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Encapsulation of flaxseed oil within native and modified lentil protein-based microcapsules

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ABSTRACT

The physical properties of lentil protein-based maltodextrin microcapsules with entrapped flaxseed oil was investigated using native (n-LPI) and pre-treated (heated, un-hydrolyzed (u-LPI); and heated, hydrolyzed (h-LPI)) lentil proteins and as a function of oil load (10, 20 and 30% of total solids). Specifically, the moisture, water activity, surface oil and entrapment efficiency (EE) were assessed, along with droplet size and emulsion morphology of all formulations. Moisture (<6%) and water activity (<0.2) of all capsules were characteristics of dried powder ingredients. Light microscopy imaging of the emulsions, revealed that the h-LPI had slightly larger oil droplets than the n-LPI and u-LPI, which both appeared similar. Findings were confirmed by light scattering, where droplet sizes were 6.7, 4.2 and 4.2 μm for the h-LPI, u-LPI and n-LPI stabilized emulsions, respectively. Overall capsules prepared from h-LPI showed significantly higher surface oil and lower EE than both the n-LPI and u-LPI materials. Furthermore, as the oil content increased, overall surface oil became higher and EE became lower. Based on testing, capsules prepared using n-LPI with 10% oil loading was found to have the lowest surface oil content (~3.7%) and highest EE (~62.8%) for all formulations, and was subjected to an oxidative storage stability test over a 30 d period vs. free oil. The encapsulation process proved to be effective at lowering the production of primary and secondary oxidative products than free oil.

1. Introduction

Flaxseed oil represents a rich plant source of essential omega-3 fatty acids (e.g. α-linolenic acid [ALA]) which provide numerous beneficial health effects on coronary heart disease, some types of cancers, and neurological and hormonal disorders (Abuzaytoun & Shahidi, 2006; Łukaszewic, Szopa, & Krasowska, 2004). Despite this, its incorporation into products by the food industry has been limited due to its low oxidative stability and off flavors caused by lipid rancidity. The latter is typically induced by processing and storage conditions, which results in reduced product shelf life (Lee & Ying, 2008). Flaxseed oil's sensitivity to oxygen entails that a means of protection must be introduced so that humans can obtain the beneficial effects of the oil (Lee & Ying, 2008; Łukaszewic et al., 2004).

Encapsulation technologies have been employed to provide protection to sensitive materials from the harsh conditions during processing and storage as well as the harsh conditions of the human gastrointestinal tract (GI). Encapsulation is defined as the process whereby solids, liquids, or gaseous materials are enclosed in small sealed capsules which can be formulated proteins, polysaccharides and/or lipid based materials (Augustin & Hemar, 2009; Champagne & Fustier, 2007; Desai & Park, 2005; Fang & Bhandari, 2010; Kailasapathy, 2009; Lee & Ying, 2008). Encapsulation also provides a means to mask the taste and smell of the oils within food formulations to maintain the product's sensory appeal and consumer satisfaction (Champagne & Fustier, 2007).

Due to their amphiphilic nature (i.e., having both hydrophilic and hydrophobic groups), proteins make ideal encapsulation agents for oils due to their emulsifying potential. Proteins migrate to the oil–water interface, re-orient themselves such that hydrophobic moieties are oriented inwards to the oil phase and hydrophilic groups are oriented outwards to the aqueous phase, and then form a viscoelastic film around the enclosed oil droplets (Can Karaca, Low, & Nickerson, 2011a; Dalgleish, 2004). The film provides stabilizing electrostatic repulsive (depending on pH and salts) and steric forces to resist phase separation (Can Karaca et al., 2011a; Dalgleish, 2004). For encapsulation, stabile oil-in-water emulsions are dried through various processes to yield a dried powder. Plant proteins as encapsulating agents for oil has been recently been reviewed by Nesterenko, Alric, Silvestre, and Durrieu (2013). The use of such proteins could represent a competitive advantage to ingredient suppliers and food manufactures due to significant growth towards specialized markets, such as those who restrict the use of animal-derived proteins based on religious beliefs or dietary preferences.

The emulsifying properties of various protein isolates derived from chickpea, lentil, faba bean, pea and soy produced by both isoelectric precipitation and salt extraction were recently explored by Can Karaca et al. (2011a). The authors' findings indicated that overall isolates produced

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by isoelectric precipitation lead to better emulsifying properties for the legume proteins than those extracted using salts, and that both lentil and chickpea displayed comparable properties to soy, and better emulsifying potential than pea and faba bean. The authors further optimized lentil and chickpea protein-stabilized emulsions based on pH, protein concentration and oil content (Can Karaca, Low, & Nickerson, 2011b). The formation of stable emulsions is a pre-requisite to having good surface coating of the oil droplets and higher entrapment efficiencies (Nesterenko et al., 2013). Partial enzymatic hydrolysis represents another means of potentially improving the emulsifying properties of plant proteins. However, this effect is highly dependent on the protein source and the processing conditions used (Panyam & Kilara, 1996). For instance, Karayannidou et al. (2007) and Guan, Yao, Chen, Shan, and Zhang (2007) reported that trypsin-treated sunflower protein isolate (DH ~10%) and oat bran protein (DH ~4–8%), respectively showed improved emulsifying properties relative to the non-hydrolyzed material. In contrast, Govindaraju and Srinivas (2006) reported that the hydrolysis of arachin protein (DH ~19%) using papain, alcalase and fungal protease resulted in a significant decrease in emulsifying potential. Also, Avramenko, Low, and Nickerson (2013) investigated the emulsifying properties of lentil proteins as a function of the degree of hydrolysis (4, 9 and 20%) following exposure to trypsin/heat, relative to un-hydrolyzed. The authors reported that overall, the emulsifying properties were reduced when treated with trypsin relative to its native form, and that no difference in effect was evident between DH 4 and 20%. However, the potential use of modified proteins, particularly plant proteins (e.g., lentils) as encapsulating agents has largely been unexplored.

Lentils (*Lens culinaris*) are primarily comprised of the storage proteins: albumins and globulins, along with minor amounts of prolamins and glutelins (Bhatty, Slinkard, & Sosulski, 1976; Boye, Fatemeh, & Pletch, 2010; Swanson, 1990). The globulins, which make up the majority of the protein content in lentils (~47% of total seed proteins), comprise of two main proteins: legumin (11S) and vicilin (7S) (Bhatty et al., 1976; Boye et al., 2010; Swanson, 1990). Legumin is comprised of a hexameric quaternary structure with acidic (molecular weight (MW) of ~40 kDa) and basic (MW of ~20 kDa) subunits; whereas the 7S vicilins are comprised of a trimeric structure with a MW of 175–180 kDa (Boye et al., 2010; Swanson, 1990). There is also a third globulin protein, known as convicilin that has a subunit MW of 71 kDa and a MW of 290 kDa in its native form (Boye et al., 2010; Swanson, 1990).

Limited information exists relating to the potential of partially hydrolyzed proteins (especially from plant sources) for use as encapsulating agents where it is hypothesized that the smaller, more open proteins may lead to better barrier properties to oxygen. The overall goal of this research was to investigate the potential use of native and pre-treated (heated/un-hydrolyzed and heated/hydrolyzed) lentil proteins, in the formulation of wall materials that entrap flaxseed oil, as a means of improving their protective ability against oxidation. Findings will provide new insight on the potential impact on benefits of protein modification for encapsulation processes.

2. Materials and methods

2.1. Materials

Whole green lentil seeds (CDC Greenland) and flaxseed oil were provided by the Crop Development Centre (Saskatoon, SK, Canada) and Bioriginal Food and Science Corp. (Saskatoon, SK, Canada), respectively. The following materials were purchased from Bio-Rad (Mississauga, ON, Canada): Bio-Rad Broad Range Marker, Bio-Rad Tris–HCl gel (15% T), Coomassie blue R-350 and Laemmli Sample Buffer. Hexane was purchased from the Fischer Scientific (Ottawa, ON, Canada). Butylated hydroxytoluene (BHT), malondialdehyde (MDA) (1,1,3,3-tertraethoxy-propane), picrylsulfonic acid (trinitrobenzene sulfonic acid (TNBS)), pyridine, soluble potato starch, 2-thiobarbituric acid (TBA) and trypsin (10,600 units/mg) were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). The following chemicals were purchased from VWR (Edmonton, AB, Canada): acetic acid, chloroform, dimethyl sulfoxide (DMSO), hydrochloric acid, isopropanol, n-butanol, potassium iodide, sodium dihydrogen phosphate, sodium dodecyl sulfate, sodium hydroxide, sodium monohydrogen phosphate, sodium thiosulfate, and sulfuric acid. All chemicals used in this study were of reagent grade except for sodium dodecyl sulfate which was ultrapure. The water used in this research was produced from a Millipore Milli-Q™ water purification system (Millipore Corp., Milford, MA, USA). Maltodextrin (DE 11, M100™ Maltrin) was donated by the Cargill Inc. (Cargill Texturizing Solutions, Cedar Rapids, IA, USA).

2.2. LPI preparation

Whole green lentil seeds were ground into a fine flour using a food processor (Cuisinart mini-prep plus grinder) (~1 min), followed by an IKA A11 analytical mill treatment (IKA Works Inc., Wilmington, NC, USA) (~1 min). The flour was then defatted by stirring in hexane (1:3 [*w*/*v*], flour: hexane) for 40 min; this procedure was repeated two additional times.

Protein isolates were prepared from the defatted flour based on methods of Papalamprou, Doxastakis, and Kiosseoglou (2010) and Can Karaca et al. (2011a). In brief, 100 g of defatted flour was mixed with water at a 1:10 (*w*/*v*) ratio. The pH of the resulting suspension was adjusted to 9.00 using 1.0 N NaOH followed by mechanical stirring at 500 rpm for 1 h at room temperature (21–23 °C). The mixture was then centrifuged at 5000 ×*g* at 4 °C for 20 min using a Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA) to collect the supernatant. The resulting pellet was re-suspended in water at a ratio of 1:5 (*w*/*v*), adjusted to pH 9.00 using 1.0 N NaOH, stirred for 1 h at room temperature, followed by centrifugation (5000 ×*g*, 20 min, 4 °C). Supernatants were pooled and adjusted to pH 4.50 with 0.1 M HCl to precipitate the protein (Bora, 2002). The LPI was washed with water, frozen at −30° C within a chest freezer and then freeze dried using a temperature difference of 35 °C for 24 h (FreeZone 6 freeze dryer, Labconco Corporation, Kansas City, MO, USA) to produce a dry powder. The powder was stored at 4 °C for later usage within a walk-in cold laboratory. Proximate composition for the resulting LPI was conducted according to AOAC Official Methods 925.10 (moisture), 923.03 (ash), 920.85 (lipid), and 920.87 (crude protein by using %N × 6.25) (AOAC, 2003). Carbohydrate content was determined on the basis of percent differential from 100%. Proximate analyses were performed on three separate protein isolate preparations with each preparation analyzed in duplicate (n = 2).

2.3. Preparation of the protein wall material for encapsulation

Three different LPI solutions were produced: (a) native LPI (n-LPI); (b) unhydrolyzed, heated LPI (u-LPI); and (c) hydrolyzed, heated LPI (h-LPI). All solutions were prepared in triplicate.

The *n-LPI solution* was prepared by dissolving the freeze dried LPI powder in 100 mL of 35 mM sodium phosphate buffer (pH 7.8) at a protein concentration of 4.0% (*w*/*v*), and then allowed to stir overnight at 4 °C to help promote protein solubility.

The *u-LPI solution* (unhydrolyzed, heated) was initially prepared in a similar manner as the n-LPI solution. However the u-LPI solution was then heated in a similar manner as the hydrolyzed material, except no enzymes were present. In brief, the n-LPI solution was heated at 37 °C for 1 h within a shaking (90 rpm) water bath (PolyScience, Niles, IL, USA), and then transferred in 10 mL aliquots (9 in total) into separate test tubes. These tubes were then heated at 85 °C for 20 min in the same water bath, cooled to room temperature and frozen (−30 °C) for later use.

The *h-LPI solution* was prepared following a similar method as the u-LPI solution, except after the 1 h heating period at 37 °C, trypsin was