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Preparation and characterisation of protein based nanocapsules of bioactive lipids

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ABSTRACT

In the present work nanocapsules based on egg albumin and soy protein encapsulants were prepared. The droplet-size of the synthesized emulsions was determined soon after their preparations and soy protein emulsions had lower particle-size [(196.4 ± 0.139) nm] in comparison to albumin emulsions [(205.2 ± 0.102) nm]. The dried emulsions were subjected to morphology, encapsulation efficiency, diffraction pattern, release and stability studies. The encapsulation technique was successful in terms of production of lipid loaded egg albumin and soy protein nanocapsules with encapsulation efficiencies (90.4 ± 0.224)% and (91.5 ± 0.315)% respectively. Diffraction patterns suggested a considerably high lipid affinity of soy protein as compared to albumin. Moreover release rate was found to be higher for albumin than soy protein nanocapsules, though both followed non-Fickian diffusion pattern. Stability in terms of fat retention was higher for soy protein than albumin nanocapsules with respect to prolonged period of exposure to humid atmospheres.

1. Introduction

Nanoencapsulation has been employed by the food industry to deliver sensitive nutraceuticals through food without detracting from its sensory properties (Couvreur et al., 1995). Encapsulating bioactive ingredients in various matrices also has the potential to deliver these components to various parts of the gastrointestinal tract (Champagne and Fustier, 2007). For different bioactive products to deliver a health benefit, it is important that these ingredients reach the desired site of action within an appropriate time span when consumed. The process for nanoencapsulation of lipids consists of two steps; first is the emulsification of a core material lipid, with a dense solution of a wall material in water. The second step is drying of the emulsions to remove the aqueous dispersion media.

The technique of hydrocolloid formation is mostly used for flavour encapsulation. In general, starch, carbohydrates or various biopolymers have been used as encapsulating materials. However food proteins have apparently not been used extensively for the purpose of encapsulation (Kim et al., 1996). Because of their different chemical make-up, ability to self-associate and interact with a variety of different types of substances, proteins have excellent functional properties such as solubility, viscosity, emulsification, and film-forming properties which would make them capable of being used in encapsulation. The resulting steric-stabilizing layer immediately protects the oil droplets against re-coalescence and thereafter provides physical stability to the emulsion during processing and storage (Dalgleish, 1997). Due to their functional properties proteins are of interest for nutrient encapsulation. However, little work has been reported on the nanoencapsulation of bioactive lipid compounds using egg albumin and soy protein as encapsulant. Although there has been extensive research on the binding of flavour compounds with such proteins (Guichard, 2002) there is not adequate mention in literature about their application in the encapsulation of nutraceutical products.

Soy protein has gained considerable attention for its potential role in improving risk factors for cardiovascular disease. Egg albumin again was used to prepare microspheres containing aceclofenac by heat denaturation method (Deveswaran et al., 2010). The prepared microspheres showed long term stability both in terms of particle-size and drug content. However, recently, it has been shown that the rate of release of sunflower oil from a composite matrix of egg albumin and cassava starch depended on solvent type, the relative humidity and temperature under which the film was dried (Wongsasulak et al., 2004).

The major challenge in developing a protein encapsulant for a nanoencapsulation system is its extreme instability. It is widely known that the chemical and mechanical stresses produced during the nanoencapsulation process and the release period exert damaging effects on the conformational and biological integrity of the

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protein (Park et al. 1998; Van de Weert et al., 2000; Schwendeman, 2002). In addition, if the proteins are exposed to the acidic and/or hydrophobic environments for a long period of time, they undergo irreversible aggregation and/or degradation and non-specific adsorption and become unavailable for release (Zhu and Schwendeman, 2000; Lu and Park, 1995). This protein instability issue is closely related to the incomplete release issue.

In the present study, an attempt was made to evaluate the efficacy of nanoencapsulation of bioactive lipid with two different protein encapsulants, soy protein and egg albumin. The prepared nanocapsules were compared with respect to their structural morphology, diffraction patterns, *in vitro* release potential, and storage stability. The functional attributes of each variety of nanocapsules could be designated to the respective encapsulant used. The protein encapsulation of bioactive lipids could be very useful in developing specialty food products where delivering the good lipids forms a desired criteria.

2. Materials and methods

2.1. Materials

Soy protein acid hydrolysate was purchased from Fluka analytical, Sigma–Aldrich, Co., St. Louis, MO, USA. Egg albumin was purchased from B.V. Bio-Corp Private Ltd., Ambadwet, Pune, India. All other reagents were of analytical grade and procured from Merck India Ltd., Mumbai, India.

2.2. Methods

2.2.1. Emulsion preparation

20% (w/v) aqueous solutions of soy protein hydrolysate and egg albumin were prepared separately in distilled water. Emulsions were prepared by mixing each of the two aqueous solutions of the proteins with linseed oil, added drop wise, while constantly stirring to get a 10% core material content. Here core to coating material ratio was maintained at 1:2 weight ratio, which would entail complete encapsulation of the core material (optimized after repeated experiments). Prior to the optimisation process, the prepared emulsions were individually treated against several salt stressed situations and it was observed that breaking of emulsions occurred not before 90 days on subjecting to a sodium chloride stressed situation of 100 mM concentration. The optimized formulation after the stress treatments was used for synthesis of the protein emulsions.

For developing the emulsions, the fat phases were blended with the aqueous solutions using high speed stirrer (OMNI International GLH; Model: GLH-220, Ser. No. 76014) at 30,000 rpm for 1 min, to give pre-emulsions. The resultant emulsions were further homogenized at 8000 rpm with 6 re-circulations using the homogenizer (Type: RQ-127A, Remi Motors Ltd., Mumbai-53, India) at 1.1 Amp and 220 V.

2.2.2. Size reduction, particle-size analysis and microscopic observation of the emulsions

The resulting coarse pre-emulsions were immediately passed through high pressure homogenization (NanoDebee [8799], B.E.E. International Inc., Easton, MA 02375, USA) with a hydraulic pressure of 3000 psi and a homogenization pressure of 40,000 psi and 5 cycles at 5 °C. The particle size distribution and mean droplet diameter of the emulsions were measured using dynamic light scattering technique (Nano-ZS, Malvern Instruments, Worcestershire, UK). Mean particle diameters were reported as *Z*-average diameters or the scattering intensity-weighted mean diameter. Samples were diluted prior to making the particle size measurements to avoid multiple scattering effects, using a dilution factor of 1:10 sample-to-deionised water. Data reported is a mean of 3 consecutive readings.

The emulsions were observed under optical microscope (Leitz Laborlux, 12 POL S, Germany). Two slides were prepared. A drop of each emulsion was placed on each of the two slides and then air dried. The slides were then observed under polarized light.

2.2.3. Powder production

Both the emulsions were initially frozen with liquid nitrogen (−196 °C) and then stored overnight at −70 °C. Next day the frozen emulsions were freeze-dried to allow the highest amount of freezable water to crystallize (Cerimedo et al., 2008). An Eyela Freeze Dryer (Type – FD-5N, Ser. No.-10160657, AC100 V, and 50/60 Hz 500 W, Tokyo RIKAKIKAI Co. Ltd., JAPAN] was used for freeze drying which was operated at −20 °C and a chamber pressure of 13.3 Pa. The dried emulsions were crushed with mortar and pestle to get uniform powder-like products.

2.2.4. Efficiency of encapsulation and quality of encapsulated oil

The efficiency of the two protein encapsulants, was determined by estimating the amount of fat encapsulated (Tan et al., 2005). Both types of nanocapsules were washed with hexane (HPLC grade) to remove the non-encapsulated oil from the surface of the nanocapsules. The washing procedure was repeated twice. The solvent was removed by vacuum drying and amount of oil not encapsulated was determined. To measure the amount encapsulated, oil was extracted by acid hydrolysis followed by extracting the oil with hexane. Hexane was then evaporated and internal oil was scaled. Encapsulation efficiency was calculated using the following formula:

$$\text{Encapsulation efficiency} = \frac{\text{Total oil} - \text{Surface oil} \times 100}{\text{Total oil}}$$

Free oil content was then calculated as percentage taking into account the total oil.

A fatty acid compositional analysis of the extracted oil was conducted by means of gas chromatography (AGILENT; Model: 6890N; FID detector; DB Wax column) to ascertain the quality of the encapsulated oil.

2.2.5. Morphology observation

The overall surface morphologies of the dried capsules were observed using scanning electron microscopy (S-3400M, Hitachi, Japan) with Ion Sputter (E1010). Prior to observation, samples were mounted on metal grids, using double-sided adhesive tapes and coated by gold under vacuum.

2.2.6. Crystallinity

Samples were analysed for their crystallinity by X-ray diffraction (XRD). A diffractometer (XPERT-PRO from Panalytical Diffractometer) using Cuα ($\lambda = 1.5406$) as X-ray source. $K_{\alpha 1 \alpha 2 \beta}$ radiation from copper was used at 40 kV and 30 mA. $K_{\alpha 2}/K_{\alpha 1}$ ratio was 0.50000. A scanning velocity of 1°/min from 2° to 80° was maintained. Experiments were performed at ambient temperature (25 °C).

2.2.7. Release study

Measured amount of dried capsules were placed in a glass bottle containing 100 ml dissolution media consisting of phosphate buffered saline (PBS) of pH 7.4. It was incubated in a shaking water-bath at 37 °C, and 100 rpm. At definite time intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours definite amount of aliquots consisting of unhydrolyzed capsules and released oil were withdrawn, filtered and the oil was extracted with hexane. The solids were added back to the reaction mixture. An equal volume of dissolution media was added back to maintain a constant volume. Released oil