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Influence of different antioxidants, packaging material and storage conditions on stability of encapsulated colour pigment of ripe pumpkin (*C. maxima* sp.)

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Abstract
The general objective of this study was to evaluate the stability of carotene colour pigment extracted from ripe pumpkin (*Cucurbita maxima* sp) in encapsulated form. The encapsulated carotenoids contained yellowish orange colour pigment along with carrier material. The stability of pigment in the freeze dried powder in different glass vials under ambient (18-27 ˚C), refrigerated (4-7 ˚C) and freeze (-18 ˚C) conditions was studied at storage intervals of 0, 30 and 60 days. Finally, the carotene retention of the encapsulated pigment was estimated. The preservation of encapsulated carotenoid powder with BHT @ 0.02 and 0.015 percent in amber glass vials under freeze storage showed maximum retention of colour pigment in comparison to refrigerated and ambient conditions.

Keywords: Encapsulated carotenoids, freeze dried powder, storage, preservation

Introduction

The colour of raw and processed food products is extremely important. If the food does not taste good, people will not try it again but if it does not look good, they may not even try it at all. Food colours are often added to change the appearance of food by reducing colour loss that often happens from exposure to air, light, extreme temperature conditions or otherwise storage conditions. Food colourants are classified into two main classes *viz.* synthetic and natural (Mortensen, 2006) [9]. In the last 20 years, synthetic colourants have been increasingly perceived undesirable by consumers (Downham and Collins, 2000) [3]. The current consumer preference for naturally derived colourants in food is because of their health promoting properties. Natural colours are also called as bio-colours due to their biological origin and are found in the form of bio-pigments in nature (Mortensen, 2006 [9] and Chattopadhyay *et al.*, 2008) [2]. Most often the natural colourants are extracted from plant material such as fruits, vegetables, seeds, bark, leaves, roots, stems, flowers and whole plant (Rani *et al.* 2004) [11]. Among these natural sources, fruits and vegetables contain abundant amount of colour pigments such as anthocyanins, chlorophyll and carotenoids which impart various hue depending on the source (Sahar *et al.* 2012) [15]. Carotenoids are some of the most vital coloured pigments accounting for the attractive yellow orange colour of a variety of fruits and vegetables. Colouration of fruits and vegetables depends on their maturity, concentration of carotenoid isomers and food processing methods. The occurrence of carotenoids in plants are not as a single compound. Most of the carotenoids are bound with chlorophyll and a combination of carotene-chlorophyll and xanthophyll-chlorophyll. The binding of carotenoids to chlorophylls can give rise to a variety of attractive colours in fruits and vegetables (Wieruszewski, 2000 [20] and Rao and Rao, 2007) [13]. However, as fruit matures, the chlorophyll content decreases and this results in coloured carotenoid pigments which are fat soluble (Simpson, 1985) [18]. *β*-carotene occurs as an orange pigment while α-carotene is a yellow pigment in many fruits and vegetables (Takyi, 2001) [19]. The yellow orange coloured vegetables like carrot, tomato, cantaloupe and pumpkin have been found to be rich in carotenoids. Besides these the green leafy vegetables like spinach, lettuce and mint are also good source of carotenoids (Holden *et al.*, 1999) [4]. According to Khoo *et al.* (2008), orange coloured underutilized fruits contain high amount of *β*-carotene while Levy *et al.* (1995) [7] observed low amount of *β*-carotene in some of the orange coloured fruits.

However, Scott *et al.* (1996) [16] and Krinsky and Johnson (2005) [6] have also found yellow orange coloured fruits such as papaya and persimmon as the major source of carotenoids in human diet. Carotenoids are one of the valuable bio-active constituent of ripe pumpkin which provide natural yellow orange colour pigmentation. Extraction of such type of natural colour pigments from plant sources give crude pigment which are generally unstable in nature. Therefore, encapsulation is done to preserve the extracted colour pigment. The key steps for encapsulation of extracted carotenoid pigment by drying are the choice of a suitable wall material with good emulsifying properties and good film forming properties during dehydration (Madene *et al.*, 2006) [8]. Research workers have made attempts to improve the stability of natural pigments during their extraction, processing and storage. Various workers reported that suitable antioxidants and storage conditions have positive effect on retention of colour pigments in food products. Therefore, the present study was aimed with objectives to evaluate i. stability of encapsulated carotene pigment extracted from waste of *C. maxima* species of pumpkin under ambient (18-27 ˚C), refrigerated (4-7 ˚C) and freeze (-18 ˚C) conditions at different storage intervals (0, 30 & 60 days) and ii. effect of different antioxidants and packaging material on quality of freeze–dried encapsulated carotene pigment.

Materials and Methods

Present study was conducted in the Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India. In order to preserve the encapsulated carotenoids extracted from waste of *C. maxima*, two different antioxidants such as Butylated Hydroxy Toluene i.e. BHT @ 0.01, 0.015, 0.02% and ascorbic acid @ 0.01, 0.03, 0.05% were tried. Encapsulated carotenoid powder was packed in transparent glass vials (TGV), Transparent Glass Vials wrapped with Aluminium Laminated Foil (TGV+ALF) and Amber Glass Vials (AGV). The vials of 3 gram (g) capacity were used and tightly sealed with rubber lid after filling the encapsulated carotenoid powder. To examine the effect of temperature on encapsulated carotenoids, these glass vials were stored under ambient (18-27 ˚C), refrigerated (4-7 ˚C) and freeze conditions (-18 ˚C) for evaluation of carotene colour pigment stability at different storage intervals (0, 30 and 60 days).

Evaluation of Carotene Colour Pigment Retention in Encapsulated Carotenoids

The content of carotenoids in microcapsules was tested during storage. The retention of carotene colour pigment in terms of percentage, which is defined as the ratio between the content of carotenoids that retained in the microcapsule after storage and the original content of carotenoids in the microencapsule was used to evaluate the storage stability of carotene microcapsules (Shu *et al.*, 2006) [17].

$$\text{Carotene Colour Pigment Retention (\%)} = \frac{\text{Carotene in encapsulated powder after storage}}{\text{Carotene in encapsulated powder before storage}} \times 100$$

For determination of carotene content, a known weight of sample (2-5 g) was dissolved in the solvent (acetone) and ground till the whole colour was extracted, then the liquid was transferred into a separating funnel. Separated coloured portion was collected after adding petroleum ether and 5 percent sodium sulphate solution. The final volume was made up to 25 ml. The optical density (OD) was taken at 452 nm and the reading was compared with the standard curve (Ranganna, 2009) [10]. The quantity of carotene pigment was calculated by using the following formula:

$$\text{mg of carotene per 100 g of sample} = \frac{\text{Concentration of carotene in solution from standard curve} \times \text{Final volume} \times \text{Dilution}}{\text{Weight of sample}} \times 100$$

Results and Discussion

The data presented in Table 1, 2 and 3 highlight the effect of antioxidants and packaging material on percent carotene colour pigment retention of encapsulated pigment under ambient (18-27 ˚C), refrigerated (4-7 ˚C) and freeze storage (-18 ˚C) conditions, respectively. Among various treatments, BHT at concentration of 0.02 percent (A_4) closely followed by A_3 (BHT@0.015%) recorded the mean maximum carotene colour retention (%) irrespective of the packaging material and storage conditions. A highly significant decrease in carotene colour pigment retention was observed during a storage period of 60 days under ambient conditons (Table 1). The initial value was found to decrease from 100 to 26.57 percent in TGV, 55.47 percent in AGV and 48.96 percent in TGV+ALF during 60 days of ambient storage. Out of different packaging material, the mean maximum (77.00%) carotene colour pigment retention was seen in AGV packed colour pigment followed by TGV+ALF (72.99%) and TGV (58.52%). As per the interaction of different antioxidant (A) and storage interval (I), a significant decrease from 100 percent to 64.85 percent at 30 days and 42.30 percent at 60 days was recorded during ambient storage. The data indicated in Table 2 showed a significant decrease in percent carotene colour pigment retention during 60 days of storage under refrigerated conditions (4-7 ˚C). The initial value was found to decrease from 100 percent to final value of 35.15 percent in TGV, 67.03 percent in AGV and 59.89 percent in TGV+ALF after 60 days of refrigerated storage. Among different packaging material, mean maximum (84.69%) colour retention was noticed in AGV followed by TGV+ALF (80.31%) and TGV (65.19%). The interaction of antioxidant (A) and storage interval (I) revealed a significant decrease from an initial value of 100 percent to 54.02 percent after 60 days of storage (Table 2). The effect of antioxidants and packaging material (Table 3) on carotene colour pigment retention (%) of encapsulated pigment under freeze storage (-18 ˚C) reflected a decrease from 100 percent to 37.97 percent in TGV, 68.91 percent in AGV and 64.38 percent in TGV+ALF during 60 days of freeze storage. Out of different packaging material, AGV showed the mean maximum (85.88%) colour retention followed by TGV+ALF (82.44%) and TGV (67.07%). The combined effect of antioxidant (A) and storage interval (I) indicated a significant decrease at 30 (100 to 78.31%) and 60 days (100 to 57.09%) of freeze storage. The interaction of antioxidant (A), packaging material (P) and storage interval (I) also revealed a significant effect on percent colour retention of encapsulated carotenoids under all storage conditions.

It can be seen from the data presented in Table 1, 2 & 3 that the incorporation of BHT for preservation of encapsulated colour pigment gave better results as compared to ascorbic acid. This effect of BHT could be due to its higher antioxidant activity to inhibit oxidation of carotenoids. The present results are supported by the findings of Alkesh (2005) [1] for preserving carotene colour pigment extracted from orange peel, paprika and carrot. The present study advocates the use of BHT@0.02% and Womeni *et al.* (2013) [21] also have suggested the use of 200 ppm of BHT as an antioxidant for stabilization of crude soybean oil in order to suppress the major oxidation reactions. The data also reflected that there was a significant decrease in carotene colour pigment retention of encapsulated pigment with the advancement of storage. This might be due to the oxidative degradation of colour pigment with passage of time. Similarly, a decreasing trend in percent carotene colour pigment retention of lycopene during storage was noticed by Ranveer *et al.* (2015) [17]. Amber glass vials (AGV) were found to be the best packaging material on the basis of maximum percent of carotene colour pigment retention of encapsulated carotene which could be due to the photo protective effect of brown coloured vials against isomerization (Sachindra and Mahendrakar, 2010) [14].

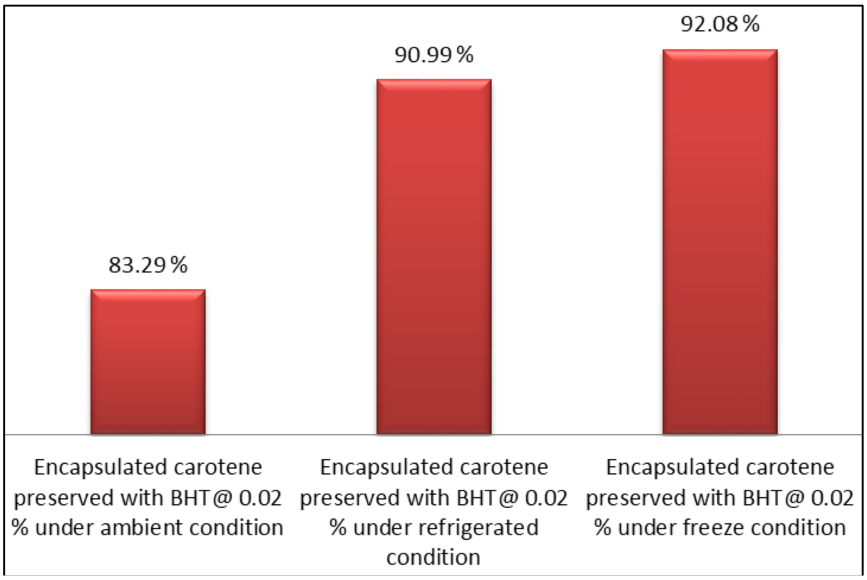

Fig 1: Effect of best antioxidant treatment on carotene colour pigment retention (%) of encapsulated carotenoids during storage.

Table 1: Effect of antioxidants and packaging material on carotene colour retention (%) in encapsulated carotenoids during ambient storage

AS	**Ambient Storage (AS)**											
P/A	**TGV (P_1)**			**Mean (A)**	**AGV(P_2)**			**Mean (A)**	**TGV+ALF (P_3)**			**Mean (A)**
	Storage in days (I)				**Storage in days (I)**				**Storage in days (I)**			
	0	**30**	**60**		**0**	**30**	**60**		**0**	**30**	**60**	
A_1	100.00	34.67	15.11	49.93	100.00	60.04	37.74	65.93	100.00	54.67	28.96	61.21
A_2	100.00	47.70	21.85	56.52	100.00	75.03	55.71	76.91	100.00	69.88	48.98	72.95
A_3	100.00	57.59	26.80	61.46	100.00	84.50	65.27	83.26	100.00	78.66	59.10	79.25
A_4	100.00	57.65	26.90	61.51	100.00	84.55	65.33	83.29	100.00	78.73	59.17	79.30
A_5	100.00	43.85	19.72	54.52	100.00	69.52	48.71	72.74	100.00	64.78	42.35	69.04
A_6	100.00	50.74	23.37	58.04	100.00	77.50	57.74	78.41	100.00	71.60	52.05	74.55
A_7	100.00	50.81	23.45	58.09	100.00	77.62	57.80	78.47	100.00	71.66	52.11	74.59
Mean (I)	100.00	49.00	26.57		100.00	75.54	55.47		100.00	70.00	48.96	
Mean (P)	58.52				77.00				72.99			
A	Interaction A x I											
	0	30	60	Mean (A)	Factor		$CD_{0.05}$		A_1 =	Without antioxidant (Control)		
A_1	100.00	49.79	27.27	59.02	P (Packaging)		0.12		A_2 =	BHT (0.01%)		
A_2	100.00	64.20	42.18	68.79	I (Storage Interval)		0.12		A_3 =	BHT (0.015%)		
A_3	100.00	73.58	50.39	74.66	A (Treatment)		0.18		A_4 =	BHT (0.02%)		
A_4	100.00	73.64	50.47	74.70	PxI		0.20		A_5 =	Ascorbic acid (0.01%)		
A_5	100.00	59.38	36.93	65.44	PxA		0.30		A_6 =	Ascorbic acid (0.03%)		
A_6	100.00	66.61	44.39	70.33	IxA		0.30		A_7 =	Ascorbic acid (0.05%)		
A_7	100.00	66.70	44.45	70.38	PxIxA		0.53					
Mean (I)	100.00	64.85	42.30									

Table 2: Effect of antioxidants and packaging material on carotene colour retention (%) in encapsulated carotenoids during refrigerated storage

RS	Refrigerated Storage (RS)											
P/A	TGV (P_1)			Mean (A)	AGV(P_2)			Mean (A)	TGV+ALF (P_3)			Mean (A)
	Storage in days (I)				Storage in days (I)				Storage in days (I)			
	0	30	60		0	30	60		0	30	60	
A_1	100.00	46.17	28.11	58.09	100.00	71.54	48.50	73.35	100.00	66.00	40.61	68.87
A_2	100.00	58.70	33.90	64.20	100.00	86.64	67.43	84.69	100.00	81.70	60.80	80.83
A_3	100.00	68.60	39.31	69.30	100.00	96.05	76.78	90.94	100.00	89.94	69.77	86.57
A_4	100.00	68.71	39.43	69.38	100.00	96.13	76.85	90.99	100.00	90.03	69.85	86.63
A_5	100.00	55.36	32.70	62.69	100.00	80.94	60.43	80.46	100.00	74.55	53.25	75.93
A_6	100.00	62.65	36.27	66.307	100.00	88.91	69.56	86.16	100.00	82.50	62.44	81.65
A_7	100.00	62.74	36.35	66.36	100.00	89.00	69.65	86.22	100.00	82.57	62.52	81.70
Mean	100.00	60.42	35.15		100.000	87.03	67.03		100.00	81.04	59.89	
Mean (I)	65.19				84.69				80.31			
A	Interaction A x I											
	0	30	60	Mean (A)	Factor		CD 0.05		A_1 =	Without antioxidant (Control)		
A_1	100.00	61.24	39.073	66.77	P (Packaging)		0.09		A_2 =	BHT (0.01%)		
A_2	100.00	75.68	54.043	76.57	I (Storage Interval)		0.09		A_3 =	BHT (0.015%)		
A_3	100.00	84.86	61.953	82.27	A (Treatment)		0.16		A_4 =	BHT (0.02%)		
A_4	100.00	84.96	62.043	82.33	PxI		0.14		A_5 =	Ascorbic acid (0.01%)		
A_5	100.00	70.28	48.793	73.03	PxA		0.25		A_6 =	Ascorbic acid (0.03%)		
A_6	100.00	78.02	56.090	78.04	IxA		0.25		A_7 =	Ascorbic acid (0.05%)		
A_7	100.00	76.10	56.173	78.09	PxIxA		0.43					
Mean (I)	100.00	76.16	54.02									

Table 3: Effect of antioxidants and packaging material on carotene colour retention (%) in encapsulated carotenoids during freeze storage

FS	Freeze Storage (RS)											
P/A	TGV (P_1)			Mean (A)	AGV(P_2)			Mean (A)	TGV+ALF (P_3)			Mean (A)
	Storage in days (I)				Storage in days (I)				Storage in days (I)			
	0	30	60		0	30	60		0	30	60	
A_1	100.00	48.71	30.82	59.84	100.00	73.61	50.28	74.63	100.00	68.05	42.80	70.28
A_2	100.00	61.35	36.73	66.03	100.00	88.56	69.36	85.97	100.00	83.13	62.77	81.97
A_3	100.00	71.64	42.21	71.28	100.00	97.54	78.42	91.99	100.00	91.80	71.88	87.89
A_4	100.00	71.79	42.35	71.38	100.00	97.69	78.55	92.08	100.00	92.23	71.80	88.01
A_5	100.00	57.92	35.61	64.51	100.00	82.57	62.72	81.76	100.00	76.44	71.74	82.73
A_6	100.00	65.56	38.97	68.18	100.00	90.50	71.43	87.31	100.00	84.43	64.75	83.06
A_7	100.00	65.7	39.10	68.27	100.00	90.65	71.58	87.41	100.00	84.57	64.91	83.16
Mean (I)	100.00	63.24	37.97		100.00	88.73	68.91		100.00	82.95	64.38	
Mean (P)	67.07				85.88				82.44			
A	Interaction A x I											
	0	30	60	Mean (A)	Factor		CD 0.05		A_1 =	Without antioxidant (Control)		
A_1	100.00	63.46	41.30	68.25	P (Packaging)		0.07		A_2 =	BHT (0.01%)		
A_2	100.00	77.68	56.287	77.99	I (Storage Interval)		0.07		A_3 =	BHT (0.015%)		
A_3	100.00	87.00	64.17	83.72	A (Treatment)		0.12		A_4 =	BHT (0.02%)		
A_4	100.00	87.24	64.23	83.82	PxI		0.10		A_5 =	Ascorbic acid (0.01%)		
A_5	100.00	72.31	56.69	76.33	PxA		0.18		A_6 =	Ascorbic acid (0.03%)		
A_6	100.00	80.16	58.38	79.52	IxA		0.18		A_7 =	Ascorbic acid (0.05%)		
A_7	100.00	80.31	58.53	79.61	PxIxA		0.31					
Mean (I)	100.00	78.31	57.09									

It was noticed that among different storage conditions (ambient, refrigerated and freeze), freeze storage exhibited the maximum retention of carotene colour pigment in powder when preserved with BHT @ 0.02 percent in encapsulated powder while minimum was observed under ambient conditions (Fig. 1).

Conclusion

The encapsulated carotenoid which have potency as bioactive compounds could be stored under suitable conditions to avoid significant pigment degradation. This study can be a part of promoting natural food colour pigments to be functional food ingredient. Among various antioxidants and packaging material, BHT @ 0.02 and 0.015 percent in amber glass vials exhibited maximum retention of colour pigment in encapsulated carotene powder during storage. The freeze storage condition showed maximum colour or carotene retention (85.88%) in encapsulated pigment followed by refrigerated and ambient conditions.

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