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Short communication

Nanoencapsulation of date palm pit extract in whey protein particles generated via desolvation method

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ABSTRACT

An alkaline solution of whey protein isolate was charged with absolute ethanol resulting in precipitation of whey protein particles. The vacuum-dried particles were then dispersed either in water or aqueous ethanol. Heat-treatment of whey proteins before desolvation process decreased the mean size of particles when dispersed in aqueous ethanol from 280 nm to 183 nm. The range and mean size of particles prepared from heat-treated protein solution when dispersed in water were 41–212 nm and 103 nm, respectively. Date palm pit aqueous extract was encapsulated inside the particulating heat-treated whey proteins during the desolvation stage with encapsulation efficiency of ~78%. Extract-loaded particles had mean size of 163 nm in alcoholic dispersion and 92 nm in water dispersion. Scanning electron microscopy imaging showed spherical nanoparticles aggregated in dry state. Fourier transform infrared spectroscopy suggested that extract and whey proteins did not covalently bind. Heat-treatment of whey proteins before desolvation resulted in the absence of denaturation endotherm in differential scanning calorimetry curve of extract-free particles. Extract loading in particles interrupted the continuity of protein matrix causing the occurrence of mild glass transition phenomenon in extract-loaded particles when heated.

1. Introduction

Nanoparticles when used in biological and food systems may provide superior characteristics to microparticles including unique quality, improved sensory properties, extended flavor perception, better mouthfeel, transparent appearance and enhanced processability (Moraru et al., 2003). Therefore, nanobioparticles prepared from generally recognized as safe (GRAS) biopolymers are increasingly nominated for various applications in biomedical, pharmaceutical and nutraceutical industries remembering their acknowledged biocompatibility.

Polyphenols act as metal scavengers, antimutagenes, and antimicrobial agents (Proestos et al., 2005). Their consumption may reduce the risk of some chronic diseases such as cancer, cardiovascular disease, chronic respiratory disease and diabetes (Arts, Van de Putte, & Hollman, 2000; Scalbert & Williamson, 2000). However, incorporation of polyphenols into foods may cause quality defects such as astringent taste and increased haze in beverages. They can act as substrates for browning reactions (Lesschaeve & Noble, 2005) resulting in undesirable color changes in food products. Also, polyphenols and other bioactive substances may undergo degradation and/or deterioration by food processing operations and storage (e.g. heating, acidification, light and oxygen) or in the gastrointestinal tract (acidic pH, enzymes, presence of other nutrients). These drawbacks impose limits for application and potential benefits of these nutraceuticals (Bell, 2001). Nanoencapsulation of bioactive molecules within appropriately structured carriers may overcome these problems without adverse effect on sensory characteristics and appearance of final product.

Desolvation technique is a straightforward, rapid and easily applicable method to carry out the whole nanoparticle preparation procedure in one pot. This technique requires only two miscible solvents without involvement of destructing factors such as high shear rate, heating and sonication that damage the tertiary structure of proteins. As well, the procedure does not include toxic reagents and surfactants (Bilati, Allémann, & Doelker, 2005). Gunasekaran, Ko, and Xiao (2007) used acetone to desolvate β-lactoglobulin and then crosslinked the generated nanoparticles by glutaraldehyde–ethanol mixture. Recently, fish oil was encapsulated in zein nanoparticles desolvated by water from aqueous alcoholic solution (Zhong, Tian, & Zivanovic, 2009). Gülseren, Fang, and Corredig (2012a) generated protein nanoparticles by adding ethanol as antisolvent to an alkaline solution of whey proteins, followed by resolvation of particles through diluting the suspension by aqueous buffers. The procedure was later used to encapsulate zinc chloride within whey protein particles (Gülseren, Fang, & Corredig, 2012b). To the best of authors' knowledge there is no report in the literature on encapsulation of polyphenols in protein nanoparticles prepared by antisolvent (desolvation) method. The objective of the present study was therefore to encapsulate the aqueous extract of date palm pit as a rich source of phenolic compounds within whey protein nanoparticles obtained through desolvating by ethanol. The obtained extract-loaded

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particles are expected to be easily added to beverages with minimum influence on sensory characteristics of food product.

2. Materials and methods

2.1. Materials

Sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol and Folin and Ciocalteau's phenol reagent (mixture of phosphomolybdate and phosphotungstate) were purchased from Merck (Darmstadt, Germany). Lactose- and fat-free whey protein isolate (WPI) with 92% protein content was a kind gift from Arla Food Ingredients (Viby J, Denmark). Bi-distilled water was used throughout the study.

2.2. Preparation of date palm pit extract

Date palm fruit (Kabkab variety) pits were washed and air-dried at 50 °C for 4 h. The dried pits were milled using a heavy-duty grinder to pass 1 mm screen, afterwards, 1 L boiling water was added to 50 g pit powder and extraction was carried out at 30 °C for 7 h while shaken at 100 rpm. The mixture was filtered through a series of Whatman filter papers in order to remove all suspended materials. The extract was freeze-dried and kept in dark glass bottles at −80 °C until use.

2.3. Measurement of phenolic content

Total phenolic content was measured using the Folin–Ciocalteau method (Singleton & Rossi, 1965). Pit extract (30 μL) was mixed with 2.37 mL deionized water and 150 μL Folin–Ciocalteau's phenol reagent and allowed to stand at room temperature for 7 min, then 450 μL sodium bicarbonate (20% w/v) was added to the mixture. After standing for 70 min at room temperature, absorbance was measured (Spectrophotometer BioQuest CE2502, Cecil Instruments Ltd., UK) at 760 nm. Results were expressed as mg gallic acid equivalents (GAE)/100 g sample (Shui & Leong, 2006). Polyphenol content of extract was 1582 mg gallic acid equivalent per 100 g dry weight at pH 6.25.

2.4. Preparation of extract-free and extract-loaded particles

WPI was dissolved (3% w/v) in 10 mM NaCl solution by stirring at 500 rpm at room temperature for 2 h; sodium azide (50 mg/L) was added to prevent microbial growth. The solution was stored at 4 °C for 12 h and filtered through 0.45 μm PVDF syringe filter (Whatman, Germany) prior to use. Protein solution was heat-treated at 60 °C for 30 min (Qi & Onwulata, 2011) and its pH was adjusted to 9.0 with 2 M NaOH. The pH adjustment led to smaller particles based on preliminary experiments. The solution was then charged with ethanol at a rate of 1 mL min^{-1} while stirring at 500 rpm until became turbid. The rate of ethanol addition was controlled carefully since it influences the size of generated particles (Langer et al., 2003). The amount of ethanol added was approximately 3.3 mL per mL protein solution. Nanoparticle suspension was centrifuged at 18,000 ×*g* (refrigerated centrifuge model RS-20IV, Tomy Seiko Co., Ltd., Tokyo, Japan) for 10 min and obtained supernatant was used in measurement of encapsulation efficiency. The resulting nanoparticles were then vacuum dried at 60 °C and stored at −80 °C until analyses.

For preparation of pit extract-loaded particles, WPI solution was supplemented before pH adjustment with 0.045 g or 0.06 g extract powder to obtain 1:20 or 1:15 mass ratio of extract to WPI, respectively. The whole procedure for preparation and separation of nanocapsules was performed the same as particles.

2.5. Particle size measurement

Size and polydispersity of particles and capsules were determined by using a dynamic light scattering particle size analyzer (ZetaPALS, Brookhaven Instruments Co., NY, USA). For this purpose, dried samples were either dispersed in 10 mL ethanol at pH 9.0 or bi-distilled water with pH 9.0 at ratio of 1:200 (w/v) and shaken continuously at room temperature for 12 h using orbital incubator (Stuart®, S150, Guill Bern Corporation Inc., Philippines) to allow complete hydration. To investigate the influence of heat-treatment of WPI on particle size, a sample was also prepared from non-heated WPI following the same procedure as described. Particle size measurements were carried out at 25 °C with laser beam operated at 657 nm and scattering angle of 90°. Each sample was read three times. Average sizes reported are the volume-averaged diameters.

2.6. Scanning electron microscopy

The morphology of dry extract-free and extract-loaded nanoparticles was observed with a scanning electron microscope (SEM, KYKY-EM3200, KYKY Technology Development Ltd, China) operated at 24 kV. The surfaces of particles were sputtered with gold, observed and photographed.

2.7. Particle yield and encapsulation efficiency

The weight of dried pellet of extract-free and extract-loaded particles was used for calculating the particle yield as follows:

$$\text{Particle Yield}(\%) = \frac{\text{weight of dry pellet}}{\text{total weight of extract and WPI used for particles preparation}} \times 100.$$

The efficiency of extract entrapment in particles was calculated as the difference between phenolic content added to WPI solution before desolvation stage and the content remained in the centrifugal supernatant. To determine the holding degree of encapsulated polyphenols within particles, precipitated pellet was dispersed both in 70% ethanol and bi-distilled water, mildly shaken for 30 min and re-precipitated through centrifugation after which the supernatant was used for polyphenol measurement.

2.8. Fourier transform infrared (FTIR) spectroscopy and thermal analysis

FTIR spectra of pit extract, WPI, extract-free and extract-loaded particles were obtained with a Perkin Elmer 2000 FT-IR spectrometer (Perkin Elmer Co., MA, USA) using the KBr disk method. Spectra were obtained in transmission mode from 450 to 4500 cm^{-1} wavenumber range.

The thermal analysis of samples was performed by using a calibrated differential scanning calorimeter (Star System DSC1, Mettler Toledo, OH, USA). For this purpose, each specimen (5 mg) was heated under nitrogen stream (20 mL/min) from 25 to 150 °C at rate of 10 °C min^{-1}.

2.9. Statistical analysis

The data, reported as mean ± standard deviation, are from experiments conducted in triplicate. One-way analysis of variance (ANOVA) was performed using SPSS (ver. 16) software. ANOVA was used to check the assumptions of variance homogeneity and normality and compare the treatment means. Differences among mean values were examined by the least significant difference (LSD) and Duncan's test at $P<0.05$ significance level.