



## Storage Stability and Antioxidant Activity of Encapsulated Carotenoid from Pumpkin Pulp

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The study was undertaken to microencapsulate carotenoid pigment extracted from pumpkin pulp by spray drying method. Carotenoid concentrate was used as the core substance with two wall materials namely, maltodextrin and maltodextrin + gum arabic. Higher amount of carotenoid was retained when the pulp was emulsified with maltodextrin + gum arabic, spray dried at an inlet air temperature of 200°C with atomizer pressure of 5 bar. Total carotenoid content was found to be maximum in Arjun variety of pumpkin (334.21 µg.g<sup>-1</sup>) and the colour values L\*, a\* and b\* were 58.68, 25.25 and 68.86, respectively. The microencapsulated carotenoid powder characteristically possessed 2.91 % moisture, 250 µg. g<sup>-1</sup> of total carotenoid content, 67.22 % encapsulation efficiency, 71 % solubility and 44.31 yellowness value (b). The stability of encapsulated carotenoids coated with maltodextrin and maltodextrin + gum Arabic (2:1) during 60 days storage was evaluated. Maltodextrin + gum arabic showed better encapsulation efficiency and helped to protect carotenoids during storage. Radical scavenging activity studies revealed significant antioxidant activity of microencapsulated powders after storage. The pumpkin pulp carotenoids microcapsules described in this study represents promising food additive for incorporation into functional foods due to antioxidant content.

**Key words:** Pumpkin, Carotenoids, Spray drying, Maltodextrin and Gum arabic

Carotenoids are natural pigments, synthesized by plants and are responsible for the bright colours of various fruits and vegetables. There are several carotenoids present in the foods that we eat, and most of these compounds have antioxidant activity. Among all, β-carotene is the most common carotenoid available in fruits and vegetables. Mixtures of carotenoids or in association with other antioxidants (e.g. Vitamin-E) can increase their activity against free radicals. Carotenoids (including β-carotene) can promote our health when consumed as dietary foods. At present, there is no officially recommended dietary intake of carotenoids, but recommended dietary intake of vitamin A is about 1-3 mg per day of retinol equivalent (Murkovic *et al.*, 2002).

Pumpkins, are the fruits of different species of the genus *Cucurbita*, are cultivated worldwide for their pulp and seeds for human nutrition, either for direct consumption or for preparation of other foods such as syrups, jellies, jams and purees. Pumpkin is rich in β-carotene, responsible for the yellow or orange colour and also rich in carbohydrates and minerals. β-carotene in plants that have a pleasant yellow-orange colour is a major source of vitamin A (Mukesh *et al.*, 2010). Incorporation of β-carotene rich materials in the human diet is therefore considered as a cost-effective approach to vitamin A related health problems. Besides the pro-vitamin A activity studies

have also indicated that consumption of carotenoids lowers the risk of degenerative and cardiovascular diseases, cataracts, muscular degeneration as well as certain types of carcinomas.

Different cultivars and species of pumpkin contain different carotenoids. Murkovic *et al.* (2002) found that a wide range of varieties of pumpkins contains common carotenoids: β-carotene (600-7400 µg/100g). Hence, there is a considerable industrial interest in extracting carotenoids from pumpkins to develop functional food ingredients.

Several research reports on the extraction of carotenoids from different fruits and vegetables are available, research work on carotenoids from pumpkin pulp is inadequate. The objectives of this study was to determine the carotenoid composition in different variety of pumpkin and to evaluate the storage stability of carotenoids extracted from pumpkin pulp.

### Material and Methods

Pumpkin was purchased from the orchard, Horticulture College of Research Institute, TNAU, Coimbatore. The varieties used for the study are (Mahyco Pumpkin hybrid-1)MPH-1, (Mahyco Pumpkin hybrid-3)MPH-3 and Arjun. The pumpkins were peeled and deseeded, and then the pulp was ground by the mixie for the extraction of carotenoids.

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### Estimation of Physicochemical Properties of Pumpkin

Properties such as moisture content, protein and total carbohydrate were determined following the method described by Ranganna (1986). The colour of the pumpkin pulp was measured by using CIELAB scale at 10° observer at D<sub>65</sub> illuminant. The total solids present in pumpkin pulp extract and carotenoids emulsion were determined by using digital hand refractometer (ATAGO, Tokyo, Japan).

#### Preparation of carotenoid extract

Samples were extracted with a mixture of acetone and petroleum ether with a ratio of 1:1 at room temperature. This step was repeated until the ground pumpkin flesh becomes colourless. The crude extracts were filtered, evaporated in rotary evaporator and re-suspended in petroleum ether (Ren and Zhang, 2008).

#### Preparation of wall material for encapsulation of carotenoid from pumpkin pulp

The extracted and concentrated carotenoids from the pumpkin pulp were used as a core material. Gum Arabic (GA), and maltodextrin (MD) were used as wall materials for encapsulating carotenoid extract from pumpkin pulp. 300g of maltodextrin (DE = 20) was dissolved slowly in 500 ml of distilled water at 60° C by continuously stirring and final volume was made up to 1000 ml by adding distilled water to get 30 per cent maltodextrin solution (Sheikh et al., 2006). 2:1 ratio maltodextrin and gum arabic was dissolved slowly in 500 ml of distilled water at 60° C by continuously stirring and final volume was made up to 1000 ml by adding distilled water to get 30 per cent maltodextrin + gum arabic solution.

#### Spray drying

A lab model spray drier (M/s Labultima, Mumbai, India) which, is a vertical co-current type having water evaporation capacity of 1 kg/h was used for this experimental study. Carotenoid powder were microencapsulated by wall materials maltodextrin and maltodextrin + gum arabic at the inlet air temperature of 200°C with the atomizer pressure of 5 bar.

#### Packaging and storage

Microencapsulated powders obtained from both collection bottles were mixed and packed in Aluminium foil pouches and sealed air tight using the hand sealer (Make: Sevana, India). Sealed bags were stored at room temperature (28 ± 2°C), for studying the stability of the microencapsulated carotenoid powder.

#### Determination of carotenoid

The total carotenoid was directly quantified as per the procedure of Lucia *et al.* (2012). The maximum absorbance in the visible range of 453 nm against a blank of petroleum ether on spectrophotometer (Make: Systronics, Ahmedabad) was measured. The amount of the extracted pigment was calculated by the formula given below:

$$\text{Total Carotenoids } (\mu\text{g/g}) = \frac{AxV(ml) \times 10^4}{A_{1cm}^{1\%} \times P(g)} \dots\dots(1)$$

Where,

A = Absorbance

V = Total extract volume, ml

P = Sample weight, g

$A_{1cm}^{1\%}$  = 2592 (β-carotene extinction coefficient in petroleum ether)

#### Determination of antioxidant activity

##### Free radical scavenging activity (DPPH Assay)

Stock solutions of samples were prepared by dissolving 10 mg of encapsulated carotenoid extract in 10 ml of distilled water to give concentration of 1mg/ml. Then the samples were prepared at the concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml. Six milligram of DPPH was dissolved in 100 ml methanol. The solution was protected from light by covering the test tubes with aluminum foil.

Antiradical activity was measured by a decrease in absorbance at 517 nm of a solution of colored DPPH in methanol brought about by the sample. Decrease in the absorbance in the presence of sample extract and standard at different concentrations was noted after 30 min using UV-visible Spectrometer and IC50 (Inhibitory concentration to scavenge 50 per cent free radicals) is also determined. A blank reading was taken using methanol instead of sample extract. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. IC50 value denotes the concentration of sample required to scavenge 50 % of the DPPH free radicals.

The capability to scavenge the DPPH radical was calculated using the following equation.

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100 \dots\dots\dots(2)$$

Where,

A<sub>0</sub> = Absorbance of the control

A<sub>1</sub> = Absorbance of the sample

IC50 was calculated from equation of line obtained by plotting a graph of concentration versus per cent inhibition (Jasim *et al.*, 2010).

#### Statistical Analysis

Statistical analysis was done to study the effect of different parameters on all dependent variables by using the statistical software AGRSS. Analysis of variance (ANOVA) was performed to determine the significant effect. All the treatments and their interactions were compared at P ≤ 0.01 level and 0.05 level using the Least Significant Difference (LSD) test.

## Results and Discussion

### Physicochemical properties of fresh pumpkin pulp

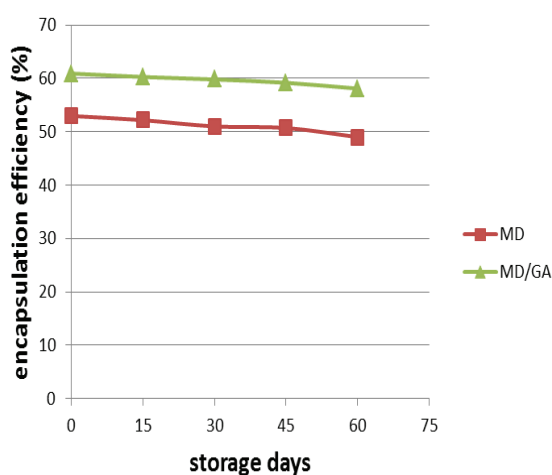
The fresh pumpkin pulp of the varieties MPH-1, MPH-3 and Arjun were analyzed for its physicochemical properties and the results are presented in the Table 1.

**Table 1 Physicochemical properties of different varieties of pumpkin pulp (fresh)**

Properties	MPH-1	MPH-3	Arjun
Colour (L*)	66.72	51.43	58.68
(a*)	23.77	19.00	25.25
(b*)	51.79	55.13	68.86
Total carotenoid ( $\mu\text{g. g}^{-1}$ sample)	210.03	238.56	334.21
Moisture content w.b (%)	88.46	87.01	87.12
Protein (%)	0.89	0.84	0.78
Carbohydrate (%)	6.67	7.11	6.54
Total soluble solids ( $^{\circ}$ Brix)	8.2	8.2	8.5
Water activity ( $a_w$ )	0.93	0.91	0.91

Values are mean  $\pm$  SD of two samples of each cultivar, analyzed individually in triplicate

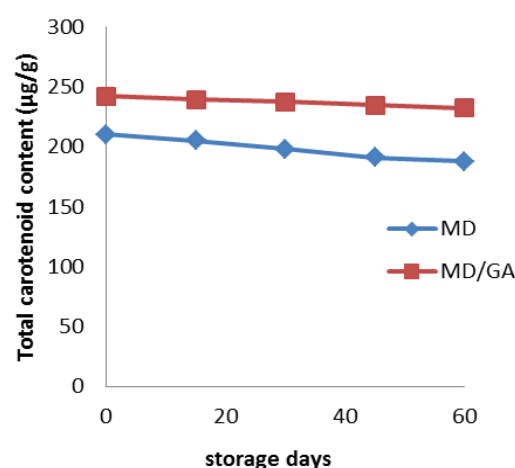
The extraction of carotenoids using the three different varieties of pumpkin viz MPH-1, MPH-3 and Arjun, revealed that the colour values of the Arjun variety of pumpkin was comparatively higher than MPH-1 and MPH-3 varieties. Also the 'b' value *i.e.*



the yellowness of the Arjun variety was found to be higher (68.86) than other varieties. Total carotenoid content was also found to be higher in the Arjun variety pumpkin (334.21  $\mu\text{g/g}$  sample) compared to MPH-1 and MPH-3 varieties (210.03 and 238.56  $\mu\text{g/g}$  sample). Kothainayaki (2013) reported similar concentration of total carotenoids content (341.06  $\mu\text{g/g}$  sample) for Arjun variety.

#### Storage stability of encapsulated total carotenoid content

The concentrated carotenoid extract was stored in amber coloured bottle and kept as control. The microencapsulated carotenoid powder was stored in aluminium foil pouches for studying the stability characters. The stability character for control and sample were recorded at fifteen days interval for 60 days of storage. The result of the storage stability is presented in the Fig. 1 and 2. It was observed that the concentrated pumpkin pulp carotenoid extract stored at  $28 \pm 2^{\circ}\text{C}$  in the amber coloured bottle resulted in fungus attack after fourteen the days of storage.



**Fig. 1. Encapsulation efficiency and total carotenoid content of powder formulation**

It was also observed that after sixty days of storage of encapsulated carotenoid powder in aluminum foil pouches, the maximum total carotenoid content of 232.01  $\mu\text{g/g}$  (retention of 82.07 per cent) was recorded in the maltodextrin + gum arabic coated microencapsulated powder, which was followed by maltodextrin coated microencapsulated powder with total carotenoid content of 187.78  $\mu\text{g/g}$  (retention of 70.74 per cent).

It is observed from the Fig. 2 that the moisture content of the microencapsulated carotenoid powder slightly increased during the storage period of two months at room temperature ( $28 \pm 2^{\circ}\text{C}$ ). The increase in moisture content might be due to the exposure of microencapsulated powders to atmospheric conditions during collection, filling and sealing. A similar result of increase in moisture content was reported by Mishra *et al.*, (2002) for apple powder during storage. There was no significant change in encapsulation efficiency during the storage period. It

was also seen that yellowness of carotenoid ('b' value) decreased during the two months storage period. This might be due to the oxidative degradation of active components present in the microencapsulated powder. The highest retention of total carotenoid content in maltodextrin + gum arabic might be due to the addition of maltodextrin which decreased the glass transition on gum arabic matrix. A similar result was observed by Ximena *et al.* (2011) in degradation of beta-carotene in amorphous polymer matrix.

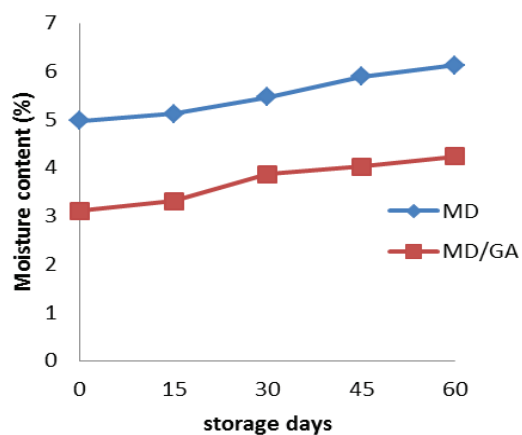
The following regression equations were found to fit the stability data in microencapsulation of carotenoid powder: For maltodextrin + gum arabic coated micro encapsulated carotenoid powder

$$y = -0.162x + 241.8 \quad R^2 = 0.994 \quad \dots (3)$$

For maltodextrin coated microencapsulated carotenoid powder (minimum retention of total carotenoid)

$$y = -0.388x + 210 \quad R^2 = 0.989 \dots (4)$$

These regression equations could be used to predict the total carotenoid content of micro encapsulated carotenoid powder at any desired storage period within the range studied.



#### Antioxidant activity of encapsulated powder

From the Fig.3, it was observed that the antioxidant activity has increased for all concentration tested. The extract of concentration of 10µg/ml showed a per cent inhibition of 33.4 and for 80µg/ml it was 86.7.

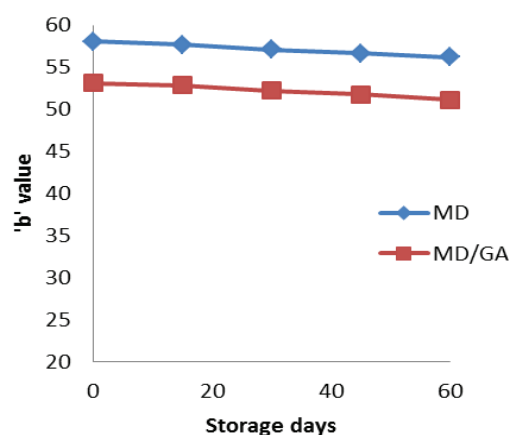


Fig. 2. Variations in moisture content and 'b' value of encapsulated carotenoid powder during storage period

In free radical scavenging activity, 2,2-diphenyl-1 picrylhydrazyl (DPPH) is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by decreasing its absorbance at 517 nm. The decrease in absorbance of DPPH radical is caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. The similar result was observed by Nayan *et al.* (2011) in the *Cassia fistula* flowers extract.

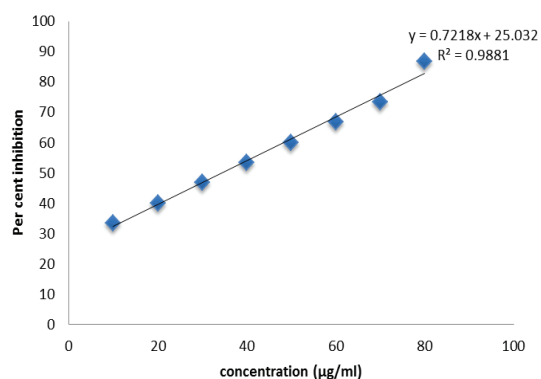


Fig. 3. DPPH free radical scavenging activity of encapsulated carotenoid powder

The following regression equation was found to be fit for the encapsulated carotenoid powder:

$$y = 0.721x + 25.03 \quad R^2 = 0.988 \dots (5)$$

IC50 value was found to be 34µg/ml, indicating 34 µg of encapsulated carotenoid powder was required to scavenge 50 % of the DPPH free radicals.

#### Conclusion

Pumpkins are rich sources of carotenoids. The total carotenoid concentration varied considerably within pumpkin cultivars, with 'Arjun variety' being the richest one. Pumpkin pulp carotenoids extract were only stable for fourteen days in amber bottles at room temperature. Spray dried pumpkin pulp carotenoids powder was characterized by high storage stability. Maltodextrin + gum arabic as a wall material gave the highest encapsulation efficiency and total carotenoid content at the end of sixty days of storage period. Pumpkin pulp carotenoids microcapsules described in this study represent a promising food additive for incorporation into functional foods, due to their antioxidant content and presence of bioactive components beneficial for human health.

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