Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Use of chickpea protein for encapsulation of folate to enhance nutritional potency and stability

Isuru R. Ariyarathna, D. Nedra Karunaratne[*]

Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka

ARTICLE INFO

Article history:
Received 20 April 2014
Received in revised form 13 January 2015
Accepted 12 April 2015
Available online 18 April 2015

Keywords:
Chickpea protein
Isoelectric point
Folate
Protein microencapsulate
Nutrient delivery
Thermal stability

ABSTRACT

Proteins have been used to produce capsules and shell materials for food applications. The use of protein in food applications is beneficial from the point of view of biocompatibility, non-toxicity as well as nutritional advantage. The procedures used for the preparation of protein capsules have yielded micro sized encapsulates. This study reports a simple method to produce a protein microencapsulate for carrying nutrients such as vitamins in food preparations. The protein used in this study was isolated from chickpeas. Based on the amphoteric property of the protein, folate (vitamin B9) was encapsulated efficiently from a homogenous protein folate solution mixture, by acidification to the isoelectric point of the protein to obtain the microencapsulate. This yielded a free flowing powder after freeze drying. The encapsulation efficiency and loading capacity were calculated to be 62.19 ± 2.05% and 10.18 ± 0.89%, respectively. The release of the folate was monitored at pH values ranging from 2 to 8. In all cases gradual release was observed. Encapsulation imparted greater stability to folate relative to unencapsulated folate. Therefore protein encapsulation is beneficial for improving the stability of folate in processed food items. This simple method for production of protein microencapsulate can be used to prepare various types of nutriment and drug encapsulations.
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1. Introduction

Delivery of small molecules using microparticles or microencapsulates has been the focus of interest in the areas of pharmaceuticals and nutrient supplements. The vehicle used for delivery plays an important role in determining the protection of the cargo, the delivery properties such as controlled or sustained release and the site of delivery. Carriers ranging from synthetic polymers to natural biopolymers have been investigated for their versatility in delivery. Protein has been reported as a good delivery vehicle for the formation of microcapsules in the food industry where whey proteins, soy proteins, vegetable proteins and zein proteins have been studied to a great depth for microcapsule formation (Chen et al., 2006; Wang et al., 2011; Lazko et al., 2004; Augustin et al., 2006). There are reports of protein microencapsulates as drug delivery vehicles where proteins such as gelatin, albumin, gliadin and legumin were used as the carrier (Jahanshahi and Babaei, 2008). The advantage of protein carriers as vehicles is in their biocompatibility and biodegradability. They are GRAS (generally recognized as safe) in food applications and do not cause toxicity or side reactions. Protein applications in the food industries are generally limited to soybean seeds, while other cereal proteins are less used. Chickpea (*Cicerarietinum L.*) is a cereal grown extensively in different parts of the world. Among cereals, the amount of protein present in chickpea is only less than that present in soybean (Jukantil et al., 2012; Londhe et al., 2011). Chickpea protein contains major fractions of 11S legumins and 7S vicilins and minor fraction of 2S albumin. The protein digestibility percentage of chickpea protein ranges between 95 and 96 and the isoelectric point pH of the protein is 4.5 (Chang et al., 2012; Mansour, 1996).

* Corresponding author. Tel.: +94 81 2394450; fax: +94 81 2388018.
E-mail address: nedrak@pdn.ac.lk (D. Nedra Karunaratne).
http://dx.doi.org/10.1016/j.fbp.2015.04.004
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Folate is a vitamin required for DNA synthesis with the added advantage of use in tumor therapy as a targeting agent towards cancer cells and reduction in neural-tube defects (Crott et al., 2001; Wals et al., 2007; Daly et al., 1997). Also it has been recorded that folate reduces the plasma homocystine levels of patients with coronary heart diseases (Malinow et al., 1998). Folate deficiency can lead to several disorders in humans such as birth defects and also disparities in hair, skin and fingernail pigmentation (Bender, 2003; Shils et al., 2005).

The main objective of this study was to prepare a protein microencapsulate incorporating folate. Folate was encapsulated or encased in protein as the biopolymer for the purpose of improving the stability of folate towards heat and light. Also the encapsulation may ameliorate the bioavailability and adsorption of folate in the gut (Brouwer et al., 2001). The effect of changes in pH of the gastrointestinal tract towards the release of folate from the protein microencapsulate could contribute to sustained or controlled release of folate.

2. Materials and methods

NaOH, concentrated sulfuric acid, HCl (analytical grade), folic acid, Hexane (chemical grade), KBr, NaCl, KH_2PO_4, $CaCl_2$, KCl and pepsin were purchased from Sigma Chemicals. Chickpeas were purchased from a supermarket.

2.1. *Folate encapsulation using chickpea protein*

Chickpea protein was isolated using alkaline extraction and freeze dried according to a previous study (Sanchez et al., 1999). Isolated chickpea protein (0.1 g) was dissolved in 50 ml of 1 M NaOH solution in a beaker. Then 0.1 g of folate was dissolved in the protein solution. The resulting solution was acidified to the isoelectric point of the protein (pH 4.5) using 1 M HCl solution under rapid stirring until a precipitate was observed. After centrifugation and removal of supernatant, precipitate was freeze dried in a 600 ml LABCONCO flask for 24 h using an Edwards freeze dryer. Procedure was performed in triplicate to obtain three different encapsulated samples. The samples obtained were free-flowing powders.

2.2. *IR analysis*

FTIR spectra were obtained by using KBr pellets on a Shimadzu IR Prestige-21 instrument. Each KBr pellet was made by mixing sample in fused KBr keeping the ratio 1:100, respectively. IR analyses were carried out for folate, isolated chickpea protein and for the encapsulate.

2.3. *Determination of encapsulation efficiency and loading capacity*

To determine the folate encapsulation efficiency the supernatant from the centrifugation of the encapsulated protein was collected for each of the three samples. The amount of folate in the supernatant was quantified using UV atomic absorbance spectroscopy by a Shimadzu-1800 instrument. The following equation was used in the calculation:

$$\text{Encapsulation Efficiency\%} = \frac{[F_{tot} - F_{sup}]}{F_{tot}} \times 100$$

where, F_{tot} and F_{sup} are moles of total folate and moles of folate in supernatant respectively.

To determine the moles of folate entrapped in the protein microencapsulate, the loosely bound folate was removed by washing the protein encapsulate in deionized water. Then the encapsulate was broke open with 10 M NaOH solution to release the entrapped folate. The released folate concentration was measured by UV spectroscopy using a standard folate calibration curve of 1, 5, 10, 15, 20, 25 mg l^{-1}.The wavelength of maximum absorption (λ max) was observed at 284 nm and selected for the estimation.

Loading capacity was determined using the following equation.

$$\text{Loading Capacity\%} = \frac{\text{Entrapped folate amount}}{\text{Weight of spheres used}} \times 100$$

2.4. *Size analysis of the folate encapsulate*

Size distribution of the encapsulate was determined under two different wet conditions, one in a 4.5 pH buffer and other in ethanol. A dispersion of encapsulate (0.2 g) in a buffer solution (10 cm^{-3}) was prepared, 2 cm^{-3} of that was placed in the sample cell holder in a CILAS particle size analyzer and operated. Another dispersion of encapsulate (0.2 g) in an ethanol solution (10 cm^{-3}) was placed in the wet dispersion unit of the Laser Particle Sizer ANALYSETTE 22 Micro Tec plus instrument and operated under optimal ultrasonic intensity of 40 kHz.

2.5. *Surface analysis of the folate encapsulate*

The detailed outer topography of the encapsulate was studied by SEM. SEM was carried out by mounting the microencapsulate in to specimen stub of a SU 6600 Hitachi scanning electron microscope operated at 5.0 kV. Micrographs were prepared using an industry standard 4 × 5 Polaroid film.

2.6. *Determination of swelling property*

The swelling properties of folate encapsulate was investigated at 25 °C. Dry spheres (0.1 g) were placed in 50 ml test tube containing 30 ml of pH 4.5 HCl solution. In order to determine the degree of swelling, spheres were separated, periodically, from the dispersing medium and their surfaces were lightly dabbed with a soft tissue paper to remove excess of free dispersing medium. Then their weight was recorded and encapsulated sample was reintroduced to the dispersing medium. The swelling ratio was calculated by the formula, Swelling ratio = $\frac{[W_w - W_d]}{W_d}$

where, W_w and W_d were the weight of wet folate encapsulated spheres at time t and weight of dry encapsulated sample (at time t = 0), respectively. Procedure was repeated at pH 5.5 and 6.5 HCl solutions. The same procedure was followed for the pure protein sample as well.

2.7. *Determination of releasing property of the microencapsulate*

To measure release of folate, the microencapsulate were suspended in pH 8 phosphate buffer solution, inserted in to a dialysis bag at 37 °C and dialyzed against pH 8 phosphate buffer solution. While stirring, 3 ml aliquots of the dialyzate were removed at 30 min intervals, absorbance checked at

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