Research Article

Microencapsulation of Anthocyanin Pigments of Grape Skin Using Maltodextrin and Gum Arabic by Spray Drying

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Abstract

The effects of maltodextrin (10 DE) and gum arabic as wall material on microencapsulation of anthocyanin extracts from Bangalore blue grape skin by spray drying were investigated. Solubility of the microcapsules was found to increase with the increase in the air inlet temperature at all concentrations of anthocyanin extracts. Encapsulation efficiency of the microcapsules ranged from 49.8 to 68.4 percent and the maximum efficiency (68.4%) was observed for the gum arabic coated extracts dried at 200°C. Total anthocyanin content of the microcapsules decreased with the increase in the anthocyanin concentration of the extracts. After 90 days of storage, 77.8% retention of anthocyanin content was observed in the gum arabic coated anthocyanin microcapsules spray dried at 200°C. SEM micrographs showed that anthocyanin microcapsules were spherical in shape with smooth outer surface and particle size ranging from 5-50 µm. Internal structure of the capsules revealed the presence of multi core matrix in which anthocyanin was embedded in the gum arabic and maltodextrin wall system.

Keywords: Anthocyanin, Grape skin, Microencapsulation, Gum arabic, Maltodextrin, Spray drying

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Introduction

Grapes, an excellent source of many nutrients and phytochemicals are highly pigmented with anthocyanin and owe their attractive red to purple coloration to the water soluble flavonoid group of polyphenols. Anthocyanin pigments mainly exist in the grape skins and are not quantitatively transferred to the wine or juice and considerable quantities of them are left in pomace which becomes an important source for the extraction of pigments [1]. Grape pomace have been used for the recovery of food ingredients, nutraceutical and functional food components such as anthocyanin, citric acid, hydrocolloids, grape seed oil, β-glucans, antioxidants, *etc*.[2]. The potential health benefits of anthocyanins enhance consumer interest in using it in food to replace artificial red or purple colorant [3].

A great number of literatures on anthocyanins [4-9] indicate that the anthocyanins are strong antioxidants and free radical scavengers. Epidemiological data associate anthocyanins with reduction of risk of various diseases such as visual and vascular diseases, obesity, tumors, neurological dysfunctions and some cancers [10-16].

The colour and stability of the grape skin pigment is affected by the factors like temperature, pH, oxygen, light, presence of co pigments, metallic ions, enzymes and different co-factors. [17-18]. Hence, there is a need for the protection of anthocyanin against environmental factors *viz*. oxygen, light and moisture, which contributes to its deterioration [19]. Microencapsulation is a method to preserve and enhance the stability of natural colorants by entrapping the ingredient in carrier materials that can release their contents at controlled rates under specific conditions.

The most commonly used microencapsulation technique in the food Industry is spray drying because of its continuous production and easiness of industrialization [20-21]. For microencapsulation of the food compounds gum arabic is a good choice as wall material because its properties of stable emulsions, film formation at the oil interface volatile retention and encapsulation efficiency [22-24]. Maltodextrin as a wall material for encapsulation is a good compromise between cost and effectiveness as it is available in different average molecular weights, has low viscosity at a high solid ratio and protects the encapsulated ingredients from oxidation [21, 25-26].

Reports on microencapsulation of grape skin anthocyanin in the scientific literature are scant. Microencapsulation of anthocyanin from Cranberry press cake, Concord grape juice filter trim and Rosella calcyces by spray drying has been reported. [27]. The objectives of this study were production of spray dried microcapsules from anthocyanin extracts of grape skin using maltodextrin and gum arabic as wall materials and to determine the effects of different spray drying temperatures on the encapsulation efficiency, anthocyanin content, properties of spray dried powders, their storage stability and the outer and inner structure of the encapsulated powders.

Materials and Methods

Grapes

Bangalore blue (*Vitis vinifera*) variety of grape was procured from local market at Coimbatore, Tamil Nadu, India and was used for extraction of anthocyanin. The infected and damaged grapes were removed from bunches and the good quality grapes were washed thoroughly in hot water before extraction. Grape skins were peeled manually from the fresh, cleaned grapes and were used for the extraction of anthocyanin.

Wall and core materials

Wall materials used for the study included maltodextrin (DE= 20) and food grade gum arabic both procured form M/s. Viveka agencies, Coimbatore, India. Core material used for the study was the concentrated anthocyanin pigment extract from Bangalore blue grapes stored in amber colored bottles.

Extraction of anthocyanin from grape skin

One hundred gram of grape skin (wet weight) was ground in to fine particles, in to which 250 ml of 3 per cent citric acid solution was added. The blend was continuously stirred for 20 min by keeping at a temperature of 28°C in the water bath. The solution was filtered in a Whatman #1 filter paper by using Buchner funnel with vacuum to obtain anthocyanin. The same procedure was repeated until extract become colourless [28]. The extracted anthocyanin was concentrated from 15° Brix to 20° and 25° Brix in a rotary flash evaporator (Superfit, India) at 50° C and 28 in. Hg vacuum.

Preparation of emulsions

Maltodextrin and Gum arabic were dissolved in hot distilled water to obtain 40 percent solution. Four hundred grams each of maltodextrin (DE=20) and gum arabic were dissolved separately in 500 ml of distilled water at 60° C by continuously stirring to form an aqueous solution and the volume was made up to 1000 ml by adding distilled water. The gum arabic solution was kept under ambient condition for 12 h to improve the film forming and emulsification properties. One thousand ml of anthocyanin concentrate **(**15, 20 and 25^{O} Brix**)** was added into one thousand ml of 40 percent aqueous solution of maltodextrin and gum arabic separately by stirring to form a coarse emulsion. The coarse emulsion was then emulsified in a high speed mixer for 10 min with the addition of two drops of Tween-80 to form homogeneous emulsion.

Emulsion viscosity

The viscosity of grape skin anthocyanin concentrate emulsions was determined using rotational viscometer (M/s Fungi lab, Spain). The L3 spindle recommended for high viscous foods was selected and the spindle was run at 20 rpm. After one minute of start at 20 rpm, the viscosity values attained a steady state and the apparent viscosities were recorded at a temperature of $28\pm2\ ^{o}$C.

Spray drying

The emulsion of the grape skin anthocyanin concentrate at room temperature (28 ± 2ºC), was pumped in to the pilot model spray drier (Goma Engineering Pvt Ltd, Mumbai, India) of 2 kg/h water evaporation capacity equipped with the rotary wheel atomizer operated at a speed of 18,000 rpm. The spray drier was operated at three different air inlet temperatures of 180,200 and 220^{O}C. The emulsion was fed at 2100 ml/h into the chamber atomized by hot air at a flow rate of 110 kg/h. The spray dried microcapsules were collected from the collection chamber and cyclone separator and filled in air tight self sealable aluminium foil pouches.

Encapsulation efficiency

Microencapsulation efficiency (ME) was calculated according to the procedure described by Barbosa and others (2005). Encapsulation efficiency was calculated as the ratio between the mass of anthocyanin entrapped inside the wall material to the total anthocyanin present in the spray dried anthocyanin powder.

$$\text{Microencapsulation efficiency} = \frac{\text{Total anthocyanin - surface anthocyanin}}{\text{Total anthocyanin}} \times 100$$

For the total anthocyanin (TA) determination, 1 g of anthocyanin microcapsule containing maltodextrin as wall material was homogenized with 20 ml water in a mixer to form a homogenous solution followed by extraction of anthocyanin with 20 ml of dichloromethane. The mass of the anthocyanin collected in the filtrate was quantified by a UV Spectrophotometer (Systronics, Ahmedabad, India) by reading the absorbance at 470 nm. When gum arabic was used as the wall material, 10 ml methanol was added to the solution in order to break the capsules and the anthocyanin was extracted and quantified.

Surface anthocyanin (SA) was determined by direct extraction of 1 g of anthocyanin microcapsule with 5 ml dichloromethane in a mixer for 30 s, followed by centrifugation at 3000 rpm for 10 min. After phase separation, the liquid phase containing the anthocyanin available from the wall material was collected and filtered and was quantified in a UV spectrophotometer at an absorbance of 470 nm. The determination of the anthocyanin level in the microcapsules was carried out in triplicate.

Solubility

Solubility of the microencapsulated powder was determined as described by [29]. Ten gram of microencapsulated powder was added to 100 ml of water at room temperature (28 ± 2ºC). The mixture was thoroughly mixed and centrifuged at 15000 rpm for 90 sec. The presence of sediment was the indicative factor for the solubility test. The addition of microencapsulated powder was done gradually till the formation of sediment. The total weight of microencapsulated powder added to the water for the formation of sediment was noted.

Microcapsule storage for stability studies

Microencapsulated anthocyanin pigment powder of grape skin produced under optimum process conditions was stored in aluminium foil pouches at 28 ±2ºC, to find out the stability of anthocyanins. Degradation of anthocyanin was monitored for 90 days of storage and the anthocyanin content was analysed at 15 days interval.

Structural analysis by using SEM

Scanning electron microscope (SEM) was used to investigate the characteristics outer and inner topography of the microencapsulated anthocyanin powders. A small amount of spray dried anthocyanin microcapsules were placed directly to the specimen stub using a double sided adhesive tape. Samples were coated with platinum to a thickness of about 100ºA under vacuum conditions and the coated samples were observed on a Hitachi scanning electron microscope (Model JEOL- JSM 6310) operated at 20 kV. In order to evaluate the inner structure of the encapsulated anthocyanin microcapsules samples were broken in liquid nitrogen and coated with platinum under vacuum conditions and observed by SEM and photographed.

Statistical Analysis

Factorial Completely Randomized Design (FCRD) was followed for all the statistical analysis with three repeated experimental data [30]. Experimental data were analyzed by ANOVA (Analysis of variance) method, using statistical analysis software AGRES. A probability level of $p \leq 0.05$ was considered to be significant for all statistical procedures.

Results and Discussion

The grape skin anthocyanin was extracted from the Bangalore blue grapes. According to the results of the analysis the quantity of anthocyanin extracted using citric acid was 20.2 ± 0.12 g/ kg and the colour values for the extracted anthocyanin was recorded as L*, a* and b* values and found to be 95.78 ±3.81, 25.5 ± 4.79 and 10.8 ± 1.59 respectively. The anthocyanin extract recorded the lowest pH value of 2.3, which indicated that the product was shelf stable. The total soluble solids value of anthocyanin extracted by this method was calculated as 15 ± 0.15 °Brix.

Viscosity of grape skin extracted anthocyanin emulsion

The anthocyanin extract of the Bangalore blue grapes at concentration levels of 15, 20 and 25° Brix was made into

emulsion with the addition of DE maltodextrin and gum arabic as coating agents and the viscosity of the emulsions are given in **Table 1**.

Table 1 Viscosity of grape skin extracted anthocyanin emulsion

Wall material	Viscosity (cp)		
	Concentration of anthocyanin extract (°Brix)		
	15	20	25
Maltodextrin	1405 ± 1.15	1629 ±0.58	1980 ± 1.0
Gum arabic	1905 ± 0.58	2367 ±0.59	2658 ±1.53

It was observed that the viscosity of the emulsions increased with increase in the concentration of anthocyanin in the emulsion irrespective of wall materials used. Gum Arabic as coating agent recorded higher viscosity than maltodextrin in all three levels of concentration studied. This might be due to the presence of higher amount of polymers *viz.*, D-glucouranic acid, D-galactose and L-arabinose in gum arabic used as wall material.

The emulsion of gumarabic coated anthocyanin extract recorded the viscosity of 2658 cp and the lowest viscosity of 1405 cp was recorded by the emulsion of maltodextrin at 15°Brix concentration.

Solubility

The anthocyanin extract of grapes with different concentration levels as feed solid content and maltodextrin and gum arabic as coating material were spray dried at 180,200 and 220°C air inlet temperatures and the changes in the solubility of microcapsules are given in **Table 2**.

Table 2 Solubility of microencapsulated anthocyanin powder spray dried with maltodextrin and gum arabic at 180,200 and 220°c air inlet temperature

Wall material	Inlet air temperature(°C)	Solubility (%)		
		Concentration of anthocyanin extract (°Brix)		
		15	20	25
Maltodextrin	180	83.1 ± 0.1^{r}	87.6 ± 0.1^{m}	89.2 ± 0.2^{j}
	200	85.2 ± 0.2^{q}	88.2 ± 0.5^{l}	90.3 ± 0.3^{h}
	220	86.5 ± 0.5^{p}	89.7 ± 0.15^{i}	91.1 ± 0.3^{g}
Gum arabic	180	86.9 ± 0.3^{o}	92.6 ± 0.1^{f}	93.5 ± 0.2^{e}
	200	87.4 ± 0.2^{n}	93.7 ± 0.4^{d}	94.1 ± 0.4^{c}
	220	88.6 ± 0.1^{k}	94.2 ± 0.4^{b}	95.2 ± 0.5^{a}

Different letters in the same row or column differ significantly at $p \leq 0.05$

It was observed from the table that the solubility of the anthocyanin microcapsules varied in the range of 83.1 to 95.2 per cent with the highest value registered for microcapsules obtained from the extract of 25°Brix coated with gum arabic and dried at 220°C. It was evident that the gum arabic coated samples recorded the higher solubility values than the samples coated with maltodextrin. With the increase in the air inlet air temperature at all concentrations of the extract the solubility was found to increase.

Increasing the inlet air temperature decreased the moisture content of the microencapsulated powders and the hygroscopic property of the powder decreased significantly which resulted in increase in the solubility of the microencapsulated powder. Similar results were obtained by Barbosa and others (2005) for microencapsulation of bixin powder.

Encapsulation efficiency

The variation of encapsulation efficiency values of the microencapsulated grape skin anthocyanin powder samples is presented in **Figure 1**. The encapsulation efficiency ranged from 49.8 to 68.4 per cent. It was observed that the encapsulation efficiency of the gum arabic coated grape skin anthocyanin powder was higher than the maltodextrin coated samples. Among the different treatments studied, the maximum encapsulation efficiency (68.4 per cent) was noticed for the anthocyanin powder obtained from anthocyanin concentration of 20 °Brix coated with gum arabic and dried at 200º C.

The figure also revealed that encapsulation efficiency of the microencapsulated grape skin anthocyanin powder decreased with increase in anthocyanin concentration. At increased anthocyanin concentration, the viscosity of the emulsion formed might have been high resulting in the larger dispersed particle size of the emulsion, which reduced

the surface area to volume ratio. Large size droplets of the emulsion resulted in longer time for film formation which would have resulted in loss of core material.

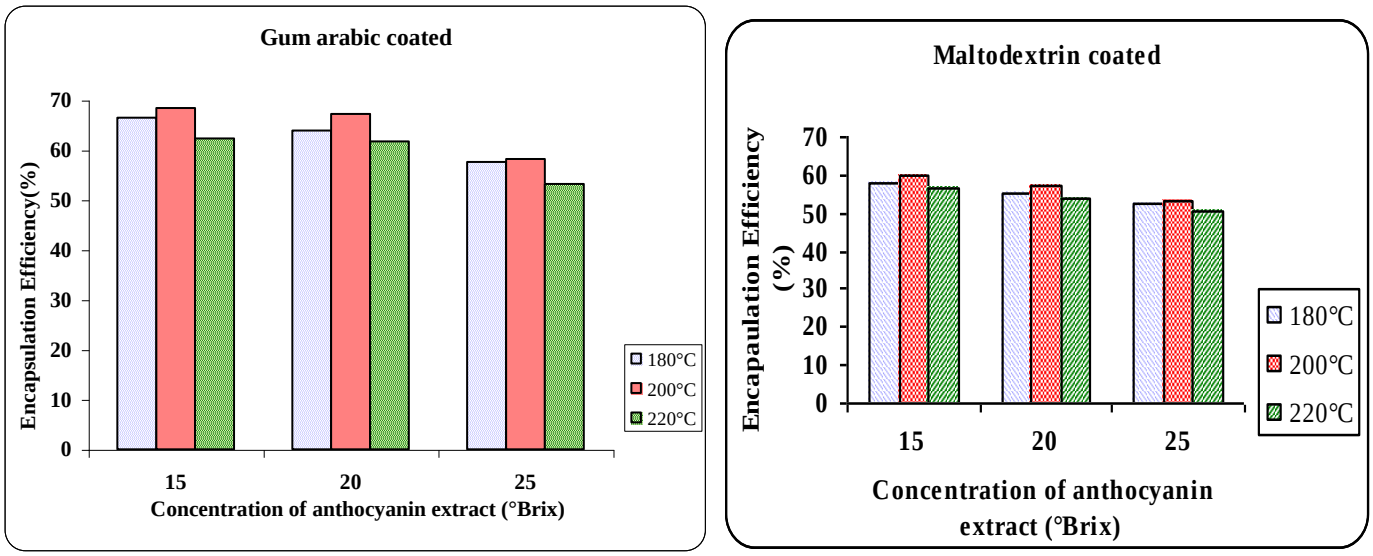


Figure 1 Effect of inlet air temperature, wall material and anthocyanin concentration on encapsulation efficiency of the microencapsulated anthocyanin powder

Increasing the concentration of the core material in the emulsion resulted in the heavy loading of the core component in the encapsulated powder which also resulted in exposure of the core component to the surface and formation of crust that decreased the encapsulation efficiency as reported by Bhandari and others (1992). Similar results were obtained from Soottitantawat and others (2003) for microencapsulated flavouring compounds *viz*, d – limonene, ethyl burate and ethyl propionateite

Increasing inlet temperature from 180 to 220º C increased the encapsulation efficiency with temperature at first, and then decreased when temperature reached 200ºC. High inlet temperature may break the balance between the rate of water evaporation and film formation, which would lead to the breakdown of wall system and microcapsules and hence a low encapsulation efficiency. A Similar work on microencapsulation of Lycopene was reported by Shu and others (2005) in which increasing air temperature from 170 to 210ºC decreased the encapsulation efficiency from 85.3 to 76.3 per cent.

Total anthocyanin content of the microencapsulated grape skin capsules

Anthocyanin is one of the pigments present in the grape skin and gives the colour to the grapes and wine product. The higher the anthocyanin content, the better the quality of microencapsulated powder and hence, the drying parameters are considered to be very important. Total anthocyanin content in the microencapsulated grape skin anthocyanin powder as affected by air inlet temperature of spray drier and the level of anthocyanin concentration in the emulsion is presented in **Table 3**.

Table 3 Effect of inlet air temperature, wall material and anthocyanin concentration on total anthocyanin content of the microencapsulated anthocyanin powder

Wall material	Inlet air temperature (°C)	Total anthocyanin (g/kg)		
		Concentration of anthocyanin extract (°Brix)		
		15	20	25
Maltodextrin	180	51.34 ± 0.02^{j}	50.45 ± 0.01^{n}	48.82 ± 0.02^{p}
	200	53.56 ± 0.01^{d}	52.56 ± 0.01^{h}	49.65 ± 0.02^{o}
	220	50.12 ± 0.02^{m}	46.34 ± 0.02^{q}	45.67 ± 0.03^{r}
Gum arabic	180	54.56 ± 0.03^{c}	53.34 ± 0.02^{e}	51.98 ± 0.02^{i}
	200	56.78 ± 0.03^{a}	55.82 ± 0.03^{b}	52.75 ± 0.05^{g}
	220	53.24 ± 0.04^{f}	50.49 ± 0.03^{k}	50.28 ± 0.02^{l}

Different letters in the same row or column differ significantly at $p \leq 0.05$

The results showed that the highest total anthocyanin content (56.78 g/kg) was observed in gum arabic coated microencapsulated powder obtained from 15° Brix of anthocyanin emulsion, spray dried at 200°C. The least total anthocyanin content (45.67 g/kg) was noticed in maltodextrin coated microencapsulated powder obtained from 25° Brix of initial anthocyanin emulsion, spray dried at 220° C using rotary wheel atomizer.

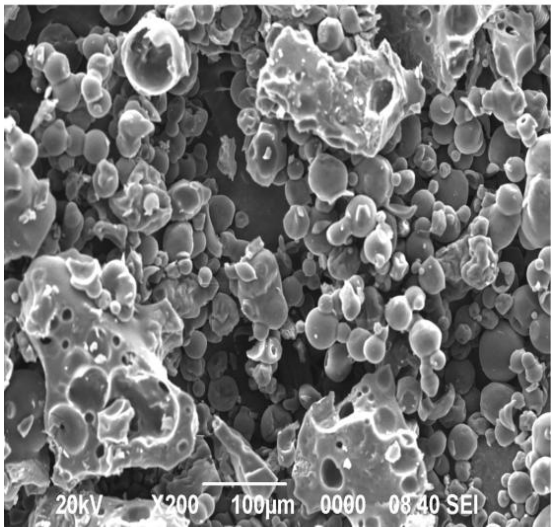
Lower viscosity of maltodextrin incorporated emulsion might have caused internal mixing during spray drying delaying the formation of semi permeable membrane, leading to a greater flavour loss during drying. The efficient entrapment was obtained from gum arabic due to its good film forming and their plasticity rather than glassy properties which prevents the cracking of the protection matrix (Sheikh and others 2006).

It was also observed from the table that the total anthocyanin content in the microencapsulated anthocyanin powder decreased with increase in anthocyanin concentration.

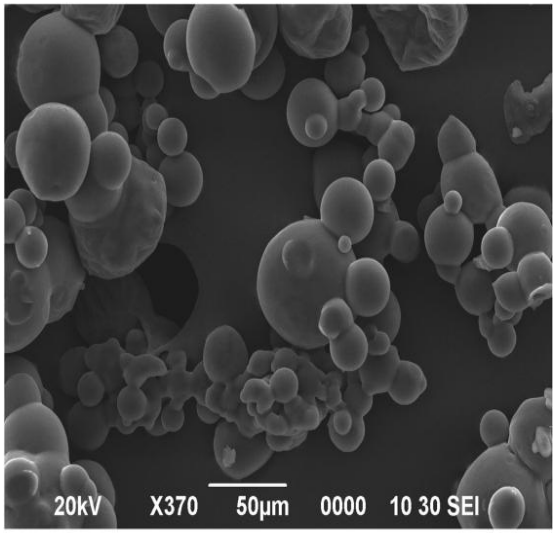
The higher anthocyanin load might have resulted in greater proportion of total anthocyanin close to the drying surface, there by shortening the diffusion path length to the air/particle interface and hence the loss of total anthocyanin in the microencapulated anthocyanin powder. It was also seen that increase in temperature from 180 to 220ºC increased the total anthocyanin content with temperature at first, and then decreased when temperature was above 200ºC. High inlet temperatures might have broken the balance between the rate of water evaporation and film formation, leading to lower encapsulation efficiency and thus causing the lesser retention of principle components.

Morphology of microencapsulated anthocyanin powder

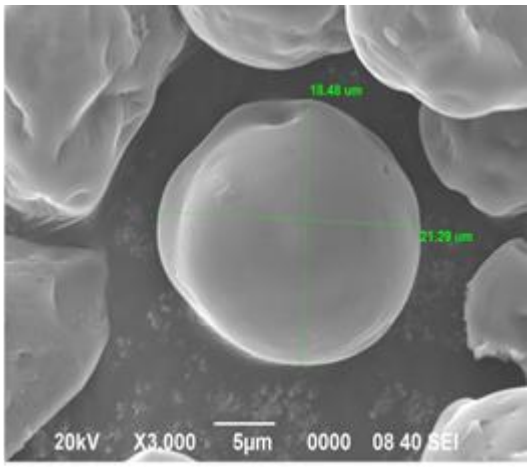
SEM micrographs of particles which were spray dried at 200^{O}C air inlet temperature with 20^{o} Brix feed solid with maltodextrin and gum arabic as wall material are presented in **Figure 2**.



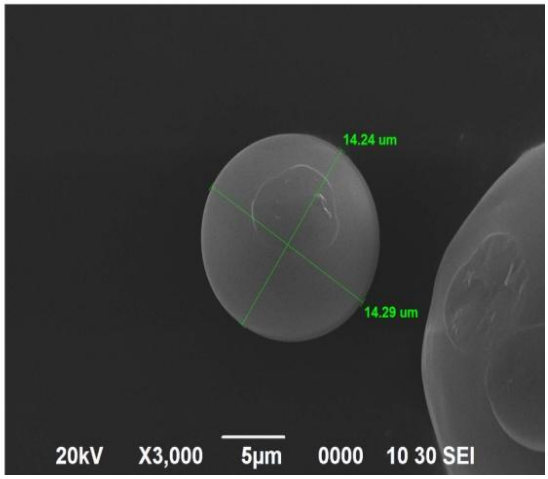
(a) Gum arabic X 200



(b) Maltodextrin X 370



(c) Gum arabic X 3000



(d) Maltodextrin X 3000

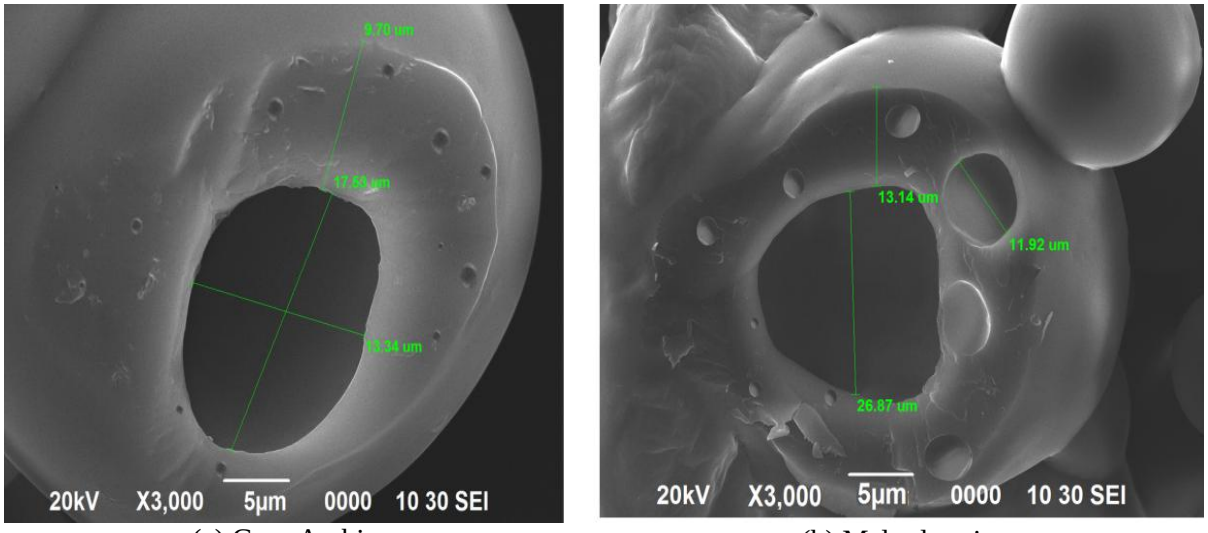
Figure 2 SEM micrographs of spray dried microencapsulated anthocyanin powder with (a) gum arabic X 200 (b) maltodextrin X370 (c)gum arabic X3000 (d) maltodextrin X 3000

It was observed that maltodextrin and gum arabic microencapsulated anthocyanin powder were found to be nearly spherical and the particle size of the powders ranged from 5 to 50 µm approximately (Figure 2a and 2b). The outer morphology of microencapsulated anthocyanin powder had a smooth surface, without cracks or faults, which suggested a high degree of microencapsulation efficiency and good protection of the core material. Maltodextrin coated powder had a particle size 14.29 µm and gum arabic coated microencapsulated powder had a particle size of 18.48 µm.

It was observed from the Figure 2 that gum arabic coated particles had superficial indentations and dents similar to honey comb like structure, which ensured greater protection to the core material. Surface indentations and dents of the gum arabic coated sample might be due to the result of shrinkage while cooling process after the drying, especially high drying rates, which were associated with small particles and usually led to rapid wall solidification. A similar result of morphology was also observed by Bertolini and others *(*2001) in microencapsulated monoterpenes using gum arabic as wall material.

Inner structure of microencapsulated anthocyanin powder

SEM images of the internal structure of the maltodextrin and gum arabic coated microencapsulated anthocyanin powder samples are presented in **Figure 3**.



(a) Gum Arabic (b) Maltodextrin

Figure 3 SEM images of spray dried anthocyanin microcapsules showing the Inner structure

It was observed from the internal structure of both samples that holes were present in the wall matrices. Generally, holes would contain droplets of flavours in the case of encapsulated liquid flavour [34]. Hence, it could be inferred that anthocyanin could be present inside the hole.

The internal structure of samples also revealed that the gum arabic coated microcapsules exhibited the multi core matrix, in which anthocyanin droplets were embedded in the wall matrix as small droplets. Internal structure with multi core matrix of gum arabic and maltodextrin coated samples might be due to the smaller dispersed particle size with incorporated anthocyanin emulsions. Formation of central void was also observed which was related to the expansion of the capsule. The mechanisms involved in the formation of void might be related to desorption of dissolved gases from the emulsion during drying and subsequent expansion, the formation of a steam bubble within the drying droplet, or incorporation of air into the liquid drop during atomization. Similar results were observed in microencapsulation of d-limonene volatile compound [34].

Storage stability evaluation

Stability of anthocyanins in spray dried microencapsulated powders was evaluated during 90 days of storage. At the end of 90 days storage period, maximum of 77.8 % retention of anthocyanin content was observed in the gum arabic coated capsules spray dried at 200° C at 15° Brix concentration (**Figures 4** and **5**).

Forty-one percent loss of anthocyanin content (59 % retention) was observed in the maltodexrin coated capsules from the 20° Brix concentration dried at220° C. Ersus and Yurdagel (2007) have reported 33% decrease in the anthocyanin content of encapsulated powders from the extracts of black carrot (maltodextrin as carrier material) after 64 days of storage period at 25°C.

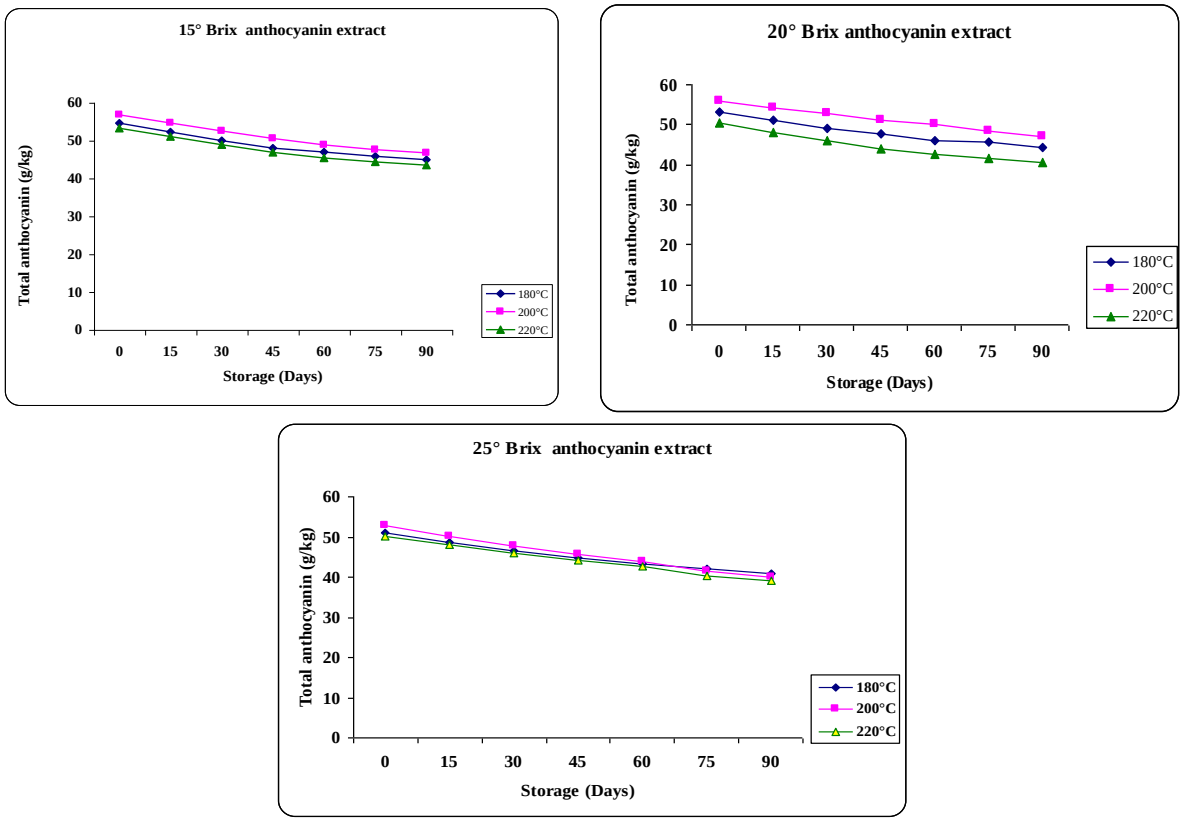


Figure 4 Stability of total anthocyanin in the gum arabic coated microencapsulated powder at 15, 20 and 25º Brix concentrations of anthocyanin extract

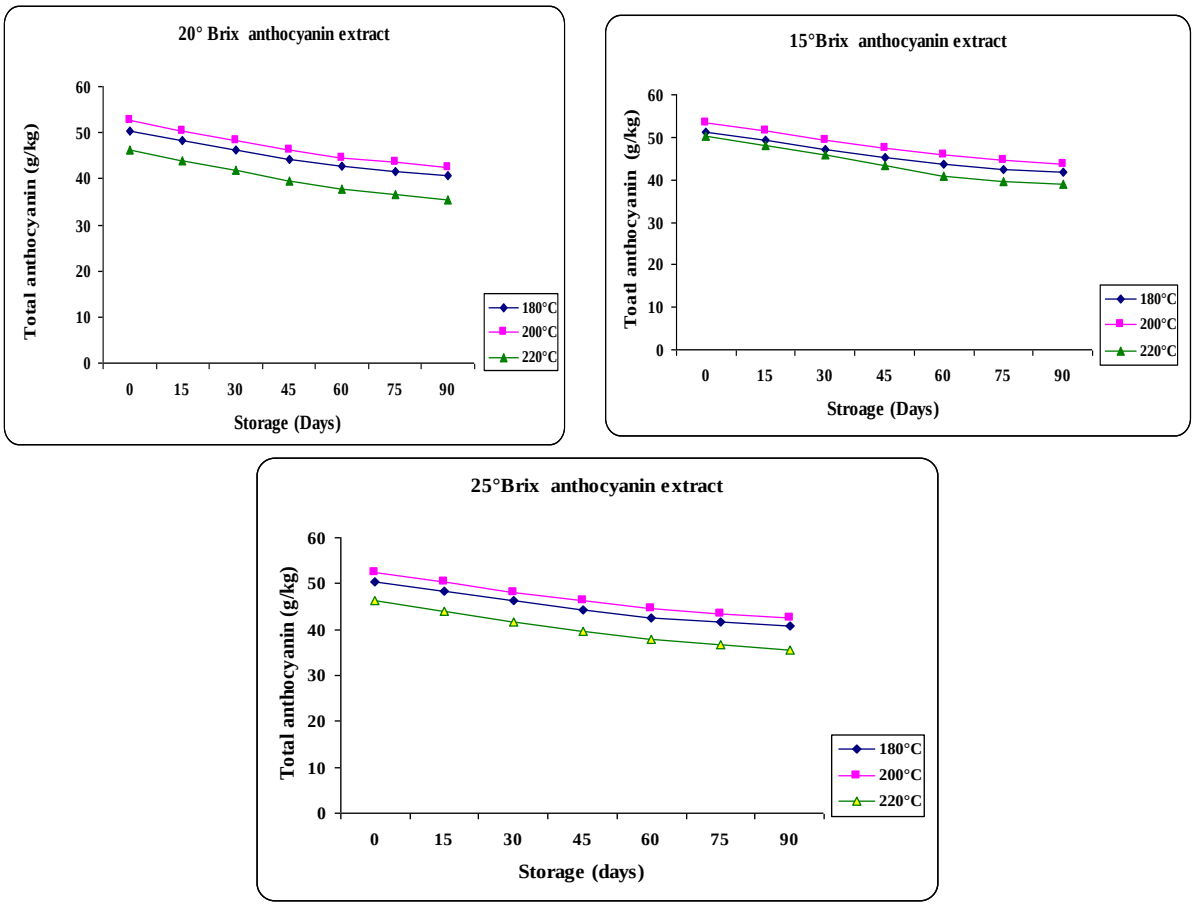


Figure 5 Stability of total anthocyanin in the maltodextrin coated microencapsulated powder at 15, 20 and 25º Brix concentrations of anthocyanin extract

Conclusion

In the spray drying of grape skin anthocyanins, higher inlet air temperatures (180-220°C) increased the solubility. Encapsulation efficiency and total anthocyanin content were observed to be higher at 200°C and then decreased. Gum arabic as wall material yielded higher solubility, encapsulation efficiency, total anthocyanin content making it as a suitable coating agent for usage in the spray drying of grape skin anthocyanin. After 90 days of storage maximum of 77.8% retention of anthocyanin content was observed in the microcapsules obtained from the anthocyanin concentrate of 20°Brix coated with gum arabic as wall material and spray dried at 220°C. Based on the quality attributes and microstructural characteristics of the microcapsules the grape skin anthocyanins can be optimally spray dried at an air inlet temperature of 200º C using the anthocyanin concentrate at 20º Brix using gum arabic as wall material.

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