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Microencapsulation of Vitamin D by Complex Coacervation Using Soy Protein Isolate and Cress Seed Mucilage

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Abstract

Vitamin D plays a significant role for human health, survival and fertility. Several studies have focused on preventing diseases such as heart, immune and skeletal disorders, and infectious using vitamin D. In this study, microencapsulation process of vitamin D by complex coacervation method was investigated using cress seed mucilage as an indigenous hydrocolloid and soy protein isolate, and effects of core to shell and protein to polysaccharide ratios were evaluated. The results showed that both parameters had significant effects on the encapsulation efficiency and loading capacity ($P < 0.05$). Using the ratios of core to shell and protein to polysaccharide of 0.3 resulted in the production of microcapsules with the best functional properties. The microencapsulation efficiency and loading were in the range of 20-90 and 2-27%, respectively. Scanning electron microscopy indicated that microcapsules were almost non-spherical and had rough surfaces. The mean particle size was 57.2 ± 1.2 . The analysis of Fourier infrared transformation spectrometry confirmed the presence of vitamin D in the produced microcapsules and interaction of cress seed mucilage and soy protein isolate. The results of this study suggested the possibility of using cress seed mucilage and soy protein as domestic and low-cost hydrocolloids for encapsulation of hydrophobic compounds.

Keywords: Cress Seed Mucilage, Microencapsulation, Soybean Protein Isolate, Vitamin D

Introduction

Vitamin D is a fat-soluble bioactive that plays an important role in bone health. Vitamin D deficiency leads to bone softness; while, its excessive intake increases the risk of hypercalcemia and kidney problems (Verkaik-Kloosterman, Seves, & Ocké, 2017). This vitamin is usually found in two forms of D₂ (ergocalciferol) and D₃ (calciferol) (Park, Garcia, Shin, & Kim, 2017). However, this vitamin is susceptible to some environmental conditions such as high temperature, oxidation and acidic media (Verkaik-Kloosterman *et al.*, 2017).

Encapsulation is a process in which bioactive compounds or cells in forms solid, liquid or gas are protected against environmental harsh conditions in a homogeneous or heterogeneous coating shell(s). Materials that are encapsulated are called cores and those used for coating are named shells (Azevedo, Bourbon, Vicente, & Cerqueira, 2014; Jafari, Assadpoor, He, & Bhandari, 2008). Encapsulation protects bioactive components from adverse conditions. In addition, sensory features are enhanced and unpleasant flavors are masked (Zhu, 2017). Coacervation method which is also called phase separation that two or more polyelectrolytes with opposite charges are mixed under suitable conditions, two liquid phases are formed, including the supernatant and mass phases (Thies, 2007). Therefore, the aims of this research were using coacervation technique for encapsulation of vitamin D by soy protein and cress seed mucilage and investigating properties of microcapsules.

Materials and methods

Extraction of mucilage was performed according to Karazhiyan *et al.* (2009). Microcapsules were prepared according to method of Kavousi, Fathi, & Goli (2017). An oil dispersion of vitamin D (10% Vitamin D in sunflower oil) was added to a 3% soy aqueous solution at 25 °C and mixed with a homogenizer at 18000 rpm for 4 min. The pH was increased with NaOH (0.1 M) to 8, then the aqueous solution containing 1% cress mucilage was added at 40 °C and the system stirred for 10 min. The pH was adjusted to 3.4 with a solution of chloric acid (0.1 M).

The yield of production of microcapsules was determined by weighing of solid particles after drying with a digital balance (0.0001 g) for a specific volume of solution. To measure the encapsulation efficiency and load of vitamin D, amount of non-encapsulated vitamin D was measured

To study the morphology of the particles, a scanning electron microscope (SEM: Philips, XL30, Holland) was used. Samples were covered with a layer of gold before imaging (Peng *et al.*, 2014). Size of microcapsules was determined by image analysis using ImageJ software for at least of 20 particles.

To evaluate chemical interactions of functional groups, FTIR spectra of CSM powder, gelatin, vitamin D and CSM-gelatin microparticle loaded by vitamin were obtained by Fourier transform infrared spectrophotometer (Jasco FTIR-680 plus, Japen) with a wavenumber range of 400-4000 cm^{-1} . The samples were mixed with potassium bromide (KBr) in ratio of 1:100 and pills were formed (Butstraen & Salaün, 2014).

Results and discussion

In order to optimize the production conditions of microencapsulation, the effects of ratio of core to shell (10, 20 and 30%) and ratio of protein to polysaccharide (0.1, 0.3 and 0.5) were analyzed.

As indicated in the Table (1), one of the important factors affecting the properties of coacervates was volume ratio of biopolymers due to its effect on changing ionic strength of the system and interaction of biopolymers (Dubin, Bock, Davis, Schulz, & Thies, 2012). The highest values of encapsulation efficiency and load are desirable. With increasing core to shell and decreasing SPI/CSM ratios the values of EE and LE increased. The formulation containing 30% core and SPI/CSM ratio of 3:10 had the highest EE and LE.

Table 1. Characteristics and their normalized values of vitamin D loaded SPI/CSM microcapsules.

No.	Core/ Shell ratio	SPI/CSM ratio	Yield (%)	EE (%)	EL (%)	N. Yield	N. encapsulation efficiency	N. encapsulation loading	Sum of normalized values
1	0.10	1	78.05±1.50	20.65±1.30	2.35±0.70	0.7911	0.222	0.074	1.087
2	0.20	1	94.80±0.40	45.60±1.20	8.95±0.10	0.949	0.50	0.333	1.783
3	0.30	1	97.83±1.34	69.80±1.40	21.57±0.80	0.975	0.783	0.783	2.542
4	0.10	0.50	99.05±4.20	46.00±2.00	4.60±0.30	1.00	0.50	0.166	1.666
5	0.20	0.50	88.90±1.80	66.25±1.50	13.18±0.16	0.901	0.727	0.485	2.114
6	0.30	0.50	93.15±0.90	84.90±0.60	25.60±0.80	0.939	0.933	0.933	2.806
7	0.10	0.30	95.30±2.10	62.90±0.90	6.50±0.60	0.947	0.694	0.231	1.873
8	0.20	0.30	91.10±2.10	85.50±1.10	17.40±0.80	0.926	0.944	0.629	2.500
9	0.30	0.30	87.02±3.40	90.60±1.20	26.60±0.70	0.891	1.00	1.00	2.891

SPI: Soy protein isolate; CSM: Cress seed mucilage; EE: Encapsulation efficiency; EL: Encapsulation loading; N: Normalized number

Morphological study

Fig. (1) showed the SEM images of optimized microcapsules. The microcapsules had dissimilar shape with irregular surface. The presence of teeth and rugged surfaces can be attributed to the drying process in the freeze dryer. Similar results were reported by Kavousi *et al.* (2017) for encapsulation of fish oil in hydrogels of cress seed mucilage and chitosan.

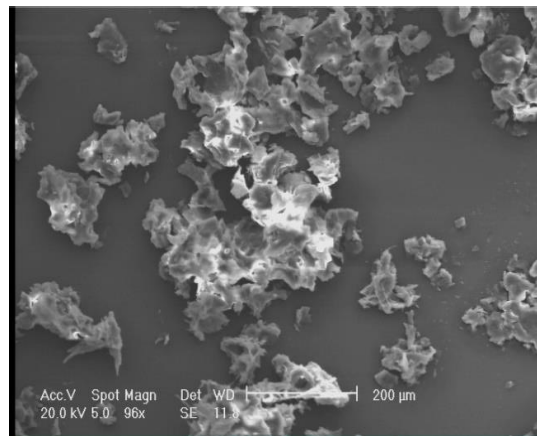


Fig. 1. Scanning electron microscopy of vitamin D loaded microcapsules.

Fourier transform infrared spectroscopy (FTIR)

The spectra obtained from the analysis of soy protein isolate-cress mucilage microcapsules containing vitamin D, CSM powder, SPI and vitamin D were shown in the Fig. (2). CSM spectrum showed peaks at 1620 and 1420 cm^{-1} that were related to the tensile vibrations of symmetric negative carbonyl (C=O). The peak in 3416 cm^{-1} was tensile vibrations of O-H. Symmetric and asymmetric peaks of the C-H group at 2924 cm^{-1} and weak tensile vibrations related to C-O at 1128 cm^{-1} , were other main peaks of the mucilage (Karazhiyan *et al.*, 2009). The infrared spectrum of soy protein had a very weak peak in 1100 cm^{-1} corresponding to bending vibrations of the C-H or PO₂ and POH of serine amino acids. Different peaks at 1450, 1230, 1530 and 1630 cm^{-1} were attributed to the C-H and N-H in amide type III, vibrations of C-H, vibration of N-H in amide type II and tensile vibrations of the carbonyl group (C=O) in amide, respectively (Schmidt, Giacomelli, & Soldi, 2005). For vitamin D spectrum, there were clear peaks at 1632 and 1167 cm^{-1} which were related to tensile vibrations of carbonyl C=O and C-O groups, respectively. In addition, the tensile vibration peaks of the C-H group were detected in 2868 and 2938 cm^{-1} , respectively (Kiani, Fathi, & Ghasemi, 2017).

It should be noted that all main peaks of ingredients were found in spectra of produced microcapsules which showed that only physical interactions occurred between materials.

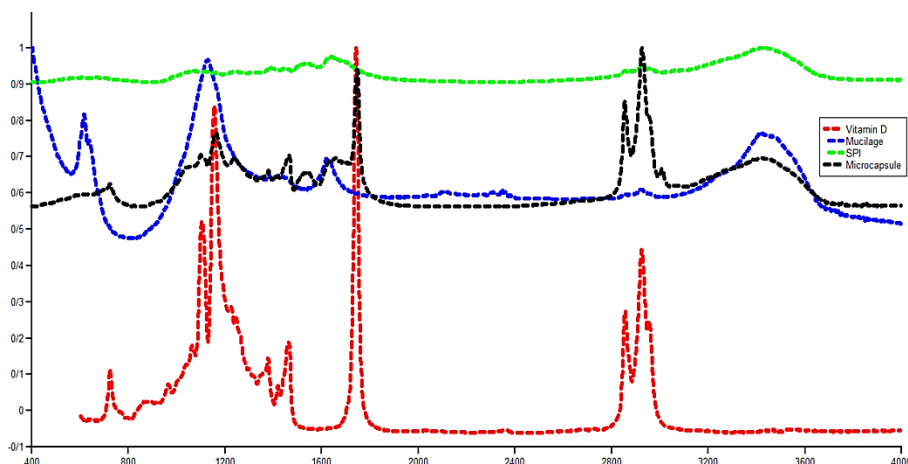


Fig. 2. FTIR spectra of vitamin D, cress seed mucilage (CSM), soy protein isolate (SPI) and produced microcapsules

Conclusions

Vitamin D plays a significant role in human health. In this study vitamin D was encapsulated using cress seed mucilage (CSM) and soy protein and its physicochemical properties were investigated. The values of encapsulation efficiency, encapsulation load and yield of production for the optimum microcapsule were 90, 27 and 88.75%, respectively. The results showed that ratio of core to shell and soy protein/CSM had significant effects ($P < 0.05$) on properties of microcapsules. Scanning electron microscopy showed that particles were not generally uniform with rough surfaces. The infrared Fourier transformation spectroscopy confirmed the presence of vitamin D in the produced microcapsules. Regarding the role of vitamin D in the health and its deficiency in many societies, the produced microcapsules can be used for enrichment of foods.

Acknowledgments

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