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Effect of high pressure treatment on rapeseed protein microparticle properties and gastrointestinal release behavior of the encapsulated peptides

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ABSTRACT

This study investigated the high pressure (HP) modifications of rapeseed protein isolate (RPI) to improve its functional properties as wall materials for rapeseed peptides (RPs) microencapsulation by spray-drying. Results indicated that the surface hydrophobicity of HP-treated RPI microparticles was increased at 400 MPa and 5–15 min but decreased when extended to 20 min. Microparticles obtained with HP-treated RPI showed lower spray-drying yield than native RPI microparticles (67 ± 1.0% and 73 ± 1.4% respectively). Conversely, HP-treated RPI (400 MPa, 15 min) formed microparticles that enabled significantly higher encapsulation efficiency of up to 94.7 ± 1.8% (91.4 ± 1.4% for control samples). Morphological characterization indicated that microparticles with 6.1–8.5 μm diameters were spherical without fissures or cracks. Release profiles revealed that the core of microparticles (especially the 400 MPa, 15 min) achieved controlled release without an initial burst when exposed to the simulated intestinal environment; meanwhile they were resistant to gastric release-stimuli, such as extremely low pH and pepsin.

1. Introduction

Recent progress in molecular biology and biotechnology has led to the development of biotherapeutics and the discovery of bioactive compounds. Peptides are potent molecules used for the treatment of various chronic and life-threatening diseases. In our previous studies, we found that rapeseed peptides had antioxidant, antitumor, and antihypertensive activities (He, Malomo, Alashi, Girgih, Ju & Aluko, 2013; He, Malomo, Girgih, Ju & Aluko, 2013). Unfortunately, such bioactive compounds are prone to undesirable inactivation or degradation under the hostile bio-environment, such as extremely low pH and proteolytic enzymes in the gastrointestinal tract (GIT) (LaVan, Lynn, & Langer, 2002). In addition, the peptides may not reach their intended destination in the GIT, which results in low bioavailability upon oral administration (De Koker et al., 2011). These limitations have necessitated the need for high oral peptide doses and/or frequent administration to obtain therapeutic efficacy; however, such practices may produce toxic and adverse effects. Aside from high and/or frequent intakes, one approach to obtain a health benefit is to enhance the stability of bioactive compounds in the GIT through controlled release for digestion and/or absorption (Augustin, Sanguansri, & Lockett, 2013). For this reason, technologies for encapsulation have been extensively investigated in the nutritional and pharmaceutical fields.

Microencapsulation permits the formation of a physical barrier between the external medium and bioactive compounds for their protection. It is often used for controlled release of active molecules, formulation stability enhancement, and flavor and taste masking. The development of protein-based microencapsulation is an interesting approach to preserve potentially active ingredients, such as peptides, or even enhance their efficacy (Goodwin, Simerska, & Toth, 2012) for different food applications. The main technique used for active material encapsulation of plant based proteins is spray-drying. Moreover, available data show that soybean and pea proteins are the most studied plant materials used for microencapsulation (Nesterenko, Alric, Silvestre, & Durrieu, 2012). Rapeseed protein isolate (RPI) is normally produced from defatted rapeseed meal and is considered a suitable source of dietary protein when compared to other legume seeds. This is due to the high bioavailability and excellent balance of essential amino acids (Fleddermann et al., 2013). Our previous report (He, He, Chao, Ju, & Aluko, 2014) confirmed that RPI has some desirable functional properties, especially water holding, gelling, and film-forming. Recently, a study used RPI as an edible film ingredient in food packages (Jang, Lim, & Song, 2011). Vegetable proteins, being relatively cheap,

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non-toxic, bio-compatible and biodegradable biopolymers, are actually becoming a realistic alternative to animal proteins. Their use as wall material for active component microencapsulation reflects this actual tendency, particularly in the nutritional, pharmaceutical and food industries (Patel, Heussen, Hazekamp, Drost, & Velikov, 2012).

Physical modification can be used to significantly improve functional properties of proteins, in order to make these native materials more suitable for current microencapsulation techniques. Many native proteins possess limited functionality, therefore a modification, such as high pressure (HP) treatment, is often performed to improve their functional properties. Recently, HP treatment has received tremendous research interest as a means of improving ingredient functionality and especially to influence protein structural changes that can enhance the formulation of new food products with desirable qualities. Previous research reports have shown that appropriate application of HP treatment could effectively change molecular structure and lead to improved gelation properties of RPI (He et al., 2014). In addition, HP treatment could promote unfolding as well as exposure of hydrophobic groups, which allows maximal protein interactions with the aqueous media for enhanced functional properties (Chao, He, Jung, & Aluko, 2013). Therefore, HP-treated RPI may be used to make an efficient encapsulation system designed for controlled release or delivery peptides.

The aim of this study was to prepare encapsulated rapeseed peptides (RPs) in the form of stable microparticles obtained by spray drying the peptides with HP-treated RPI (wall material). We expect improved physiochemical properties of the microparticles so as to withstand structural deterioration in the presence of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) coupled with prolonged RPs release profile.

2. Materials and methods

2.1. Preparation of rapeseed protein isolates

The industrially defatted rapeseed meal was supplied by COFCO East Ocean Oils & Grains Industries Co., Ltd. (Zhang Jiagang, China). The meal was milled and passed through a 15 mm screen.

The RPI was produced from defatted rapeseed meal according to a previously reported method (Yoshie-Stark, Wada, Schott, & Wäsche, 2006) with slight modifications. The defatted rapeseed meal was dispersed in deionized water (1:15, *w*/*v*), adjusted to pH 11.0 with 1 M NaOH, mixed at 45 °C for 2 h and the slurry centrifuged at 10,000*g* for 30 min. The supernatant was recovered, adjusted to pH 4.5 with 1 M HCl, allowed to stand for 1 h at room temperature followed by centrifugation at 10,000*g* for 30 min. The recovered precipitated proteins were washed with anhydrous ethyl alcohol (to remove the polyphenolic components), re-dispersed in deionized water, adjusted to pH 7.0 with 1 M NaOH and freeze-dried to produce RPI. Protein content of the RPI was determined by the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978).

2.2. Preparation of rapeseed peptides (RPs)

RPs was produced from RPI according to the method described previously (He, Malomo, Alashi et al., 2013) with slight modifications. RPI (5%, *w*/*v*, protein basis) was suspended in deionized water in a reaction vessel equipped with a stirrer, heated to 37 °C and adjusted to pH 2.0 prior to addition of the pepsin. The enzyme was added to the RPI slurry at an enzyme to substrate ratio (E/S) of 4:100, based on the protein content of the RPI. Digestion was performed for 2 h (pH maintained constant by addition of 1 mol/L NaOH), then adjusted to pH 7.5, followed by addition of trypsin. Digestion was continued for 4 h at 50 °C. After digestion, the enzyme was inactivated by immersing the reaction vessel in a boiling water bath for 10 min. The undigested proteins were precipitated by centrifugation at 8000*g* for 30 min. The supernatant was passed through ultrafiltration membranes with 1 kDa molecular weight cut-off (MWCO) using an Amicon stirred ultrafiltration cell. The permeates from MWCO membrane (<1 kDa) were collected, lyophilized, and stored at −20 °C until needed for further analysis.

2.3. High pressure processing (HP)

Prior to pressure treatment, 1% (*w*/*v*) RPI slurry was prepared in 50 mM Tris–HCl buffer (pH 7.5) with stirring at 4 °C for 12 h. For HP treatment, the RPI slurry was sealed in a polyethylene bag and then subjected to a 4-L high pressure reactor unit equipped with temperature and pressure regulation with a transmitting medium of water (High Pressure Systems, Bao Tou KeFa High Pressure Technology Co., Ltd., Baotou, China). RPI solutions were subjected to HP treatment at 400 MPa for 5, 10, 15, and 20 min. The target pressure was reached at a rate of about 250 MPa/min, and released at a rate of about 300 MPa/min. After HP treatment, the RPI solutions were freeze-dried and stored at −20 °C until needed for further analysis. The unpressurised RPI were used as experimental controls.

2.4. Spray-dried microparticle preparation

Wall/core mixtures were prepared according to the following procedure: 8% *w*/*v* protein solution based on intact (control) or modified (400 MPa for 5, 10, 15and 20 min). RPI was adjusted to pH 11 and heated to 45 °C for 2 h under constant mechanical stirring to obtain maximal protein solubilization. RPs were added at 500 g/min to the protein solution under constant stirring for proper blending of the solution mixture to obtain a 2:1 (RPI:RPs) ratio.

Freshly homogenized mixtures were spray-dried in a Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) using the following process conditions: inlet air temperature at 135 ± 2 °C and outlet at 74 ± 2 °C, drying airflow rate of 450 L/h, liquid feed flow rate of 0.350 L/h. Dry microparticles were collected from the container and stored at 4 °C. The yield of spray-drying was defined as follows:

$$\text{Spray} - \text{drying yield}\ (\%) = \frac{M_P}{M_{RPI+RPs}} \times 100$$

where M_p — the mass of collected powder and $M_{RPI\ +\ RPs}$ — the initial mass of solid content added to form the mixtures including rapeseed protein isolate and peptides.

2.5. Encapsulation efficiency (EE)

2.5.1. Protract specification curve

A 300 mg portion of rapeseed peptide (RPs) was dissolved in artificial gastric juice and made up to 100 mL in a volumetric flask. Aliquots of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL each of the resultant solution were pipetted into 50 mL volumetric flasks and made up to the mark with simulated gastric fluid. The absorbance of each concentration was measured at a wavelength of 214 nm to draw the protract specification curve.

2.5.2. Measurement of encapsulation efficiency

Encapsulation efficiency was measured by repeatedly washing (three times) the microparticle powders with distilled water. The powders were then filtered and dried. Dried microparticles powders were then weighed out (100 mg) and placed in a conical flask. This was followed by the addition of 50 mL of 0.5 mol/L sodium citrate solution at 45 °C, at the rate of 250 *g*/min for 10 h and then centrifuged at 10,000 *g* for 20 min. A 1 mL aliquot of the filtrate was diluted to 10 mL and the absorbance measured at 214 nm. Mass concentration of the filtrate was determined using regression equation and the following