Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 2947-2950
Received: 10-05-2019
Accepted: 12-06-2019

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Extraction, encapsulation and fortification of carotene from papaya in cooking oil

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Abstract
Fortification of food with micronutrients is considered a valid technology and strategy to provide adequate levels of the respective nutrients in the diet, and where the fortified food is highly accessible to the target population. Papaya is a common man's fruit reasonably priced and has a high nutritional value. Papaya has more carotene compared to other fruits. Hence the present study focuses on extraction of carotene, encapsulation and fortification in edible oils to address the micro nutrient malnutrition. The study indicated that the most efficient method of extraction of β carotene from papaya is by Cold extraction method using hexane as the solvent. The yield of encapsulated carotene powder from papaya 1.75 g containing 300 mg of β carotene.

Keywords: Extraction, encapsulation, fortification, carotene, papaya, cooking oil

Introduction

Micronutrient malnutrition" also known as "hidden hunger" is a global public health problem and affects the quality of human resource development and productivity of the country. Malnutrition in India, particularly among women, children and adolescents is an important issue requiring immediate attention if the country has to have inclusive growth and development. (GOI, 2017). Addressing the global challenge of micronutrient malnutrition requires the need for many strategies – both short- and intermediate-term and long-term sustainable approaches. In addition to the conventional approaches of micronutrient supplementation and fortification, promoting sustainable food based approaches to enable adequate intakes of micronutrients by much of the population. Fortification of food with micronutrients is considered a valid technology and strategy to provide adequate levels of the respective nutrients in the diet, and where the fortified food is highly accessible to the target population. Papaya is a common man's fruit reasonably priced and has a high nutritional value. Papaya has more carotene compared to other fruits (Gopalan, 2004) [3]. Hence the present study focuses on extraction of carotene, encapsulation and fortification in edible oils to address the micro nutrient malnutrition. The objectives are, (i) Extraction and encapsulation of carotenoids from papaya (ii) Fortification of carotenoids in oil and (iii) Quality analysis and shelf life of the fortified oil

Materials and Method

Standardization of Extraction method for β carotene

Different Extraction Methods

Three different methods for extraction of β carotene were identified and performed in the sunlight proof room and the amount of Carotene obtained was determined in each method.

i) Cold Extraction Method

Hundred gram of papaya was weighed and placed in a mortar and crushed with a pestle. Totally 200 ml of the solvent was measured and kept aside and from that 10 ml of solvent was added into the mortar and the sample was crushed. About 5ml of solvent was added slowly at regular intervals. The solvents were collected separately and was filtered through a filter paper and then transferred into a separating funnel.

To the separating funnel 50ml of distilled water and 50 ml of 10% NaCl solution was added. The mixture was shaken vigorously and kept aside for the layers to separate. The upper layer contained Carotene and it was collected separately. The lower layer was discarded. The extract was stored in amber colored bottles for concentration in a rotary flash evaporator.

ii) Boiling Method

For extraction, hundred gram of the sample was taken in a round bottom flask and to it 200 ml of solvent was added. The flask was placed in a heating mantle. The contents were heated to facilitate the extraction. After heating for a fixed time (30 minutes) at specific temperatures (50 ^{0}C), the extract was filtered and collected in amber colored bottles till concentration.

iii) Soxhlet Method

The sample (100 g) to be extracted is placed in the Soxhlet extractor. The extractor was connected to the condenser and the round bottom flask. The solvent was heated to reflux, the vapor travel through the passage of the extractor and the condensate dripped back down into the solid material. The solvent filled up the chamber while the material was extracted from the solid. When the solvent volume had almost filled the chamber, the solvents containing the extract flowed back into the round bottom flask through a siphon tube. The process was repeated for a number of cycles and the extract was accumulated in the round bottom flask. After complete extraction, the extract was filtered and collected in amber colored bottles.

Concentration of Carotene Extract

Rotary flash evaporator was used to concentrate the extract of the Carotene, by removing the solvent present in it. Solvent present in the solution gets vaporized due to heating and the vapor was condensed by the supplied cold water. After removal of solvent by vaporization the remaining solution was obtained as concentrate.

Estimation of β carotene

The concentrated sample was estimated for the amount of β carotene. The 10 ml of the sample were taken and made it up to 50 ml with petroleum ether in a standard flask. Ten grams of anhydrous sodium sulphate was added and kept for 30 minutes and the absorbance is read at 453 nm using petroleum as blank (AOAC 2000) [1]

$$\text{Carotene content of the sample} = \frac{\text{OD value of sample x total volume of the sample}}{\text{Weight of the sample}} \text{ X } 100$$

Standardization of Encapsulation method for β carotene

Microencapsulation of carotene was carried out in a two-step process. The first step was the emulsification of the concentrate and wall material using an emulsifier, followed by spray drying of the resultant emulsion.

Core material

The concentrated Carotene from papaya were used as core material. This core material was stored in amber colored bottles in order to protect the concentrate from light and oxygen before conducting the microencapsulation experiments.

Wall material

Carrier materials lecithin obtained from M/s Himedia Laboratories Pvt. Ltd, Mumbai.

Preparation of emulsion

The wall: core material ratio was tried in different proportions viz., 1:1, 2:1, 3:1, 4:1, 5:1, 6:1. The proportion of wall material, core material, water and emulsifier for the various trials is given in the table below.

Table 1: Proportion of contents for the development of premix.

Proportion of contents	Wall to core material ratio					
	1:1	2:1	3:1	4:1	5:1	6:1
Papaya						
Concentrate - (ml)	2000	2000	2000	2000	2000	2000
Distilled water (ml)	300	600	900	1200	1800	2000
Emulsifier Tween – 80(ml)	10	10	10	10	10	10
Wall material – (mg) (Lecithin)	0.33	0.66	0.99	1.32	1.65	1.98
Hexane (ml)	300	600	900	1200	1800	2000

Aqueous extract of the wall material was prepared by dissolving malto dextrin/ gum arabic in distilled water at 60 ^{o} C in varying proportions as given in the table. For lecithin a solvent extract using hexane was prepared.

Carotene emulsion was prepared by dissolving the aqueous extract of the wall material to the carotene concentrate. The emulsion formation and stabilization was achieved by using an emulsifying agent (Tween-80) under continuous vigorous agitation. The resultant emulsion was spray dried to obtain encapsulated powder.

Preparation of encapsulated carotene powder from papaya

Carotene emulsion (papaya) was pumped in to the spray drier equipped with the two fluid atomizers. Emulsion was fed into the main chamber through a peristaltic pump at the rate of 5 ml min^{-1}. The pressure of compressed air for the flow of the spray was adjusted to 5 bars. Air outlet temperature was maintained at 80°C and inlet air temperature was 160^{0} C. The microcapsules were collected and stored in PET bottles till use.

Fortification of encapsulated powder in cooking oil

The Recommended Dietary Allowance (2010) for β carotene is 4800 µg per day. The RDA for oil is 25 ml per day. The thumb rule of fortification is the Fortified food should provide only 1/3 of the RDA. This was followed and accordingly 25 ml of oil should provide 1600 µg per day. The following table gives the proportion of encapsulated powder and oil for fortification.

Method of Fortification

The method followed for fortification is the premix method, where in a premix of the fortificant was developed and this was dissolved in the oil to be fortified.

Development of Premix

For the preparation of the premix 100 ml of the oil/ fat was taken. The proportion of encapsulated powder, oil, temperature for the development of premix is given in the table below.

Table 2: Proportion of ingredients and temperature for the development of premix

Oil / source of βcarotene	
Gingelly oil / Mustard oil	
Encapsulated powder (L) –g	0.42

Encapsulated powder (L)- Lecithin

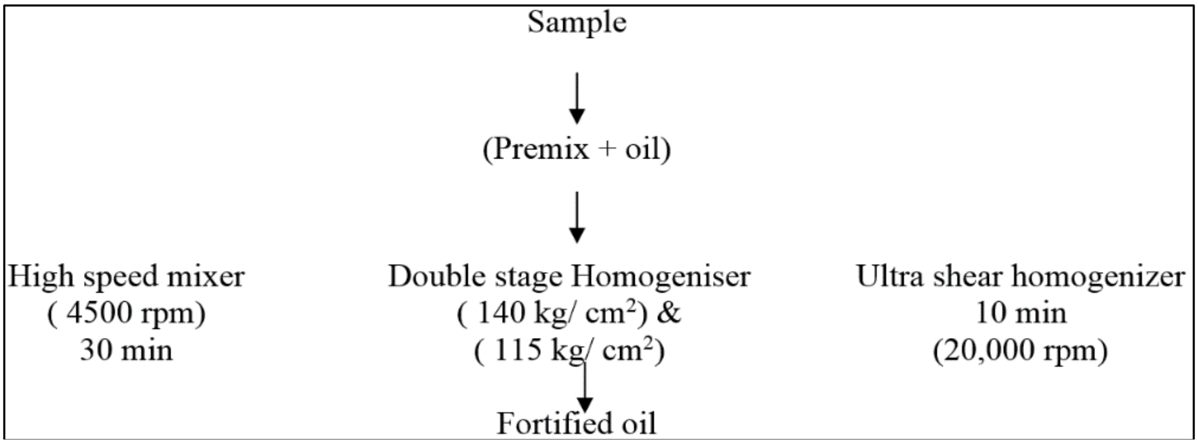
Preparation of premix

The oil was heated to 50 0 C temperature followed by the addition of the encapsulated powder. It was then thoroughly mixed in a magnetic stirrer for 30 minutes for complete dissolving of the powder in the oil and thus the premix was obtained

The effect of temperature in the dispersion efficiency of the encapsulated powder was studied and presented in the table below.

Addition of premix to the oil

The premix thus obtained was dissolved to 900 ml of the oil (Mustard/gingelly/ghee). For complete dispersion of the fortificant, this oil was subjected to thorough mixing in a high speed mixer, homogenizer and ultra shear homogenizer with varying time as given below



Results and discussion

Table 3: Efficiency of extraction of β carotene by different methods and solvents

Extraction Method	Sample (10 Kg)	Acetone		Hexane		Acetone : Hexane (50:50)		Ethanol		Ethyl acetate	
		A* (mg)	E* (%)	A* (mg)	E* (%)	A* (mg)	E* (%)	A* (mg)	E* (%)	A* (mg)	E* (%)
Cold	Carrot	436	73	450	75	425	71	412	69	400	67
	Papaya	301	71	330	78	294	69	278	66	275	65
Traditional boiling	Carrot	253	42	265	44	246	41	241	40	232	39
	Papaya	245	58	250	59	238	56	229	54	220	52
Soxhlet	Carrot	290	48	300	50	285	48	270	45	260	43
	Papaya	270	64	285	67	264	62	260	61	254	60

A* - amount of β carotene, E* - extraction efficiency

From the above table, it could be inferred that the maximum extraction efficiency of β carotene was obtained in cold extraction method, in which hexane is used as a solvent.

2. Recovery of encapsulated powder and its carotene retention

The recovery of the encapsulated powder and β carotene retention in the powder were analysed. Based on the results the best suited wall to core material ratio and the suitable wall material was screened for fortification.

Table 4: Recovery of encapsulated powder and its carotene retention

Source	Proportion	Lecithin	
		Powder (g)	β carotene (mg)
Papaya	1:1	0.66	100
	2:1	0.99	150
	3:1	1.32	200
	4:1	1.65	250
	5:1	1.98	300

From the above table, it is concluded that, the most suitable ratio of wall material to core material was 5:1. This is because, apart from the highest recovery of encapsulated powder (2.5 g) irrespective of the wall material and the source of carotene, it retained the highest amount of β carotene content. Hence this powder was used for further studies

3. Quality Analysis of the Fortified Oil

Physical properties of the fortified oil

The physical properties of the fortified oil namely the refractive index, bulk density and smoke point of the maltodextrin encapsulated gingelly/ mustard oil and ghee were estimated and presented in the table below.

Table 5: Physical Properties of the Fortified Oil

Sample/ Property	High speed mixer	Homogeniser	Ultrashear homogeniser
Gingelly oil			
Encapsulated Papaya powder (MD)			
Refractive index	1.4728	1.4021	1.3512
Bulk density	0.9239	0.9229	0.9205
Smoke point	160 ºC	151 ºC	145 ºC
Mustard oil			
Encapsulated Papaya powder (MD)			
Refractive index	1.4755	1.4740	1.4725
Bulk density	0.9263	0.9245	0.9200
Smoke point	228 ºC	220 ºC	211 ºC

From the table it could be observed that, the bulk density and refractive index was more for the oil that had been subjected to high speed mixer, when compared to homogenizer and Ultra shear homogeniser. This has also reflected upon the sharp decrease in the smoke point of the oils in homogenizer and ultra shear homogenizer. As a result these two oils will reflect poor cooking quality and the chances for increase in rancidity is also more.

Therefore it was concluded that, Oil samples (Gingelly/ mustard) added with encapsulated papaya powder with lecithin as wall material, fortified by premix method and homogenized by high speed mixing is the best suited method for fortification of β carotene in oil.

Shelf Life Studies of the Fortified Oil

The standardized fortified oil (i.e) Gingelly/ Mustard oil and ghee fortified with encapsulated carrot/ papaya powder using malto dextrin as wall material, fortified by premix method was subjected to shelf life studies in three different packaging material namely PET jars, Opaque Bi axially Oriented Polypropylene packs and (Opaque BOPP) and HDPE packs and stored at room temperature. The retention of B carotene was analysed during storage and the results are presented below.

Table 6: Retention of Carotene in different packaging material during storage (mg/litre)

Sample	PP pouches		PET Bottles		Opaque BOPP	
	0th day	60th day	0th day	60th day	0th day	60thday
Carotene fortificant from Papaya						
Mustard oil	64.162	64.132	64.162	64.146	64.162	64.158
Gingelly oil	64.128	64.084	64.128	64.109	64.128	64.122

The above table vividly indicates that the maximum retention of β carotene was found in Opaque BOPP packs irrespective of the oil and the fortificant even at 60 days of storage at ambient conditions.

Summary and Conclusion

The most efficient method of extraction of β carotene from papaya is by Cold extraction method using hexane as the solvent The amount of β carotene present in the 4.0 litres of extract is 330 mg. Effective concentration of the extract was achieved by rotary flash evaporator. The most suitable wall material for encapsulation is lecithin in the ratio 5:1 Spray drier with an inlet temperature of 160 ^{0}C and Outlet temperature of 90 ^{o}C was most effective to obtain the encapsulated powder. The yield of encapsulated powder contained was 1.75 g containing 300 mg of β carotene. In terms of addition of encapsulated powder one litre of oil contained 0.42 g of papaya powder. The most suitable method of fortification is premix method followed by high speed mixing. Storage stability – 60 days in Opaque BOPP packs.

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