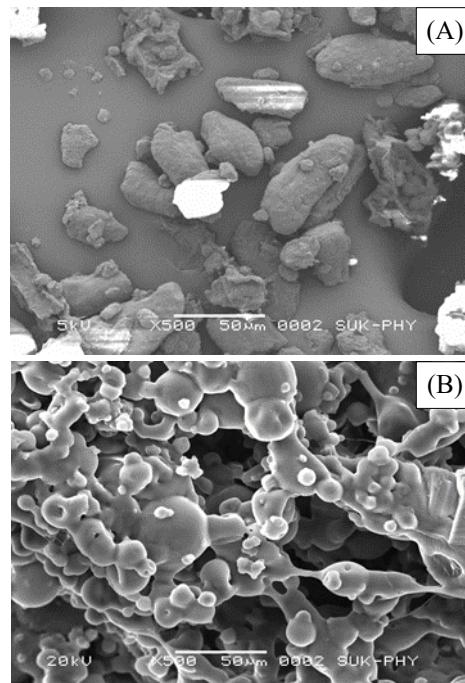


Figure 2. SEM image of anthocyanin (A) without encapsulation and (B) encapsulated with maltodextrin

3.3 Stability of anthocyanin during different storage conditions

Anthocyanin with and without encapsulation was stored at different storage conditions. The effect of storage conditions on retention was presented in Figure 3, whereas its kinetic parameters for the stability of anthocyanin are shown in Table 3. The results indicated that the anthocyanin without encapsulation was degraded with the increase in storage period in all storage conditions. The highest anthocyanin retention was recorded in dark conditions storage (44.29%) followed by refrigerated condition (43.91%), whereas the highest degradation occurred in the sample stored in the presence of sunlight (80.35%). This data was well exhibited with regression coefficients (R^2) i.e., 0.964, 0.963 and 0.825 when samples were stored in dark, refrigerated conditions and in the presence of sunlight, respectively. The highest retention was in a dark condition which was also represented by the highest half-life (6.57 weeks) and D value (9.48 weeks).

In the case of encapsulated samples, more than 90% retention was recorded in all storage conditions. The highest anthocyanin retention i.e., 96.25% was in

Table 3. Kinetic parameters for the degradation of anthocyanin during storage

Storage condition	Variation Kinetics		R^2		$t_{1/2}$ (Week)		D Value (Week)	
	WE	E	WE	E	WE	E	WE	E
Open	$y = -0.659x + 73.47$	$y = -0.116x + 100.7$	0.811	0.948	5.44	74.03	7.85	106.81
Airtight	$y = -0.815x + 98.17$	$y = -0.053x + 100.4$	0.961	0.972	5.94	180.11	7.93	259.84
Sunlight	$y = -0.639x + 74.75$	$y = -0.118x + 100.4$	0.825	0.932	5.54	64.02	7.99	92.36
Dark	$y = -0.721x + 101.1$	$y = -0.065x + 100.5$	0.964	0.965	6.57	131.35	9.48	189.50
Room Temperature	$y = -0.647x + 83.18$	$y = -0.082x + 99.93$	0.899	0.962	5.76	142.44	8.30	205.50
Refrigerated	$y = -0.692x + 95.05$	$y = -0.040x + 100.2$	0.963	0.982	6.30	245.77	9.09	354.57

WE = Without Encapsulation, E = Encapsulation

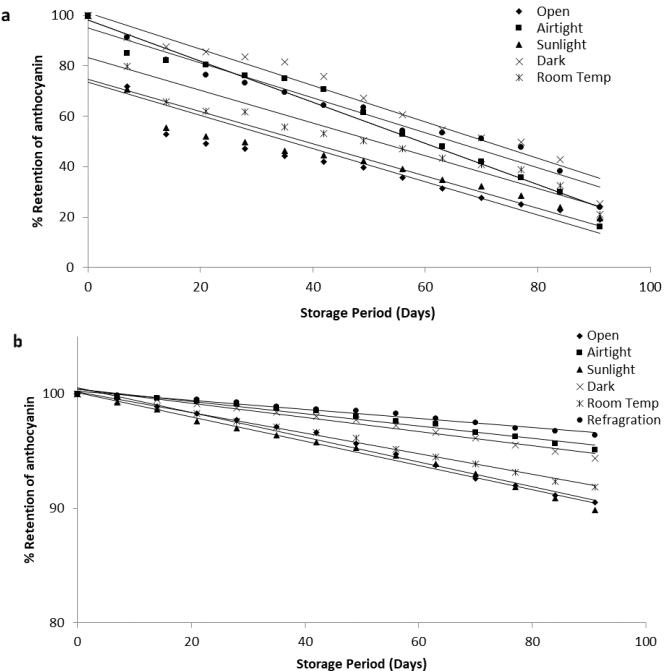


Figure 3. Effect of different storage conditions on % retention of anthocyanin (a) without encapsulation and (b) encapsulated

refrigerated storage and lowest in presence of sunlight i.e., 89.87% and this data was well supported by R^2 of 0.982 and 0.932, respectively. Whereas refrigerated storage shows 245.77 weeks half-life and 354.57 weeks D value.

Different factors affecting on colour and stability of anthocyanin include pH, temperature, structure and concentration, oxygen, light, metallic ions, enzymes, ascorbic acid, sugar, proteins and sulphur dioxide (Mazza and Miniati, 1993; Rodriguez-Saona *et al.*, 1999; Nayak and Rastogi, 2011). Microencapsulation by spray drying is an economical method for the preservation of natural colourants by enclosing the ingredient in a coating material (Cai and Corke, 2000).

3.3.1 Oxidative stability

The anthocyanin samples with and without encapsulations were stored in open and airtight conditions to study the oxygen stability (Table 3). The 90 days storage of encapsulated samples at airtight conditions showed 94.37% retention and the sample without encapsulation showed only 19.10% retention

which is well supported by R^2 values of 0.972 and 0.811, respectively. The results showed that better retention was recorded in airtight storage conditions of both anthocyanin samples with and without encapsulation. It was also observed that the encapsulated sample showed more stability than without encapsulation anthocyanin, which concludes that microencapsulation gives better stability to anthocyanin. The encapsulation improves the oxidative stability of edible oils (Sharma *et al.*, 2019). The encapsulation gives better stability of anthocyanin, which generally degradation due pH, temperature, the presence of light, metal ions, oxygen enzymes, ascorbic acid, sugars and their degradation products, proteins and sulphur dioxide (Fernandez-Lopez, 2013). However Antonio-Gomez *et al.* (2021) reported the high oxidative stability of microencapsulated anthocyanins of chagalapoli fruit.

3.3.2 Light stability

The anthocyanin samples with and without encapsulations were stored in the presence of sunlight and in dark conditions to study the light stability (Table 3). It is a well-known fact that sunlight significantly affects the stability of anthocyanin. This is confirmed by a lower R^2 value (0.825) of anthocyanin samples without encapsulation and stored in presence of light, while the encapsulated sample stored in presence of sunlight had a better R^2 value (0.932). However, encapsulated samples stored in dark conditions showed the highest R^2 value i.e., 0.965 and showed 95.12% retention of anthocyanin as compared to samples without encapsulation and stored in the presence of sunlight showed only 19.65% retention. A higher retention rate of microencapsulated anthocyanin than native anthocyanin was recorded in blueberry when stored in the presence of sunlight (Xu *et al.* 2019). Also, Gomes *et al.* (2021) reported that microencapsulation of the strawberry extracts with inulin obtained by spray and freeze-drying improved the stability of anthocyanins in sunlight.

3.3.3 Temperature stability

The anthocyanin samples with and without encapsulations were stored in ambient and refrigerated

conditions to study the temperature stability (Table 3). In refrigerated conditions, better stability of anthocyanin in both samples i.e., with and without encapsulation with R^2 values of 0.982 and 0.963, respectively were recorded than that of ambient condition. In both ambient and refrigerated conditions, the stability of the encapsulated sample was higher than in the ambient condition. After microencapsulation, Anthocyanin was found to have greater heat resistance than blueberry anthocyanin at different temperatures (Xu *et al.*, 2019).

Sample without encapsulation showed lower R^2 values when stored in open (0.811), in presence of sunlight (0.825) and stored at room temperature (0.899), which indicates degradation of anthocyanin was more in these conditions. Contrary, R^2 values of encapsulated anthocyanin at the same conditions i.e., stored in open, in presence of sunlight and at room temperature were 0.948, 0.932 and 0.962, respectively. This indicated that the encapsulation gave better protection against oxidation and degradation due to the sunlight and temperature.

4. Conclusion

The encapsulation study reported that the higher encapsulation efficiency (EE) and encapsulation yield (EY) were obtained when the core to wall ratio was 1:3, the Inlet temperature of 140°C and the feed rate of 2mL/ mins. The storage study showed the highest retention of anthocyanin in encapsulated samples stored under refrigerated conditions with an R^2 value of 0.982, a half-life of 245.77 weeks and D value of 354.57 weeks. The outcome of these studies will be beneficial to the Kokum processor to earn additional benefits from the rind of kokum. This will give an idea for the encapsulation of anthocyanin and also help to improve the storage stability of anthocyanin.

Conflict of interest

The authors declare no conflict of interest.

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