The Bioscan 9(4): 1459-1464, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

MICROENCAPSULATION OF â-CAROTENE AND ITS UTILIZATION IN POMOGRANATE READY TO SERVE BEVERAGE

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KEYWORDS
Microencapsulation
â-carotene
Maltodextrin freeze drying
Pomogranate
RTS beverage
Total antioxidant activity

Received on :
25.08.2013

Accepted on :
20.10.2014

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ABSTRACT
Microencapsulation of β-carotene with maltodextrin of 25 and 36.5 DE was carried out and utilized in the pomogranate RTS beverage to enrich in β-carotene. The freeze dried microencapsulated β-carotene with maltodextrin of 36.5 DE resulted 25% surface β-carotene and 80% microencapsulation efficiency and 75% retention in β-carotene content of RTS beverage during storage. The total antioxidant activity (TAA) of β-carotene enriched pomogranate RTS beverage at 5gm/lit of RTS resulted highest level of TAA with 14.82 mMol/L.

INTRODUCTION

Currently, there is a trend towards a healthier way of living, which includes a growing awareness by consumers of what they eat and what benefits certain ingredients have in maintaining good health. Preventing illness by diet is a unique opportunity for innovative functional foods (Sheehy and Morrissey, 1998; Hilliam, 1996). These products often present new challenges to the food engineer. Existing and new ingredients need to be incorporated into food systems, in which they slowly degrade and lose their activity, or become hazardous, by oxidation reactions. Ingredients can also react with components present in the food system, which may limit bioavailability, or change the color or taste of a product. In many cases, microencapsulation can be used to overcome these challenges.

Microencapsulation by spray-drying has been successfully used in the food industry for several decades (Gouin, 2004), and this process is one of the oldest encapsulation methods used since the 1930s to prepare the first encapsulated flavors using gum acacia as wall material (Shahidi and Han, 1993). Important applications are to coat colorants, flavors, vitamins, and other sensitive food ingredients in order to increase their shelf life (Shahidi and Han, 1993; Dziezak, 1988).β-carotene has been found in most yellow, orange, dark green leafy vegetables and fruits such as kale, pumpkin, spinach, papaya, apricots and peaches. Carrots are said to be a major source of β-carotene. Carotene, a yellow orange pigment, is present in the chromoplasts in fresh carrot. Maintenance of the naturally colored pigments in stored foods has been a major challenge in food processing (Ihl *et al*., 1998; Clydesdale *et al*., 1970). The high degree of unsaturation in β-carotene structure renders it extremely susceptible to oxygen. Stability and retention of labile biomolecules during drying and the later storage are often dependent on their encapsulation in an amorphous matrix formed during dehydration processes (Maa *et al*., 1997, Maa *et al*. 1998 (a) and Maa *et al*. 1998 (b). Maltodextrins are hydrolyzed starches produced by partially hydrolysis of starch with acid or enzymes. Hydrolyzed starches have the advantages of being low cost, bland in flavor and good flavor protection against oxidation. Hydrolyzed starches are reported to improve shelf life of orange oil (Anandaraman and Reineccius, 1986) and carrot carotene (Wagner and Warthesen, 1995).

Encapsulation is a potential approach to transform liquids into stable and free flowing powders which are easy to handle and incorporate into dry food systems. Spray drying is the most common method of drying encapsulation as the cost of drying is 30 to 50 times less than freeze-drying which is used in the flavor industry. Spray drying provides a very large surface area which enhances oxidation, if the wall material is not thick or dense enough to provide a good oxygen barrier. Generally the wall must comprise at least 80% of the total weight which reduces product delivery efficiency (Moreau and Rosenberg,

1996).

The present study was undertaken to evaluate maltodextrin of various DEs (Dextrose Equivalent) as encapsulating agent and to compare the effect of spray drying and freeze drying as the process of different time-temperature regimes that can lead to different stability of products due to different wall thicknesses and densities, with the following objectives to formulate stable emulsion (feed mixture) of core material (β-carotene) in the wall solution (maltodextrin) and to study physic-chemical properties of microencapsulated β-carotene powder.

MATERIALS AND METHODS

The research work was conducted in the Neutraceutical Laboratory of Food Science and Technology department of College of Food Technology, Parbhani. Maltodextrin (DE 25 and DE 36.5) was obtained from Sahyadri Agrofoods Pvt. Ltd., Faltan, Dist. Satara (M.S.), India. The pure Trans β-carotene was obtained as a complimentary sample from Valentine Agro foods Ltd., Roha, Dist. Raigad (M.S.), India. The emulsifier Tween 80 was obtained from Qualigens Fine Chemicals, A Division of Glaxo India Ltd., Dr. Annie Besant Road, Mumbai (M.S.), and India.

Pomegranate fruits *(Var. Bhagva)* were procured from local market of Parbhani. Fruits were graded and damaged and infected fruits were sorted out. Food grade citric acid was obtained from Thomas Baker (Chemicals) Pvt. Ltd., Mumbai (M.S.), India.

Preparation of feed mixtures

Pure trans â-carotene was added to the solution of maltodextrin system-maltodextrin (39.92%) + Tween 80 (40% w/w) in distilled water (60%) in a ratio of 1:800 w/w, on dry basis respectively. Tween 80 was added as an emulsifier (Barbosa *et al.,* 2005).The mixture was then vigorously homogenized to obtain an aqueous emulsion (feed mixture) and immediately fed to the dryer.

Preparation of sample

The encapsulation of β-carotene was done by spray drying as well as freeze drying as per the method of Ozcelik *et al*. (2009).

Spray drying

The feed mixtures were spray dried in a LSD 48 Mini spray dryer (Jay Instruments and Systems Pvt. Ltd., New Mumbai). The inlet and outlet air temperatures were maintained at -170 ± 5 C and 95 ± 5 C respectively with air pressure of 5 kgf/ cm^2. The feed rate was kept at 5 ml per min. with an air flow rate of 30 ml/min.

Freeze drying

The Heto Power Dry LL 1500 (Heto Power Lab, Denmark) freeze drying was used for freeze drying of feed mixtures. The Heto Power Dry LL 1500 which operated at temperature of the condenser plate of -110 C and at a minimum chamber pressure of 4×10^{-4} mbar was used. The main drying was performed without shelf temperature control

and the chamber pressure ranged from 0.1 to below 0.01 mbar, which corresponds to ice temperatures from -45 C to below -60 C, respectively. The temperature of the secondary drying was 25 C and the whole process lasted 48 hr. The dried emulsions were broken and homogenized into powder by use of a mortar and pestle and subsequently washed with hexane (HPLC grade) to remove non-encapsulated surface carotene until negligible absorbance was detected at 452 nm. The washed powder was further dehydrated under vacuum over $MgClO_4$. It was veriûed that during the homogenization of the freeze-dried samples no appreciated losses of encapsulated β-carotene had occurred. The treatments A, A_1, B and B_1 with varying combinations were as follows;

Sample	Combinations
A	Spray drying with 25 DE maltodextrin
A_1	Freeze drying with 25 DE maltodextrin
B	Spray drying with 36.5 DE maltodextrin
B_1	Freeze drying with 36.5 DE maltodextrin

The microencapsulation of β-carotene was done as follows;

The aqueous slurry of maltodextrin was made with distilled water and tween (39.92% + 0.08%) and pure trans β-carotene in maltodextrin system in the ratio of 1:800 w/w. Homogenized the mixture to form a stable emulsion and then spray dried as well as freeze dried to obtain microencapsulated β-carotene powder respectively.

Storage of β-carotene powder

The microencapsulated powders were collected, kept in plastic bags wrapped with aluminum foil and stored in desiccators containing silica gel at room temperature. Aliquots of about 0.4 g of the dried samples were distributed into glass vials (3 mL capacity) and exposed to atmospheres of saturated salt solutions of 11% RH ($LiCl_2$), 32% RH ($MgCl_2$) and 44% RH (K_2CO_3) into evacuated desiccators at 25ºC (Greenspan, 1977). At selected times samples will be removed and analyzed for total β-carotene content of powder in order to study β-carotene retention.

Utilization of microencapsulated β-carotene in pomegranate RTS

The pomegranate RTS beverage was prepared with 10% juice, 10% TSS and 0.3% acidity. The powder was utilized in β-carotene enriched pomegranate RTS in order to enrich the antioxidant activity of pomegranate juice along with the potent vitamin A activity of β-carotene. Sample B is added in β-carotene enriched pomegranate RTS @ 1.25, 2.5 and 5 g/lit of RTS for sample C_1, C_2 and C_3 respectively. One sample was taken as control (C). This level of addition of microencapsulated

Table 1: Yield, surface β-carotene content, microencapsulation efficiency and moisture content of microencapsulated β-carotene powder

Parameter (%)	A	A_1	B	B_1	S.E ±	C.D at 5%
Yield on dry basis	82.30	89.89	80.90	88.01	1.658	5.399
Surface β-carotene	27.30	25.00	22.00	20.00	1.154	3.759
Microencapsulationefficiency	72.73	75.00	77.94	80.00	2.121	6.907
Moisture content	2.74	1.73	3.10	2.20	0.219	0.715

* Each value represents the average of three determinations.

Table 2: Particle size distribution of microencapsulated β-carotene powder

Samples	Range of particle diameter in microns (μ)				
	75-105 (%)	106-150 (%)	151-250 (%)	251-300 (%)	301-500 (%)
A	8	42	38	6	6
A_1	5	24	45	16	10
B	5	48	35	7	5
B_1	8	30	51	7	4

* Each value represents the average of three determinations.

Table 3: Bulk density and water solubility of microencapsulated β-carotene powder

Samples	Water solubility index (%)	Bulk density (g/mL)
A	97.25	0.817
A_1	92.82	0.699
B	98.43	0.805
B_1	95.46	0.670
S.E ±	1.472	0.057
C.D at 5%	4.792	0.187

* Each value represents the average of three determinations.

Table 4: Retention of β-carotene in microencapsulated form during storage

Storage study	% Retention of ²-carotene		
	11 % RH	32 % RH	44% RH
Sample A			
0	100	100	100
10	88	87	85
20	80	75	75
30	75	70	65
40	73	65	55
50	70	65	45
60	68	63	35
Sample A_1			
0	100	100	100
10	90	88	85
20	85	80	80
30	80	75	70
40	75	75	60
50	75	73	50
60	73	70	40
Sample B			
0	100	100	100
10	90	88	85
20	85	85	75
30	85	80	70
40	80	77	60
50	75	75	50
60	75	70	40
Sample B_1			
0	100	100	100
10	92	88	85
20	85	85	75
30	85	80	65
40	80	75	55
50	77	75	50
60	75	70	45

* Each value represents the average of three determinations.

β-carotene was selected to reach a safe upper level for supplementation of ²-carotene which is 7 mg/day, recommended by The British Expert Committee on vitamins and minerals (EVM).

Proximate analysis

Analysis of total and surface carotene of microencapsulated β-carotene powder

The method of Desobry *et.al* (1997) was used to analyze the total carotene and surface carotene.

Physico-chemical characteristics of microencapsulated β-carotene powder

Moisture

Moisture of the sample was determined by using A.O.A.C (1990).

Particle size distribution

It was determined using Laboratory test sieve (AS 200 digit, ASTME 11, Retsch, Germany).

Water solubility of microencapsulated β-carotene powder

Water solubility index (WSI) was determined using the method described by Anderson (1969).

Bulk density of powder

The bulk density of microencapsulated β-carotene was measured by method of Hall and Hedrick (1971).

Estimation of β-carotene content of β-carotene enriched pomegranate RTS

Estimation of β-carotene was done after extraction of the sample with diacetone alcohol and petroleum ether and further purification with diacetone alcohol, methanolic KOH and distilled water. The resulting solution was filtered with anhydrous sodium sulphate and read on spectrophotometer at 450 nm against petroleum ether as a blank (Ranganna, 1986).

Total antioxidant activity of β-carotene enriched pomegranate RTS

The FRAP method was developed to measure the Ferric Reducing Antioxidant Power/ability of plasma at low temperature (Benzie and Strain, 1996). An intense blue colour is formed when the Ferric-tripyridyltriazin (Fe^{3+} TPTZ) complex is reduced to the Ferrous (Fe^{2+}) form and absorption at 593 nm was recorded.

Sensory analysis

The sensory evaluation of β-carotene enriched pomegranate RTS was carried out by a 10 member trained panel comprised of postgraduate students and academic staff members of the faculty who had some previous experience in sensory evaluation of fruit and vegetable products. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. Judgments were made through rating products on a 9 point Hedonic Scale with corresponding descriptive terms ranging from 9 'like very much' to 1 'dislike very much' (Amerine, 1965).

Statistical analysis

The analysis of variance of the data obtained was done by using Completely Randomized Design (CRD) for different treatments as per the methods by Panse and Sukhatme (1967). The analysis of variance revealed at significance of $P < 0.05$ level, S.E. and C.D. at 5 % level is mentioned wherever required.

Table 5: β-carotene content and total antioxidant activity of β-carotene enriched pomogranate ready to serve

Samples	β-carotene content (μg/100 mL)	Total antioxidant activity (mMol/L)
C	Nil	1.80
C1	160	4.92
C2	320	8.03
C3	650	14.82
S.E ±	0.165	0.595
C.D at 5%	0.539	1.938

C, C_1, C_2 and C_3 are the samples containing 0, 0.25, 2.50 and 5 g of microencapsulated β-carotene powder in 1 lit of Pomegranate RTS beverage.

RESULTS AND DISCUSSION

Yield, Surface β-carotene, Microencapsulatation efficiency and moisture content of microencapsulated β-carotene powder

The data on the yield of microencapsulated β-carotene powder and its properties are presented in Table 1. It was observed that the yield of microencapsulated β-carotene powder on dry weight basis varied from 18.01 to 89.89%.The production of microencapsulated β-carotene powder by spay drying resulted in less yield compared to that from freeze drying. This could be probable due to greater losses occurred during spray drying process resulting into less recovery of microencapsulated β-carotene powder. Among the two levels of maltodextrin as a wall material the yield of microencapsulated β-carotene powder was highest in the sample containing maltodextrin 36.5 DE and this might have due to lowering of molecular weight of DE of maltodextrin increased. Wagner and Warthesen (1995) reported the change in the stability of á and β-carotene in spray dried carrot powder when hydrolyzed starch were encapsulated with various dextrose equivalents and observed that the hydrolyzed starch of 36.5 DE was superior to 4, 15 and 25 DE in improving the retention of β-carotene.

The microencapsulated β-carotene powder contained some β-carotene material on outer surface which must have been minimized to protect it from oxidation. It was observed that freeze drying process with maltodextrin of 36.5 DE provided the most efficient encapsulation process with only 20% of surface β-carotene as compared to 27.3, 25.0 and 22.0% for samples A, A_1 and B respectively. The process of microencapsulation of β-carotene with maltodextrin of 36.5 DE and spray drying were the most optimum conditions with regard to surface β-carotene content on the powder. The results were significant ($P < 0.05$). Wagner and Warthesen (1995) in a study on carrot juice spray dried powder reported decrease in the carotene degradation rate and similar surface carotene on increasing the proportion of carrier while encapsulating the β-carotene with starch of 36.5 DE. The result of present investigation coincides with those of above researchers.

The microencapsulation efficiency was observed maximum (80%) in the freeze dried sample containing maltodextrin of 36.5 DE and was significantly higher than rest of the samples. These results are in close agreement to that of Huezo *et.al* (2004) on microencapsulation by spray drying of multiple emulsions containing carotenoids. The moisture content of spray dried sample were 2.74 and 3.10% for samples A and B and that of freeze dried samples were 1.73 and 2.20% for samples A_1 and B_1 respectively which indicated that the spray dried microencapsulated β-carotene powder exhibited highest moisture content than freeze dried one. Among the samples containing matodextrin of 36.5 DE the freeze dried one contained higher moisture than the spray dried one which might be due to lowering of glass transition temperature as molecular weight decreased; the higher DE maltodextrin has higher hygroscopy. Wagner and Warthesen (1995) reported the similar results.

Particle size distribution of microencapsulated â-carotene powder

The data on particle size distribution of microencapsulated â-carotene powder are presented in Table 2. It was observed that freeze dried microencapsulated had wider particle size distribution compares to spray dried microencapsules. The most predominant diameter of microencapsulated β-carotene powder was in the range from 106 to 250 micron (μ) with the weight percentage of 69.83 and 81 particles within this range of samples A, A_1 and B, B_1 respectively. Spray dried microencapsulated β-carotene powder has the range 106-150 microns (μ) most prevalent with 42% and 48% of particles within this range for sample A and B respectively. Whereas freeze dried microencapsulated β-carotene powder exhibited the range 151-250 micron (μ) dominant for its particle with 45 and 51% of particle diameter within this range for samples A_1 and B_1 respectively. Higher particle diameter range for freeze dried microencapsulated β-carotene powder can be attributed to its complex form as they were ground after dehydration. Lokasuwan (2007) also studied the ability of native modified tapioca starch and maltodextrin as wall materials for encapsulating β-carotene and reported that modified tapioca starch had wider particle size distribution towards smaller diameter and moisture content of micromolecules were dependent of type of wall materials.

Water solubility index (WSI) and bulk density of microencapsulated β-carotene powder

As regards to water solubility index (WSI) and bulk density of microencapsulated β-carotene powder (Table 3). The Spray dried microencapsulated β-carotene powder exhibited 97.25 and 98.43% WSI for sample A and B respectively. While that of freeze dried sample varied between 92.82 to 94.46% for samples A_1 and B_1 respectively. Spray dried microencapsulated powder exhibited greater WSI as compared to freeze dried samples. This can be explained by more regular, spherical and porous particles of spray drying process. The lower values of WSI for freeze dried microcapsules may be attributed to their larger particle size as they were ground after dehydration. Among the sample microencapsulated β-carotene powder with DE 36.5 maltodextrin as a wall material exhibited more WSI than microencapsulated β-carotene powder with DE 25 maltodextrin. The results with respect to water solubility index were statistically significant. Loksuwan (2007) reported the results similar to the present findings.

Retention of β-carotene in microencapsulated form during storage

Data depicted in Table 4 on the retention of β-carotene during storage at different relative humidity indicated that Freeze drying gave the best β-carotene preservation with 73 and 75

per cent retention for maltodextrin DE 25 and 36.5 as a wall material respectively at the end of storage study. This better retention was expected, based on particle size and surface β-carotene content. For freeze dried powder slightly lower amount of surface β-carotene and non-porous structure reduced the duration of fast oxidation period. Again increasing DE of maltodextrin provided greater advantage for spray drying to increase retention of β-carotene which gave 68 and 75 per cent β-carotene retention for maltodextrin DE 25 and 36.5 as a wall material. The present findings are more or less similar to the findings reported by Sutter *et al*. (2007) and Desobry *et al*. (1997).

Spray dried microcapsules were spherical as we expected. The high ratio of surface/volume for sphere and large amount small spheres would favour oxidation of β-carotene. Whereas freeze dried powders had complex forms because they were ground after dehydration. Although they had the small wall / carotene ratio, the larger particle provided larger barrier against diffusion of oxygen into the inner carotene and also had less surface β-carotene / volume. At all relative humdities maltodetrin of 36.5 DE provided the best protection from β-carotene losses. The less effective microencapsulation properties of 25 DE maltodextrin were in agreement with results reported by O'Boyle *et al*. (1992). Maltodextrin of 25 DE may provide less carotene stability because it contains a large proportion of long chain saccharides that cause the barrier to be inflexible and were permeable to oxygen.

Total antioxidant activity of β-carotene enriched pomegranate RTS

The data on β-carotene content and total antioxidant activity of β-carotene enriched pomegranate RTS are presented in Table 5. It was clear that sample C_3 has the highest level of total antioxidant activity. It was found that control sample has the lowest total antioxidant activity. Total antioxidant activity of sample C_1, C_2 and C_3 was 4.92, 8.03 and 14.82 mMol/L respectively. Thus the addition of microencapsulated â-carotene resulted in increased total antioxidant activity of pomegranate RTS which provided the prominent health benefits. Total antioxidant activity was expressed in TEAC *i.e.* Trolox Equivalent Antioxidant Activity. Thus total antioxidant activity of sample C, C_1, C_2 and C_3 was equivalent to that of a solution 1.80, 4.92, 8.03 and 14.82 mMol/L of Trolox calculated experimentally by the FRAP method. The results obtained are in good agreement with the results reported by the Volker *et al*. (2002) and Gil *et al*. (2000).

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