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Encapsulation of Anthocyanin from Jamun Pomace Extract and its Storage Stability

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ABSTRACT: Jamun (*Syzygium cumini*), commonly known as Indian blackberry contains many natural bio-active compounds e.g. vitamin C, anthocyanin, tannins, ellagitannins, ellagic acid, galloyl-galactoside and gallic acid. In the present study, extraction of anthocyanins from jamun pomace powder were carried out with solid to solution ratio of 1:15 at temperature 45°C for 90min using two types of solvents *i.e.* aqueous and ethanol (%). Purification of anthocyanin in the extracted juice was done using chitosan. The extracts were encapsulated using different combination of sodium alginate as coating material (1.0, 2.0, 3.0 % W/V) and calcium chloride as hardening material (1, 5, 10% W/V) following liquid suspension method and evaluated in terms of encapsulation efficiency, moisture content and bulk density. The stability of anthocyanin in encapsulated material was studied during storage in different conditions of dark and light, temperature: 30±2°C and 4±2°C, PET and glass containers. The ethanolic extracts yield more amount of anthocyanin content (290.01±02 mg/100g), total phenolic content (52.8±0.26 mg GAE/100ml extract), antioxidant activity (54.56±0.54). The FT-IR analysis of the extract showed, ethanol extract retained maximum number of functional groups as compared to aqueous extract. Encapsulation of bio-active component was found best in 3.0% sodium alginate and 5.0% calcium chloride with maximum encapsulation efficiency (75.93±0.61%) and formed uniformly. The retainance of anthocyanin was observed to be higher in glass, dark condition at lower temperature (4±2°C) than light and ambient temperature condition (30±2°C).

Keywords: Jamun pomace, solvent extraction, encapsulation, anthocyanins, stability to light, temperature etc.

INTRODUCTION

Jamun (*Syzygium cuminii*) is an important indigenous minor fruit grown in India. Worldwide, total production of jamun is 13.5 million tonnes, out of which India contributes about 15.4%. India ranks second in production of jamun in the world. Jamun tree grows throughout India in both tropical and subtropical regions from the North's lower Himalayas to the southernmost point of Tamil Nadu. In India, Maharashtra is the major jamun producer followed by Uttar Pradesh, Tamil Nadu, Gujarat, Assam, Odisha. Jamun fruits are used to make a variety of culinary items. During the preparation of jamun juice, a huge amount of pomace is obtained, which is low valued by product and not being used for anything other than livestock feed or landfilling. The great majority of food industry by-products are disposed in open areas (Singh *et al*., 2006). If not handled appropriately, fruit pomace can cause a variety of environmental issues, including surface and ground-water pollution, depletion of oxygen throughout the soil as well as the infiltration of surface, soil, and groundwater (Bordiga *et al.,* 2019). Organic waste management is a critical concern for the food sector. Therefore, the recycling of agricultural and food waste is important for minimizing environment pollution.

Jamun fruits are a good source of iron, phosphorus, calcium, mineral, protein and carbohydrates. The jamun fruit is mainly used for its high vitamin C, anthocyanin content and important minerals. It has received far more recognition in traditional medicine and in modern pharmaceutical trade besides its food values. Many experts have discovered that pomace is an excellent source of fibre, total phenols, antioxidants, anthocyanin content and other nutrients which can lower free radicals, provide numerous health advantages, and aid in the prevention of different diseases (Vital *et al*., 2017).

A large number of studies are now being conducted on the extraction of bioactive components from fruit pomace, which may be exploited to increase the nutritional value of food compositions due to their strong antioxidant potential. The study of bio-active compounds from plant material mostly depends on the proper extraction method. Extraction is the first method of any medicinal plant study. The extraction of bio-active component depends primarily on extraction method, input parameters and extracts nature of the

plant part (Raza *et al.*, 2015; Balyan, 2017). The factors depending on the extraction process are matrix properties of the plant parts, solvent, temperature pressure and time.

Encapsulation process is to entrap active agents within a carrier material and it is a useful tool to improve delivery of bioactive molecules and living cells into foods. It is based on packaging of bioactive compounds in mili, micro- or nano-scaled particles which isolate them and control their release upon applying specific conditions. The unique advantage of encapsulation lies in that the core material is completely coated and isolated from external environment. The coating or shell of sealed capsules needs to be semi permeable, thin but strong to withstand the environmental conditions and it can be designed to release the bioactive compound in a targeted specific area of the human body.

Encapsulation techniques can be archived via chemical, mechanical or physical processes. Sontosa *et al.* (2017) microencapsulated black-berry pomace extract with maltodextrin using the spray dryer technique and studied the temperature and light stability. Boonchu and Utama-ang (2015) carried out microencapsulation process of red grape extract and optimized conditions for maximizing its polyphenol compounds. Rezende *et al.* (2018) used both spray drying and freeze drying to encapsulate the bioactive compounds from acerola pulp. Yamashita *et al.* (2017) carried out the experiment to microencapsulate blackberries that are rich in anthocyanin, with the help of freeze drying. Saponjac *et al.* (2017) extracted sour cherry pomace, stabilized in whey protein (WP) and soy protein (SP) by encapsulation. Soy proteins exhibited higher encapsulation efficiency (94.90%). At the end of storage period of 6 weeks the retention of polyphenols in SP and WP was similar (67.33 and 69.30%, respectively), while the content of anthocyanins has increased in SP (for 47.97%) and decreased in WP (for 1.45%). Shwetha *et al.* (2016) studied the stability of natural colorant from jamun fruit by carrying out the microencapsulation process with spray drying technique by using maltodextrin and gum Arabic and varying the core to wall material ratio.

The jamun by-products i.e., the jamun pomace and seeds are rich in many important polyphenols as bioactive components. The jamun pomace is still a rich source of anthocyanins which are unexplored and thrown as simply waste from food and pharma industries. Very few literatures have been reported about the utilization of the jamun pomace anthocyanin extraction and encapsulation process (Raza *et al.*, 2015). The present work has been undertaken to encapsulate the jamun pomace extract to preserve anthocyanin as its bioactive compound with the specific objectives of standardizing the extraction and encapsulation process and study the stability of encapsulated materials during storage.

MATERIALS AND METHODS

Core material. Fresh jamun fruits (Local var.) were procured from the local market of Bhubaneswar, Odisha. After receiving fruits were thoroughly washed with tap water followed with chlorinated water to remove adhered impurities. Then fruits were fed into pulper machine to separate the pulp from fruits. The seed and pomace were collected and dried in the cabinet tray dryer at 50° for 6-8 hours. Dried jamun pomace was separated from the seeds by gently rubbing the seeds with hands. After separating the pomace from the seed, the pomace was powdered using a grinder and then sieved with 100 µm size. Then fine powder of pomace was collected and kept in LDPE pouches for further studies.

Coating and binding material. Coating material or wall materials are used for stabilization of core material. This can be flexible, brittle, hard or thin. The coating material can be polymer, waxes, resins, proteins or polysaccharides. In this study, two chemicals e.g. Sodium alginate as coating material at 1, 2, 3 % (W/V) and Calcium chloride as hardening material at 1, 5, 10 % (W/V) were used for encapsulation. All the chemicals used in the study were of analytical grade purchased from Sigma Aldrich Co., USA.

Extraction of Jamun Pomace Powder. The extraction of phenolic substances from dried pomace was performed using both 50 % ethanol and aqueous medium (Shweta *et al.*, 2016). 10g dried jamun pomace powder was mixed with 150ml of the solvents (aqueous and 50% ethanol) in a conical flask. Then the conical flask was covered with cork and condenser and then extraction was carried out using a water bath. The temperature of the water bath was set at 45^0C and heated for 90 mins. Then evaporation process was carried out to concentrate the extracts and evaporate the solvents using a roto evaporator.

Separation and Purification of Anthocyanins. After extraction, separation process was carried out for separating the solutes from the extract. For separation the extracts were taken in the centrifuge tubes and centrifuged at 2000 rpm for 15mins. Then the extract was filtered using filter paper and supernatant was collected for further analysis. Purification of extracted pomace juice was done following the method described by Jing *et al.* (2012) with slight modifications. For this, 1.7% (w/v) chitosan was added to 50ml (pH 4.3) of pomace extract. The mixture was then adjusted to pH 3.91 with 6 mol/l hydrochloric acid. The mixture was sealed and held for 3 hours in a dark chamber at room temperature. Then the supernatant was collected for further analysis.

Extraction yield. For determination of extraction yield the extracts were kept in the hot air oven at 70°C for 24 hours. Then the weight of dried extract was taken out. The extraction yield was calculated using the following formula (Eqn. 1) (Balyan, 2017).

$$\text{Extraction yield (\%)} = \frac{\text{weight of dry extract}}{\text{weight of powder taken for extraction}} \times 100 \qquad (1)$$

Anthocyanin Content. Anthocyanin content for both aqueous and ethanolic extract and fresh jamun pomace powder were carried out (Raza *et al.*, 2015). The aqueous and ethanolic extract of samples were first volume made up to 200ml. For this, 50ml aliquot of

extract was taken, added with 0.1M HCL alcoholic and kept overnight at 4°C. Then it was filtered (using Whatman filtration paper no. 41) and aliquot was taken for measurement of absorbance using UV-spectrophotometer at 535nm wavelength. Anthocyanin content of each sample was found at three replications using the following formulae (Eqn. 2).

$$\text{Anthocyanin content} = \frac{R \times \text{volume made up} \times 100}{\text{ml of extract taken}} \quad (2)$$

Where, R= absorbance reading of extract in the spectrophotometer at 535nm wavelength

Total phenolic content. The concentration of phenolic compound was measured by taking the gallic acid as standard. The sample of 0.5ml was equalized with 7.5ml of distilled water. And 1ml of 50% Folin-ciocalteu reagent was added to the mixture. After 5mins, 1ml of 0.02g/ml Na_2CO_3, was added and kept at room temperature. The absorbance was measured using spectrophotometer at 765nm wavelength (Chandrasekhar and Raghavarao, 2014).

$$\text{Total phenolic content (mg GAE/100g)} = \frac{\text{Concentration of sample after absorbance reading}}{\text{wt. of sample taken} \times 10 \times 0.5} \quad (3)$$

Antioxidant activity. The antioxidant activity of the extract was measured with the DPPH method with slight modifications. A solution of DPPH was freshly prepared by dissolving 4ml DPPH in 2 mL methanol. The extract 2 mL and DPPH solution 4 mL was mixed together in a test tube. The test tube was then in the dark for 20 minutes at room temperature. Pure methanol was taken as blank. The decrease in absorbance was measured at 515 nm using a UV-VIS spectrophotometer (Do *et al.,* 2014). The percentage inhibition of radicals was calculated using the following formula.

$$\% \text{ DPPH} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (4)$$

Colour measurement

Colour was measured using CR-20 (Konica, Milonta, INC, Japan). Colorimeter was calibrated the control sample coordinates, where 'L' represents the lightness of colour (0=black, 100=white), -a/+a represents greenness redness and –b/+b represents blueness or yellowness. For each sample 3 colour values were taken and average L, a*, b* was determined and total colour change was calculated using the following formula (Eqn. 5).

$$(\Delta E) = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (5)$$

Where,

ΔE= total change in colour

ΔL= L-L*

Δa= a-a*

Δb= b-b*

FT-IR analysis (Fourier-transmission infrared radiation). Fourier-transform infrared spectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. It represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. As every material has unique combination of atoms, no two compounds produce the same infrared spectrum. Therefore, infrared spectroscopy result in a positive identification of every different kind of material. The FT-IR analysis of jamun pomace extract was performed and the spectrums were obtained using instrument PerkinElmer Spectrum Version 10.4.3 using software NIOS2 Main.

Encapsulation of Jamun Pomace Extract. For encapsulation of jamun pomace extract nine different combination of coating (sodium alginate) and hardening material (Calcium chloride) were taken *i.e.* Sodium alginate solution: 1, 2, 3% w/v and calcium chloride solution: 1, 5, 10% w/v. These nine combinations of checked for their encapsulation efficiency, moisture content and bulk density. Extract of jamun pomace was mixed with sodium alginate solution in 1:1 ratio using a magnetic stirrer for 15 mins to homogenise the solution. The prepared mixture was carefully dropped into the calcium chloride solution by the use of a dropper. Then the capsules were filtered using filter paper and was washed with water to get rid of any extra chemicals present on the surface. After preparation of the capsules, these were dried in a laboratory freeze dryer (MAC, MSW-137) to get rid of the excess moisture removed from it and then stored in glass and PET bottles of 200 g size.

Bulk density. The bulk density (g/ml) of the sample was determined following the method adopted by Muzaffar and Kumar (2016). For this determination, a known quantity of powder sample was freely poured into a 10ml graduated cylinder (readable at 0.1ml) and the volume occupied was noted and then used to calculate the bulk density (weight/volume) using Eqn. 6.

$$\text{Bulk density} = \frac{\text{Mass of capsule}}{\text{Volume of capsules}} \quad (6)$$

Moisture content

Moisture content of the capsules was determined by the method AOAC (2002), standard procedure following hot air oven method at 70°C for 16-18 hours.

Encapsulation efficiency

After encapsulation process the anthocyanin content that remained in the capsule and the anthocyanin content of the extract was found out. Then the encapsulation efficiency was found out as the anthocyanin remained in the capsule divided by the anthocyanin content in the extract multiplied by 100. The formula to find the encapsulation efficiency is given below (Eqn. 7).

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of anthocyanin in capsules}}{\text{Amount of anthocyanin in the extract}} \times 100 \quad (7)$$

Stability study of encapsulated materials during storage

The freeze-dried capsules were studies for their stability in different storage conditions. Equal amount of capsules were taken and kept in two types of storage containers *i.e.* polyethylene terephthalate (PET) and glass bottles of 200 g size. The stability of capsules was studied between two conditions that are light, dark and at 4±2°C, 30±2°C. For storage in light condition a setup was made with a cardboard box where a LED light of

14W was fixed. For dark condition, a cardboard box was taken and was sealed to stop any light rays from entering. For 4±2°C, the capsules were kept in the refrigerator. And for 30±2°C, it was kept in ambient condition. Both the storage containers were kept in all the conditions for the stability study.

Statistical analysis

Statistical analysis of data was carried out to establish the difference among the treatments among different parameters studied *i.e.* Encapsulation efficiency, moisture content and bulk density. Using MINITAB statistical package, one-way analysis of variance (ANOVA) was conducted and the significance of differences among treatment means was determined with CD values at a level of significance of $p < 0.05$.

RESULTS AND DISCUSSIONS

Physico-chemical properties of jamun fruit, pomace and seed

Jamun fruits were cylindrical in shape of length 19.82±0.63 mm, diameter 18.42±0.51 mm with sphericity 0.81, bulk density 478.0±2.3 kg/m^3. Moisture content of fresh jamun fruit, and seed were 80.12 ±0.58, 50.58±0.39% respectively. Percent of pomace and seed in the fruit was 10.36±0.15 and 20.39±0.27 % respectively. Anthocyanin content in jamun pomace was 527.26±2.84 mg/100g. The pH and TSS of jamun juice were 3.18± 0.02 and 14^{0}Brix respectively.

Effect of extraction solvents on different quality parameters of jamun pomace extracts

Extraction yield. The observations for extraction yield and different quality parameters of jamun pomace extract in both types of extraction solvents are shown in Table 1. Under the same extraction time and temperature, solvent and composition of sample, the ethanolic pomace extract was found to be more (16.8±0.34%) and less extraction yield was found in aqueous pomace extract *i.e* 13.6±0.35%. This is in accordance with the previous study (Do *et al.,* 2014; Chauhan and Varshneya, (2012) and it has been reported that the extraction yield increases with increase in polarity of the solvent used for extraction. Use of ethanol may facilitate the extraction of chemicals that are soluble in organic solvents.

Availability of Anthocyanin content and total polyphenols. The amount of bioactive components extracted varied with the type of solvent (aqueous or ethanolic) used for extraction. The ethanolic (50% v/v) extract showed higher amount of anthocyanin content (290.01±02mg/100g), total polyphenols (52.8±0.26mg GAE/100ml extract), antioxidant activity (54.56±0.54) as compared to the aqueous solvent i.e. anthocyanin content (152.6±0.9mg/100g), total polyphenols (50.76±0.39mg GAE/100ml extract) and antioxidant activity (48.31±0.16).This has also been established by previous study that alcoholic solvents like methanol and ethanol are more suitable than water for extracting components of medicinal plants (Chandrasekhar and Raghavarao, 2014; Balyan and Sarkar, 2017). But as methanol has many toxic effects, ethanol was preferred for extraction.

Colour of extracts. The L, a, b values of the extracts are shown in Table 1. The higher value of L was found in ethanolic pomace extract (13.23±0.98) which shows that lightness of this extract is higher as compared to aqueous extract (7.86±0.52). The higher value of 'a' was seen in aqueous pomace extract (28.63±0.37), which depicts that redness in aqueous extract was greater compared to that of ethanolic extract (9.33±0.40).

Table 1: Quality analysis of pomace extract in different solvents.

Type of solvent extraction	Extraction yield (%)	Total polyphenols (mg GAE/100ml extract)	Anthocyanin content (mg/100g)/ Tannin content mg tannic acid/100g	Antioxidant activity (%DPPH)	L	a	b
Aqueous Extract	13.6±0.35	50.76±0.39	152.6±0.9	48.31±0.16	7.86±0.52	28.63±0.37	12.06±0.01
Ethanolic Extract	16.8±0.34	52.8±0.26	290.01±0.2	54.56±0.54	13.23±0.98	9.33±0.40	3.833±0.04

Values are mean of triplicate measurements ± standard deviation (SD)

Functional group analysis of pomace extract through FT-IR analysis. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FT-IR spectrum of ethanolic jamun pomace extract was shown in Fig. 1. The absorption bands and the wave number (cm^{-1}) of dominant peaks obtained from absorption spectrum and its functional groups were presented in Table 2. The result showed that by extraction with 50% ethanol maximum number of functional groups were recovered as compared to aqueous extract of pomace. Table 2 shows the comparison of the FT-IR Absorption peak and functional groups of jamun pomace with standard Glibenclamide. Comparing the functional groups of Glibenclamide, the above-mentioned wave numbers and functional groups match the corresponding wave numbers and functional groups of the extracted products. As most of the functional groups matched with the Glibenclamide standard which is generally recommended as a standard drug for diabetic treatments.

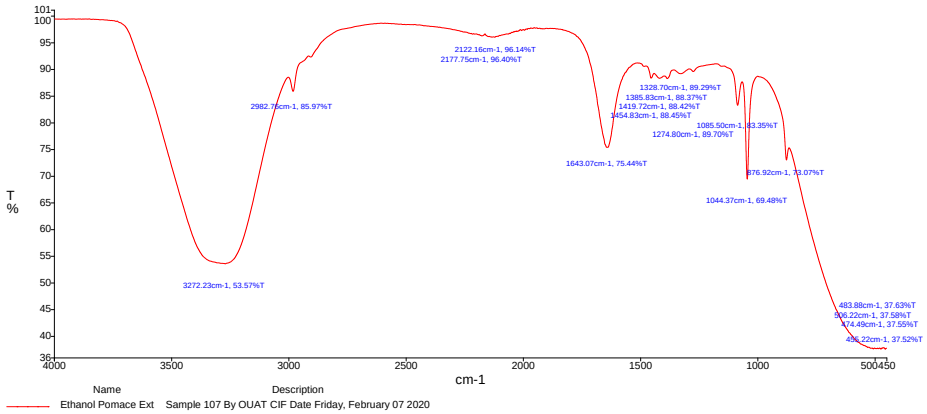

Fig. 1. FT-IR spectra of ethanolic jamun pomace extract.

Table 2: Comparison of the FT-IR Absorption peak and functional groups of jamun pomace with standard Glibenclamide.

Treatment	Wave No. of absorption peak matches with standard	Functional group
Ethanolic pomace extract	3272.23, 1643.07,1419.71, 1385.83,506.22	Alcohol, Alkene, Sulfate, Alkane, Halo compound
Aqueous pomace extract	3272.08, 2176.13, 2138.85, 2021	Alcohol, carbon dioxide, azide

Table 3: Encapsulation efficiency, moisture content, bulk density of jamun pomace and seed capsules for different combination of coating and hardening material.

Type of encapsulation materials	Encapsulation efficiency (%)	M.C.	Bulk density	Forming of capsules
1% Sod. Al. + 1% $CaCl_2$	20.53±0.36^a	1.754±0.064^a	0.340±0.034^a	Not formed
1% Sod. Al. + 5 % $CaCl_2$	21.91±0.31^a	1.891±0.043^a	0.342±0.062^a	Completely disrupted
1% Sod. Al. + 10% $CaCl_2$	20.45±0.34^a	1.923±0.018^a	0.354±0.026^a	Almost Disrupted
2% Sod. Al. + 1% $CaCl_2$	50.56±0.13^b	2.396±0.019^b	0.315±0.065^a	Partially Disrupted
2% Sod. Al. + 5% $CaCl_2$	55.32±0.42^d	2.510±0.037^b	0.322±0.038^a	Partially Disrupted
2% Sod. Al. + 10% $CaCl_2$	53.29±0.47^c	2.462±0.047^b	0.324±0.027^a	Formed uniformly
3% Sod. Al. + 1% $CaCl_2$	71.12±0.37^e	3.401±0.035^c	0.231±0.026^b	Formed uniformly
3% Sod. Al. + 5% $CaCl_2$	75.93±0.61^g	3.381±0.061^c	0.243±0.035^b	Formed uniformly and Stable/ Intact
3% Sod. Al. + 10% $CaCl_2$	73.57±0.34^f	3.492±0.031^c	0.247±0.017^b	Formed uniformly
CD (at 5 % level of significance)	1.687	0.25	0.055	---

Values are mean of triplicate measurements ± standard deviation (SD). Means with different superscripts in the same column are significantly different (P < 0.01)

Effect of coating and hardening material on encapsulation of jamun pomace and seed extract. Encapsulation of jamun pomace and seed ethanolic extract was done with different combinations of coating material as Sodium alginate (1, 2, 3 %, W/V) and hardening material as Calcium chloride (1, 5, 10 %, W/V). The effect of these materials on forming of capsules, encapsulation efficiency (EE), moisture content and bulk density are shown in Table 3. The capsules formed by 1% sodium alginate showed very low stability and minimum results in terms of encapsulation efficiency, moisture content and bulk density. This may be because less proportion of sodium alginate could not bind and encapsulate the extracts properly. When the proportion of sodium alginate is increased from 1 to 3 % the formation of capsules was better, but the encapsulation efficiency (EE) was lower (50.56±0.13 to 53.29±0.4753) as compared to 3 % sodium alginate *i.e.* 71.12±0.37-73.57±0.34 %. The variation of proportion of $CaCl_2$ as coating agent has effect on binding properties of the capsules. When $CaCl_2$ proportion increased from 1 to 5 % there is increase in encapsulation efficiency as well as uniform formation of the capsules. At 3% Sodium Alginate when $CaCl_2$ proportion increased from 5 to 10 % the encapsulation efficiency decreases indicating that 5 % $CaCl_2$ was optimum for formation of uniform capsules. The combination of 3% sodium alginate and 5% calcium chloride gave the best results in terms of encapsulation efficiency (75.93±0.61), moisture content (3.381±0.061) and bulk density (0.243±0.035) and showed stability in the formation of capsules as compared to other combinations. These are also in accordance with other research results of Shwetha and Preetha, (2016); Boonchu and Utama-ang (2015); Santosa *et al.* (2017); Saponjac *et al.* (2017); Rezende

et al. (2018) for encapsulation of anthocyanin pigments, red grape pomace, blackberry pomace, sour cherry pomace and acerola pulp respectively.

The Analysis of Variance (ANOVA) showed that there was significant difference ($p<0.05$) in the values of encapsulation efficiency, moisture content and bulk density in different combinations. The important finding was that there is significant ($p<0.05$) difference in variation of EE, moisture content between different levels of sodium alginate solutions. However, between different $CaCl_2$ proportions the values are non-significant ($p<0.05$) for a constant sodium alginate concentration.

Storage stability of encapsulated jamun pomace extract during storage. Encapsulated jamun pomace extract capsules prepared at optimum binding and coating material (*i.e.* 3% sodium alginate and 5% calcium chloride) were stored at two types of storage conditions (light, dark), storage temperature (ambient: 30±2°C, 4±2°C) and in two types of packaging material (glass and PET bottle). The quality parameters of the capsules at the initial stage (0 day) and during storage after 30 days were determined and presented in Table 4.

Table 4: Effect of anthocyanin content, total phenolic content and change in colour of capsules after 30 days of storage.

Storage condition	Anthocyanin (mg/g of capsule)	Total phenolic content (mg GAE/g)	Colour			ΔE
			L	a	b	
0 day	2.20±0.04	0.45±0.031	18.6±0.40	5.5±0.037	-8.5±0.060	
Dark + glass	**1.98±0.05**	0.40±0.023	**17.2±0.42**	1.3±0.031	-0.9±0.051	47.32±0.3
Dark + PET	1.57±0.09	0.41±0.036	16.6±0.38	1.8±0.046	-1.5±0.052	48.12±0.2
Light+ glass	1.23±0.019	0.35±0.025	14.2±0.51	1.4±0.053	-0.8±0.043	49.22±0.38
Light + PET	1.26±0.013	0.32±0.016	14.6±0.34	1.5±0.043	-1.9±0.044	49.75±0.16
30°C+ PET	1.13±0.024	0.31±0.029	21.5±0.15	2.0±0.031	-1.5±0.033	45.10±0.095
30°C+ glass	1.19±0.019	0.32±0.035	24.1±0.16	1.7±0.039	-1.2±0.022	**43.42±0.09**
4°C + glass	**1.95±0.024**	**0.42±0.015**	**17.9±0.35**	1.9±0.026	**-2.0±0.011**	47.67±0.12
4°C + PET	1.87±0.035	0.40±0.034	17.2±0.39	1.6±0.028	-1.8±0.018	47.98±0.39

Change in anthocyanin content, total phenolic content of capsules. The capsules were analysed for its anthocyanin content, total phenolic content and colour at day 30 of storage which is given in Table 3. The initial anthocyanin content of the capsule was 2.20±0.04 mg/g of sample. The degradation of anthocyanin was observed to be higher at light and ambient condition. The lower temperature (4±2°C) showed lesser degradation in the anthocyanin content *i.e.* 11.4% with respect to initial and higher degradation of 45.91% was found at ambient temperature (30±2°C) storage. A previous study observed a direct proportional relationship between hydrolysis of encapsulated material and increased temperature, resulting in a lower stability of anthocyanin. The lower temperature studied reduced the degradation of anthocyanin in extracts of jamun throughout the storage (Sharma *et al.,* 2016). There was 10% loss in anthocyanin content of capsules in dark and glass condition whereas 44.1% loss in light and glass container. Similar results were also observed in blackberry pomace extract capsules which showed greater stability of anthocyanin stored in dark condition (Santosa *et al.,* 2017). The initial total phenolic content of the capsule was found to be 0.45±0.031 mg GAE/g of capsule. After 30 days of storage, capsules stored in all the storage condition showed nearly equal values of total phenolic contents. However, the maximum was obtained from 4°C and glass condition (0.44±0.029) followed by dark and PET (0.43±0.043) and dark and glass (0.42±0.013) container. The L value (lightness) of the fresh capsule was found to be 18.6±0.40. The lightness value of capsules was decreased to (17.9±0.58) in dark and glass, (17.6±0.62) in dark and PET (16.4±0.43) in light and glass and (16.8±0.39) in light and PET conditions, whereas increased in ambient and PET (19.5±0.34) and glass (21.35±0.51) containers after 30 days of storage. In refrigerated condition the value of L almost remained constant (18.35±0.31). The 'a' value of initial capsule samples was 5.5±0.037. The 'a' value decreased in all the storage conditions and ranged between 3.3±0.041 (light and glass) to 3.7±0.049 (30°C and PET). The value of 'b' initially was -8.5±0.060. The negative value of 'b' indicates blue colour of the capsules. The value of 'b' decreased with storage period; however, the change was not significant ($p<0.05$) among different storage conditions.

Change in Colour of capsules. The variation of L values of capsules increased from the initial value in case of glass container at ambient storage and decreased from the initial value in PET and light condition. However, in the glass container and dark condition the L value of samples remained nearly same (17.2±0.42) as that of the fresh samples (18.6±0.40). The 'a' value decreased in all the storage conditions and ranged from 1.3±0.031 to 2.0 and 0.031 in dark and glass, at 30°C and PET condition. The redness of capsules increased in all the storage conditions and were nearly equal in the range -0.8±0.043 to -2.0±0.011, which was more than that of the initial value (-8.5±0.06). The net change in colour (ΔE) values were almost same in all the storage conditions. However, maximum change in colour (49.75±0.16) was observed in case of light condition in PET bottles and minimum was observed in dark condition and glass bottles. Among all of the quality parameters of the jamun pomace capsules studied during storage, it was observed that maximum anthocyanin content 1.95±0.024 (mg/g)**,** total phenolic content 0.42±0.015 (mg GAE/g) and less change in characteristics L value of colour (17.9±0.35) was observed in refrigerated storage, glass container and in dark condition (4°C + glass + dark) of storage. Therefore, any packaging container of this type will be

suitable to store jamun pomace extract encapsulated materials.

CONCLUSIONS

The ethanolic (50% v/v) extract showed higher amount of anthocyanin content (290.01±02mg/100g), TPC (52.8±0.26mg GAE/100ml extract), antioxidant activity (54.56±0.54) in jamun pomace extract. Extraction yield of ethanolic extract for both pomace (16.8±0.34%) was found to be more as compared to the aqueous extract. The FT-IR analysis showed that ethanolic extract recovered more no of functional groups in comparison to the aqueous extract. 3% sodium alginate and 5% calcium chloride gave the best encapsulation efficiency (75.93±0.61), moisture content (3.381±0.061) and bulk density (0.243±0.035) as compared to other combinations. The maximum anthocyanin content (mg/g) 1.95±0.024, total phenolic content (mg GAE/g) 0.42±0.015 and less change in characteristics L value of colour (17.9±0.35) was observed in refrigerated storage, glass container and in dark condition (4°C + glass + dark) of storage. The net change in colour (ΔE) values in all the storage conditions were almost same in all the storage conditions. However, maximum change in colour (49.75±0.16) was observed in case of light condition in PET bottles and minimum was observed in dark and glass bottles.

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Conflicts of Interests. None.

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