

# Preparation, characterization and evaluation of a vitamin-enriched dietary supplement from *Vigna radiata* L. microgreens

## Abstract

The present study attempted to develop a simple protocol for the transformation of vitamin-rich microgreens of *Vigna radiata* into microcapsules with a longer shelf life. The microencapsulation method adopted was the coupling ionotropic gelation method followed by lyophilization.

The microcapsules thus produced were characterized based on weight, swelling index, vitamin content and also, the range and magnitude of in *vitro* dissolution in simulated gastric and intestinal fluids (SGF: pH 1.2; SIF: pH 6.8, 7.4). The results showed that the microcapsules undergo high water loss during hydrogel to xerogel conversion (96.525%) and have a high swelling index (5.76) in SGF.

The release rate of the enclosed vitamins was found to be very low in gastric conditions (SGF) but high in intestinal conditions (SIF).

The timecourse of vitamin release (over 1–6 hours) was found to be linear and probably diffusion-controlled. Hence, microcapsules developed through this technology can be potentially used as natural and biodegradable dietary supplements that can replace and thereby reduce the health risk associated with artificial supplements.

GP Lekshmi

Bindu R Nair <sup>1</sup>

<sup>1</sup> Department of Botany  
University of Kerala  
Kariyavattom  
Thiruvananthapuram  
695 581, Kerala

\*Corresponding author:  
Bindu R Nair  
bindunairbot123@gmail.com

**Keywords:** Microcapsules, ionotropic gelation, lyophilization, *Vigna radiata*, microgreens

## Introduction

The increased incidence of health disorders associated with a sedentary lifestyle and an unbalanced diet is forcing people to look for special foods with health benefits beyond basic nutrition. Food or dietary supplements belong to the category of special foods and are products that are intended to supplement the diet. These are carriers for one or more dietary components or ingredients, such as vitamins, minerals, herbal or other botanical constituents and amino acids, which can be consumed in the form of capsules, pills or liquids. The use of these innovative special foods provides a unique opportunity to increase immune activity, thereby improving and maintaining overall health<sup>[1]</sup>. The development of these new supplements requires technologies for incorporating health-stimulating bioactive chemical compounds into food without any reduction in their stability, bioavailability or functionality<sup>[2]</sup>.

Microencapsulation is one such technology, useful for producing highly stable, small particles from short-shelf-life active ingredients. It can be defined as a process of encapsulating active solid particles, liquid droplets or gas within thin polymeric coatings to protect them from adverse environmental conditions<sup>[3]</sup>. The products thus formed, with an outer coating and an inner core of active materials, are called microcapsules.

Nutritional analysis of *Vigna radiata* microgreens has revealed a higher vitamin content than the mature edible counterparts, along with intense flavours, vivid colours and tender textures. However, the colour, texture, taste, odour and freshness deteriorate over the course of time<sup>[4]</sup>. The objective of the present investigation was to develop a simple microencapsulation protocol for transforming vitamin-rich, short-shelf-life microgreens of

*Vigna radiata* into longer-shelf-life, vitamin-enriched dietary supplements, followed by their characterization and evaluation.

This study was carried out to explore the feasibility of using microencapsulation technology to formulate potential natural and biodegradable supplements in the form of microcapsules, thereby reducing the health risk of artificial supplements.

## Materials and methods

### General experimental procedure

Among the several available microencapsulation methods, the ionotropic gelation method was utilized to transform microgreens of *Vigna radiata* L. to microcapsules.

### Plant material

#### Microgreens of *Vigna radiata*

(family: *Fabaceae*; common name: mung bean, green gram)

The seeds of *Vigna radiata*, collected from Agro Super Bazaar, West Fort, Thiruvananthapuram, were sown in dirt-free mixtures of sand and soil in well-aerated thermocol planting trays. Microgreens, characterized by a pair of true green leaves and pale white-green stems with a pair of cotyledons, were harvested at a height of approximately 6–7 cm within 4 days of germination as the core material for the microencapsulation process.

### Preparation of microcapsules

Microcapsules were prepared by employing the external ionotropic gelation method adopted by Gonzalez-Rodriguez *et al.* (2002)<sup>[5]</sup>, with some modifications as described below.

### Preparation of calcium-alginate hydrogels

Approximately 500 mg of fresh microgreens of *Vigna radiata* was ground into a paste, dissolved in 5 ml of distilled water and

used as the core material. In order to form hydrogels, 10 ml of an aqueous solution of sodium alginate was mixed with the prepared microgreen paste, stirred to complete dissolution for 20–30 min and kept aside for 15 min (to remove bubbles). The completely dissolved mixture was dropped into 100 ml of 0.15 M calcium chloride solution to form the hydrogels (water-containing beads).

### Standardization of sodium alginate

The amount of sodium alginate required for good-quality hydrogels (with a smooth texture and spherical shape) was standardized by varying its concentration (0.25 to 2.5% in distilled water). The quantities of the other constituents such as the microgreens (500 mg in 5 ml of distilled water) and the CaCl<sub>2</sub> solution (0.15 M) were fixed. The hydrogels formed were retained in the same solution for 30 min, and later recovered by filtration and washed twice with distilled water. The entire process was carried out at ambient temperatures.

Sufficiently hard and good-quality hydrogels obtained from the standardized procedure were used for further analysis.

### Identification of the best cationic compound

Chitosan-alginate and calcium-chitosan-alginate hydrogels were developed by dropping the suspension (500 mg of microgreens of *Vigna radiata* + 10 ml of an aqueous solution of sodium alginate) into chitosan solution (1.6 g of chitosan + 50 ml of distilled water + 50 ml of 2% acetic acid) and calcium-chitosan solution (50 ml of chitosan solution + 50 ml of 0.15 M CaCl<sub>2</sub>), respectively, and these were compared with calcium-alginate hydrogels to identify the best cation for alginate.

### Preparation of xerogels/microcapsules

The hydrogels were subjected to evaporation under ambient temperatures and the freeze drying/lyophilization (physical

microencapsulation) method was adopted to form microcapsules.

## Characterization of microcapsules

### Weight of hydrogels and xerogels

Weights were determined using an analytical balance.

### Determination of water loss for calcium-alginate microcapsules

This was carried out as per [6]. Calcium-alginate hydrogels were weighed immediately after preparation and later after being subjected to freeze drying to form xerogels. The mean water loss ( $W_1$ ) from the capsules was calculated using the following equation:

$$W_1 \% = [(W_0 - W_d)/W_0] \times 100$$

where  $W_0$  is the initial weight before drying and  $W_d$  represents the final weight after drying.

### Swelling studies

These were carried out as per [7].

The swelling properties of the calcium-alginate microcapsules were determined in simulated gastric fluid (SGF: pH 1.2) and in simulated intestinal fluid (SIF: pH 6.8, 7.4). Samples of microcapsules of known weight (25 mg) were placed in a glass vial containing 5 ml of swelling solution and these were allowed to swell at 37°C. The swollen beads were removed and weighed after 1 hour. The wet weight of the swollen beads was determined by blotting with filter paper to remove moisture adhering to the surface, immediately followed by weighing using an electronic balance.

All experiments were carried out in triplicate. The swelling index of the beads was calculated using the formula given below: Swelling index =

$$\frac{\text{Final weight of microcapsules (Wt)} - \text{Initial weight of microcapsules (W}_0\text{)}}{\text{Initial weight of microcapsules}}$$

### Estimation of vitamin content

The vitamin content of the microcapsules was determined using the digestion method.

The required amount of the microcapsules (25 mg) was incubated in 10 ml of phosphate buffer (pH = 7.4) at room temperature for 24 hours.

The suspension was then centrifuged for 30 min. The supernatant was assayed spectrophotometrically to estimate the vitamin content, except in the cases of niacin and vitamin C.

The vitamins thiamine<sup>[8]</sup>, riboflavin<sup>[9]</sup>, niacin<sup>[10]</sup>, vitamin C<sup>[11]</sup> and carotene<sup>[12]</sup> were measured. The analysis was conducted in triplicate and reported as the means of 3 replicates ± standard deviation.

### In vitro dissolution study of the microcapsules

This was carried out as per<sup>[13]</sup>.

#### Tolerance test

A tolerance test was carried out by incubating 10 mg of microcapsules in 10 ml of SGF (pH: 1.2) with 75 rpm and a temperature of 37°C. At fixed time intervals, the fluid was filtered and subjected to vitamin analysis.

#### Release profile in SIF

The dissolution studies were performed in SIF, at 2 pH values (6.8 and 7.4). Microcapsules recovered from the tolerance test in pH 1.2 were filtered out and transferred to the above solutions (pH 6.8 and 7.4) and centrifuged at a speed of 75 rpm and at a temperature of

37 ± 0.5°C. The temperature was maintained throughout the experiment and the dissolution was carried out for 4 hours.

At fixed time intervals, aliquots (5 ml) of sample were withdrawn and replaced with fresh dissolution media and the withdrawn samples were assessed for vitamin concentrations. The studies were carried out in triplicate and the means were calculated.

## Results and discussion

### Preparation of microcapsules

Microcapsules were prepared by a two-step process – preparation of hydrogels followed by preparation of xerogels.

#### Preparation of hydrogels

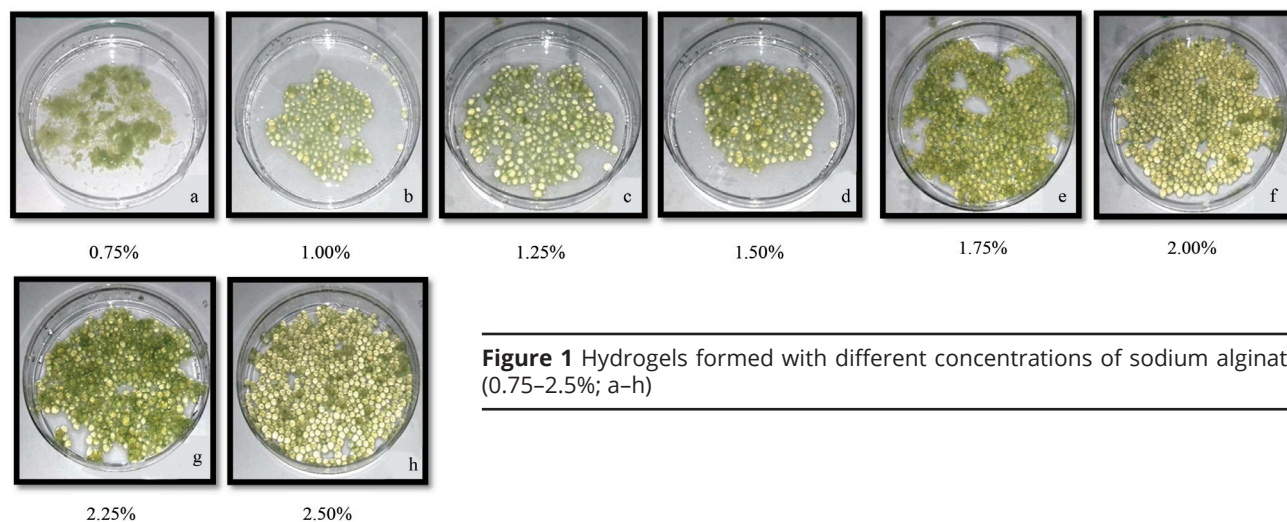
##### Standardization of sodium alginate

The polymer coat consisted of sodium alginate and calcium chloride. Standardization of sodium alginate for recovering the best formulation for the hydrogels was carried out by texture analysis.

The quantities of calcium chloride and the core material were kept fixed, while the percentage concentration of sodium alginate was varied to obtain the right texture (**Table 1**).

**Table 1** Identification of sodium alginate concentration for hydrogel preparation

Requirement	Suitable texture of calcium-alginate beads									
Sodium alginate (%)	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	2.50
CaCl <sub>2</sub>	0.15 M									
Core material	500 mg microgreens of <i>Vigna radiata</i> dissolved in 5 ml distilled water									
Bead texture	-	-	Very loose	Loose	Loose	Semi-hard	Semi-hard	Hard	Very hard	Very hard



**Figure 1** Hydrogels formed with different concentrations of sodium alginate (0.75–2.5%; a–h)

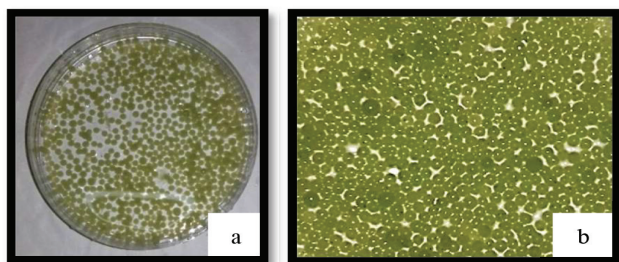
The formulations with sodium alginate at a higher concentration (1.75 to 2.5%) were not only good for cross-linking with the calcium ions in  $\text{CaCl}_2$ , but were also acceptable in terms of the recovery of the maximum number of better-quality beads, compared to formulations with a reduced concentration (1 to 1.5%). Texture analysis showed that hydrogels formed using 1.75% sodium alginate were semi-hard while the beads assumed a very hard structure as the concentration of sodium alginate was increased (2.25 and 2.5%), which could have a negative impact on the dissolution and release rate of core materials (Fig. 1).

Thus, a formulation with 2% sodium alginate was considered adequate for producing and retrieving better quality hydrogels. These hydrogels were discrete, large and of a uniform spherical size, and were free-flowing with the capacity to store water. Previous studies have reported that the size can be reduced with either higher levels of calcium chloride or by altering the reaction time<sup>[14]</sup>.

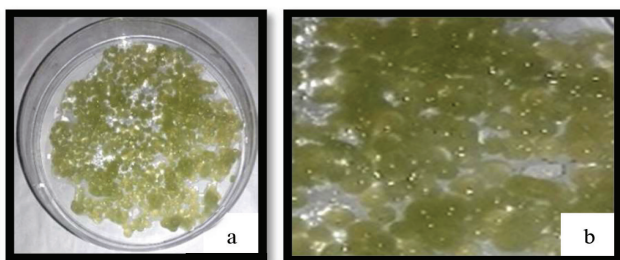
**Identification of the best cationic component**  
Calcium, chitosan and an equimolar mixture were tested as cations and the results are given in **Table 2**.

Method		Ionotropic gelation		
Hydrogels		Calcium-alginate	Chitosan-alginate	Calcium-chitosan-alginate
Coating material	Anionic component	Alginate		
	Cationic component	Calcium	-	Calcium
		-	Chitosan	Chitosan
Core material		Microgreens of <i>Vigna radiata</i> (500 mg) in distilled water (5 ml)		
Characteristics		No clumping of beads	High percentage of clumping	Low percentage of clumping
		Easy to recover (99%)	Difficult to recover (<10%)	Moderately difficult to recover (50%)
Bead texture		Small, uniform, individually separated, distinct and free-flowing particles	Coarse and unseparated	Not uniform, large, clumped particles

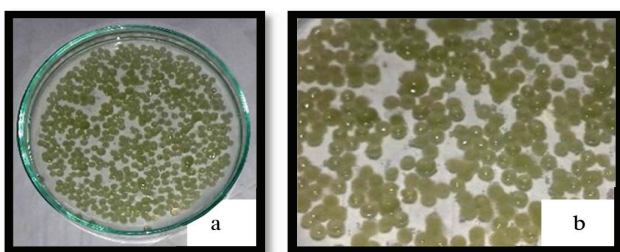
**Table 2** Identification of the best cation component for hydrogel preparation



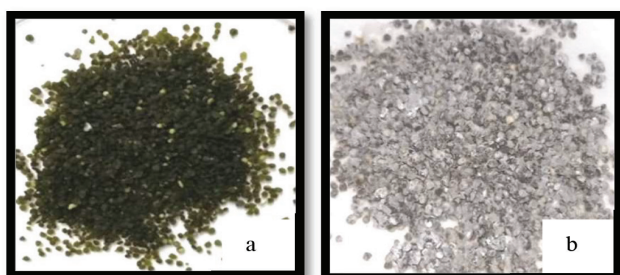
**Figure 2** Calcium-alginate hydrogels (a, b)



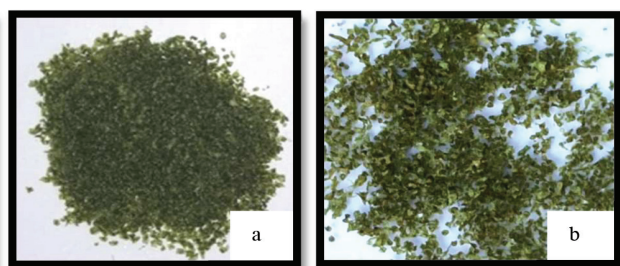
**Figure 3** Chitosan-alginate hydrogels (a, b)



**Figure 4** Calcium-chitosan-alginate hydrogels (a, b)



**Figure 5** Xerogels obtained by evaporation of hydrogels under room temperature (a) and 1 month after development (b)



**Figure 6** Xerogels obtained by the lyophilization of hydrogels (a) and 1 month after development (b)

Calcium ions were found to be the better cationic component compared to chitosan for cross-linking with alginate in order to form superior hydrogels (Fig. 2–4).

### Preparation of xerogels (microcapsules)

Hydrogels obtained from 500 mg of microgreens of *Vigna radiata*, 2% sodium alginate (10 ml) and 0.15 M CaCl<sub>2</sub> (100 ml) by utilizing the ionotropic gelation method were subjected to evaporation under ambient temperatures and lyophilization in order to form long-shelf-life xerogels (microcapsules).

Microcapsules obtained by the evaporation of calcium-alginate hydrogels under ambient temperatures became damaged after 1 month whereas microcapsules obtained by lyophilization did not show any signs of deterioration (Fig. 5 and 6). Hence, lyophilization can be utilized for the production of well-defined, discrete and small-sized capsules with a better shelf life.

### Characterization of microcapsules

A marked difference in weight (7665 mg to 266.35 mg) was observed when the hydrogels shrank in size to the xerogels, indicating a considerable reduction in water content (96.525%, Table 3).

These microcapsules were found to swell in SGF (pH 1.2) and had a swelling index of 5.76, suggesting a high water-holding capacity.

S.No	Characteristics of microcapsules	
	1	Weight of hydrogels
2	Weight of xerogels	266.35 ± 0.31 mg
3	Water loss during hydrogel to xerogel conversion	96.525 ± 0.29%
4	Swelling index	5.76 ± 0.2 (at pH 1.2)
		Complete dissolution (at pH 7.4)

**Table 3** Physicochemical characterization of microcapsules

This may be due to the penetration of water through the capsule surface causing weight gain from hydration of the hydrophilic groups, as previously described by Patel *et al.* [6].

This was followed by complete dissolution of calcium-alginate cross-linking, and thereby, release of the core ingredient in SIF (pH 7.4).

The estimation of vitamin content in the microcapsules by the digestion method showed the release of 1.634 mg, 1.78 mg, 3.92 mg, 8 mg and 0.372 mg of thiamine, riboflavin, niacin, vitamin C and carotene, respectively (Fig. 7).

### In vitro dissolution study of microcapsules

Sustained release of the vitamins from the microcapsules occurred over an observation period of 6 hours. No burst effect was observed for the microcapsules under gastric conditions and only a small fraction of vitamins was measured in the fluid (Fig. 8–12). Incubation in SGF resulted in the escape of calcium from the microcapsules and the conversion of calcium alginate into insoluble alginic beads, as

reported previously by Sabitha *et al.* [7].

Vitamin measurement following the incubation of the microcapsules in SIF (at a pH of both 6.8 and 7.4) showed controlled and sustained vitamin release over a period of 4 hours.

Thus, under *in vitro* conditions, it is evident that a major portion of the enclosed vitamins are released into the intestinal environment (SIF) compared to the gastric environment (SGF). These results indicate the swelling of capsules in the stomach and the potential for dissolution in the intestine.

It can be concluded that sodium alginate is a pH-sensitive polymer [15], which can be used to prevent core discharge in gastric fluids and to deliver the core materials specifically to the intestine. Once vitamin release was initiated, the amount released was found to increase in a linear pattern over time, indicating that the release mechanism of these microcapsules is diffusion-controlled.

The release of thiamine from the microcapsules was noted over a continuous period of 6 hours and the data are presented in Fig. 8.

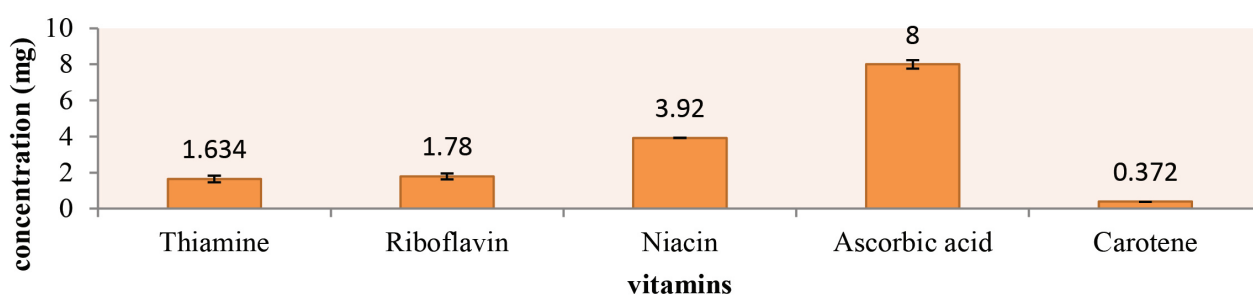


Figure 7 Vitamin content of microcapsules (digestion method)

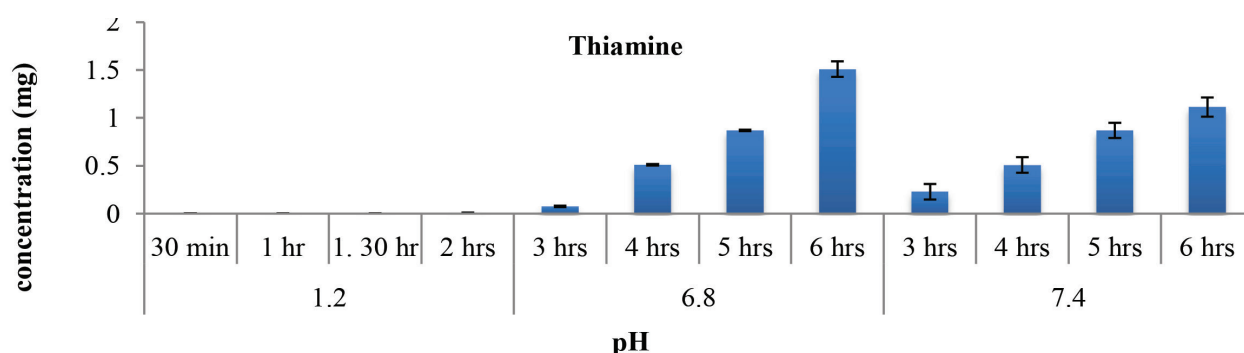


Figure 8 Release profile of thiamine from microcapsules

A negligible amount of thiamine (0.0031 to 0.0076 mg) was released when microcapsules were incubated over the first 2 hours under gastric conditions (SGF at pH 1.2). Incubation of the microcapsules over the next 4 hours in intestinal conditions (SIF at pH 6.8 and 7.4) resulted in controlled and sustained release of a greater quantity of thiamine (1.51 and 1.115 mg, respectively). The release was more controlled at the higher pH (7.4). The rate of riboflavin release from the microcapsules over a period of 6 hours is presented in Fig. 9. The amount released under gastric conditions (SGF) over the first 2 hours was negligible (0.002 to 0.01 mg), while incubation of

the microcapsules over the subsequent period of 4 hours under intestinal conditions (SIF at pH 6.8 and 7.4) resulted in controlled and sustained release up to 1.7 and 1.524 mg, respectively. The rate of niacin release from the microcapsules over 6 hours is presented in Fig. 10. The release rate under gastric conditions (SGF) over the first 2 hours was very low (0.02 to 0.04 mg), whereas incubation under SIF conditions (pH 6.8 and 7.4) over the next 4 hours resulted in high release levels (up to 3.69 and 2.391 mg, respectively). The release pattern for the microcapsules was noted for vitamin C over an incubation period of 6 hours and is shown in Fig. 11.

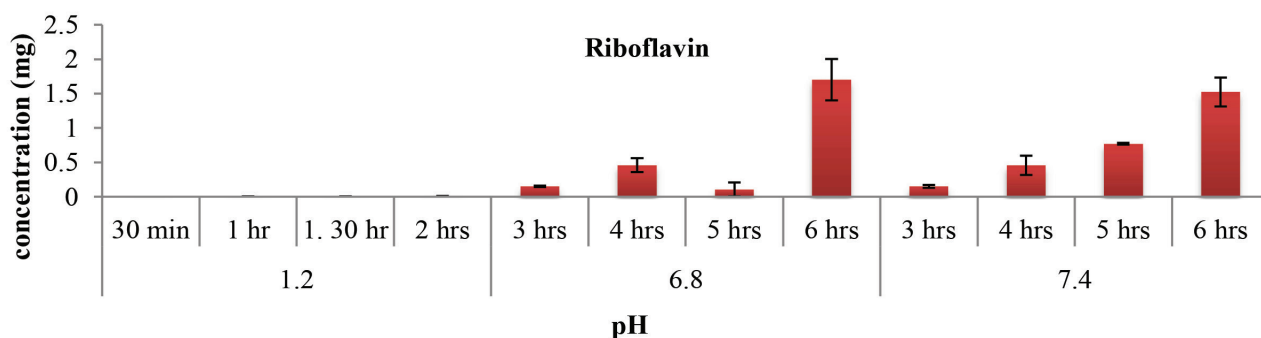


Figure 9 Release profile of riboflavin from microcapsules

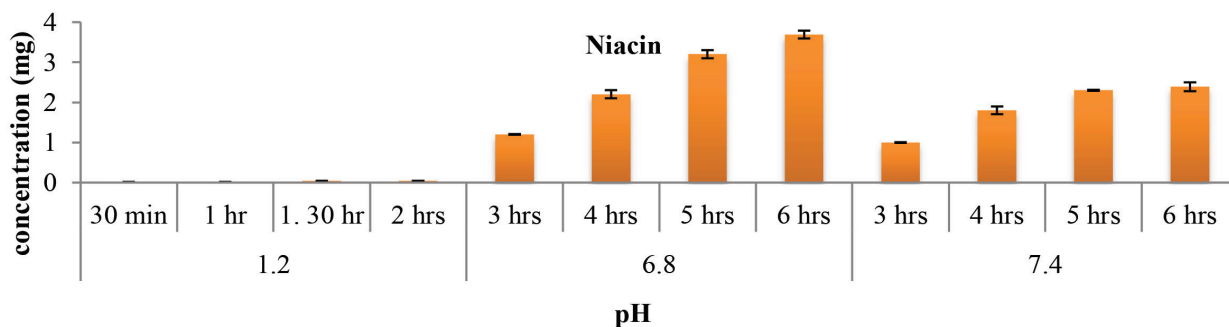


Figure 10 Release profile of niacin from microcapsules

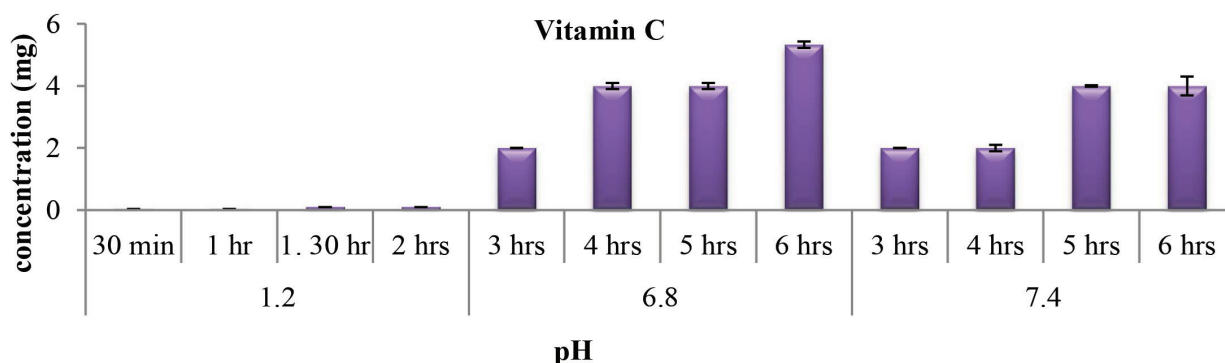
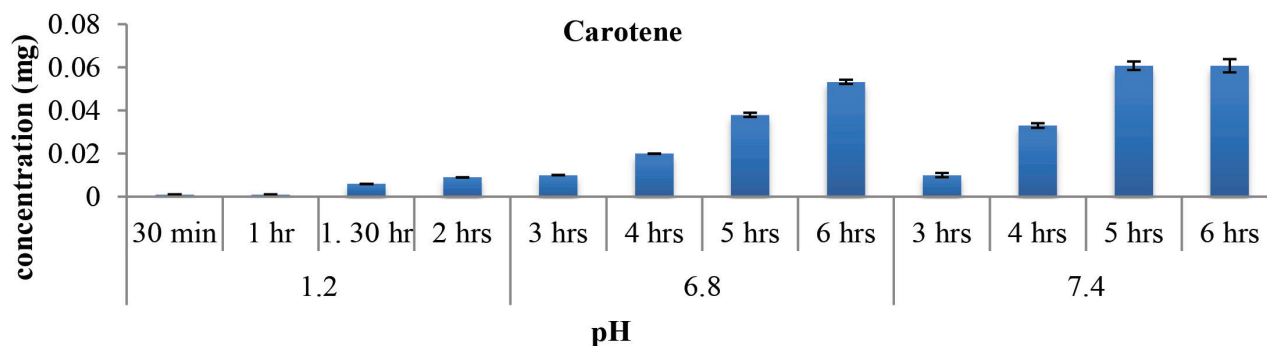


Figure 11 Release profile of vitamin C from microcapsules





**Figure 12** Release profile of carotene from microcapsules

The release rate under gastric conditions (SGF) over 2 hours was very low (0.04 to 0.1 mg), whereas under SIF conditions (pH 6.8 and 7.4) it was higher, with levels of 5.33 and 4 mg recorded, respectively. Thus, it was observed that a major portion of the enclosed vitamin C was released into the intestinal environment compared to the gastric environment.

Results obtained from the *in vitro* dissolution study regarding the estimation of carotene release from microcapsules are shown in Fig. 12.

A sustained release of carotene from the microcapsules was observed over a period of 6 hours. Only a small fraction of carotene was estimated in the SGF fluid over 2 hours (0.001 to 0.009 mg), while comparatively higher quantities of carotene (0.0532 mg and 0.0607 mg) were released into the intestinal environment (SIF at pH 6.8 and 7.4, respectively).

## Conclusion

The ionotropic gelation process followed by lyophilization, utilizing calcium and alginate as coating elements, appears to be a feasible mechanism for transforming *Vigna radiata* microgreens to natural, biodegradable and vitamin-enriched microcapsules or dietary supplements with a longer shelf life and an additional property of sustained release.

## Acknowledgements

The authors are grateful to the Head of the Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India for providing the necessary research facilities during this work.

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