Journal of Food Engineering 191 (2016) 58–66

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Optimisation of the complex coacervation between canola protein isolate and chitosan

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ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form
20 July 2016
Accepted 20 July 2016
Available online 21 July 2016

Keywords:
Complex coacervation
Canola protein isolate
Chitosan
Zeta potential
pH
Coacervate yield

ABSTRACT

The complex coacervation phenomenon between canola protein isolate (CPI) and chitosan (CS) was studied. CPI was extracted in the laboratory from canola meal and used in this study. The factors affecting the yield of CPI-chitosan complex coacervates such as CPI-to-chitosan ratio, pH and strength of the electrostatic interaction (SEI) were investigated. The thermal characteristics of the un-cross-linked and transglutaminase cross-linked complex coacervates were also determined. The optimum complex coacervation between CPI and CS occurred at the CPI-to-chitosan mass ratio of 16 and the pH range of 5.8–6.2. The peak denaturation temperature and the denaturation enthalpy of CPI in CPI-chitosan complex were higher than those of the uncompleted or free CPI indicating that the complexation made the CPI more thermally stable. The thermal stability of the coacervates was further enhanced when cross-linked with transglutaminase. The increased thermal stability of CPI in CPI-chitosan coacervate indicated that CPI-chitosan coacervates would be suitable for encapsulation of thermally sensitive food and pharmaceutical ingredients.

1. Introduction

Complex coacervation is a process in which liquid-solid phase separation occurs when two oppositely charged polymers undergo complexation in an aqueous medium. The bottom phase is rich in complex coacervates and is relatively denser. The top phase contains much lesser amount of complex coacervates and is less dense. Nevertheless, it remains in equilibrium with the dense bottom phase. Due to the concentration of complex coacervates, this bottom phase can have 10 times higher viscosity than that of the individual polymers undergoing complexation at the same concentration (Ducel et al., 2004a; 2004b). The most important industrial application of complex coacervation is in the microencapsulation of sensitive ingredients in food and pharmaceutical industries. Food-grade complex coacervates, in which both the cationic and anionic polymers are food grade, are desired due to their nutritional benefits and superior functional properties in stabilizing unstable food ingredients such as omega-3, enzymes and flavours from oxidation and/or degradation (Barrow et al., 2013; Jiang et al., 2013; Yeo et al., 2005). Due to this reason, the formation and characterization of protein-polysaccharide complex coacervates such as beta-lactoglobulin-carrageenan, gelatin-agar, whey protein-gum Arabic are researched in considerable detail (Ould Elaya and Turgeon, 2000; Singh et al., 2007; Weinbreck et al., 2004).

Complex coacervation produces biopolymer-rich and solvent-rich phases (Boral and Bohidar, 2010; Schmitt and Turgeon, 2011; Schmitt et al., 1998; Tolstoguzov, 1991). The biopolymer-rich phase consists of soluble and insoluble complexes that re-orient into either a coacervate or precipitate-type morphology depending on the strength of charge of the participating polymers. Coacervates comprised of complexed biopolymers, typically involving positively (e.g. protein) and negatively (e.g. polysaccharides) charged polyelectrolytes entrap some solvent to remain mobile (Klassen et al., 2011).

Complex coacervation between proteins from soy, pea lentil and chia seeds with polysaccharides from chitosan, alginate, gum Arabic, dextran sulphate, carrageenan pectin and chia seed gum have previously been studied to a considerable detail (Huang et al., 2012; Jun-xia et al., 2011; Elmer et al., 2011; Timilsena et al., 2016). These studies show that the complexation between protein and

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http://dx.doi.org/10.1016/j.jfoodeng.2016.07.008
0260-8774/

polysaccharide depends on the nature of the participating protein and polysaccharide and requires thorough study in each case. To date, there is very limited study on complex coacervation involving canola protein isolate (CPI), especially between CPI and chitosan. Given that large quantity of canola meal which is rich in canola protein is produced by the oil industry, its alternate use as complexing biopolymer has economic potential. In addition, study of complex coacervation between canola protein isolate and chitosan will provide greater understanding of the science underpinning the complex coacervation phenomenon between a less studied plant protein and a commercially available polysaccharide, both of which are co-products of their respective industries. Furthermore, chitosan being a sustainable, naturally-derive, high molecular weight cationic biopolymer, recognised for food neutraceutical encapsulation (Chen et al., 2006) with mucoadhesive (Sogias et al., 2008 and Sogias et al., 2012), intestinal lipid binding, serum cholesterol lowering effects (Razdan and Pettersson, 1994) properties, it is of practical interest to study the complex coacervation process between CPI and chitosan In addition to that, chitosan also has antioxidative and preservative effects in muscle foods (Darmadji and Izumimoto, 1994), an emulsifying, thickening and stabilizing agent in food industry (Shahidi et al., 1999) as well as is easily manipulated in mildly acidic conditions (e.g. dilute acetic acid).

G lobal canola production has grown rapidly over the past 40 years and it is the second largest oil crop (USDA, 2010) and its worldwide production is projected to reach 15 million metric tons by 2015 (Newkirk, 2009). To date, the protein-rich canola meal is underutilized and being sold as low cost animal feed supplement despite the fact that it contains 35–40% protein (Stone et al., 2014). Canola proteins have a good essential amino acid profile including isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine (Sosulski and Sarwar, 1973; Khattab and Arntfield, 2009) and the highest protein efficiency ratio (PER = 3.29) of all plant-based proteins (Bell et al., 2000).

Due to the nutritional values mentioned above, canola protein isolate makes a good base material to produce plant protein-based complex coacervate to be used in the food and pharmaceutical industries. According to Tolstoguzov (Tolstoguzov, 2003), protein-polysaccharide interactions play an important role in controlling food quality and texture. Besides, the commercial utilization of canola protein as major ingredient of complex coacervate and its utilization as encapsulating matrix material for sensitive food ingredient will be helpful in reducing the canola meal waste. To some extent, this will also maximize its utilization as high value nutritional ingredient in functional food formulations. This will contribute, in part, to the reduction of food wastes and helps in food security. Likewise, chitosan is the second most abundant polysaccharide in the world. Due to its abundance, non-toxicity, biodegradability and biocompatibility, good film forming properties, low viscosity and high solubility, chitosan has great potential as a microencapsulant (Peniche et al., 2003).

Canola protein isolate and chitosan are suitable to be used to form coacervates as chitosan is positively charged in a wide pH range, while canola protein isolate is amphoteric with negative charge above its isoelectric point (pI). Some proteins that undergo complex coacervation with chitosan include α-lactalbumin and β-lactoglobulin (Lee and Hong, 2009), soy globulin (Liu et al., 2011), pea protein isolate (Elmer et al., 2011) and soybean protein isolate (Huang et al., 2012).

The complex coacervation based encapsulation method has several benefits such as high pay load and high encapsulation efficiency, use of non-toxic solvent system, moderate temperature and ready availability of a large number of polymers for complexation (Arntfield and Cai, 1998; Klassen et al., 2011).

Complex coacervation process is affected by the nature of polymers, charge density, concentration and the ratio of the polymers used, pH, temperature and ionic strength (de Kruif et al., 2004; Siow and Ong, 2013). Hence, determining the optimum conditions is crucial in the complex coacervation process.

In this study, the science that delivered the optimal complex coacervation between CPI and chitosan is reported. Specifically, the charge density, ionic strength, pH and CPI-to-chitosan mass ratio were optimised to produce complex coacervates of the highest yield. The interactions between the functional groups of participating polymers were also studied. Thermal characteristics of uncross-linked and transglutaminase cross-linked CPI-chitosan coacervates were also investigated.

2. Materials and methods

Canola meal (cold pressed) was provided by Cootamundra oilseeds (New South Wales, Australia) and stored at 4 °C in air tight plastic bags until use. According to the supplier, the meal contained 36.8% proteins, 12.1% crude fats, 6.3% minerals (ash) and 9% moisture on wet basis (w/w). These values are comparable to the reported literature values of 36.1% protein, 6.3% ash and 11.4% moisture, except for crude fat (2.8%) (Khattab and Arntfield, 2009; NSWDPI, 2004).

Canola protein isolates (CPI) was extracted and purified in the laboratory. All chemicals including sodium hydroxide, citric acid, acetic acid and chitosan were purchased from Sigma-Aldrich (NSW, Australia) and were of analytical or food grade and they were used as received. The bicinchoninic acid (BCA) assay reagents were obtained from Fisher Scientific (Victoria, Australia). A low molecular weight chitosan (deacetylated chitin, poly (D-glucosamine) with viscosity of 20-300 cP was obtained from Sigma-Aldrich. Transglutaminase (as cross linker) was purchased from the Melbourne Food Depot (Melbourne, Australia).

2.1. Preparation of canola protein isolate

Canola protein isolate (CPI) was prepared by combining the methods reported previously (Klassen et al., 2011; Klockeman et al., 1997) with some minor modifications.

The canola meal (500 g) was defatted by hexane extraction using a meal-to-hexane ratio of 1:10. The extraction was carried out for 24 h by subjecting the mixture to constant mechanical stirring. The hexane containing the extracted oil was separated by filtration and the meal was further washed twice with hexane (2 × 100 mL). The recovered meal was air-dried in a fume hood for 24 h in an air-conditioned room (24 ± 2 °C). These defatting and drying protocols were replicated once to remove the fat content further.

Defatted canola meal (100 g) was dispersed in Milli-Q water at a meal-to-water ratio of 1:10 (w/w) and the pH of this suspension was adjusted to pH 12.0 using 1.0 M NaOH solution as the solubility of canola proteins in deionized water was highest at pH 12.0. The slurry was stirred at 500 rpm for 2 h at ambient temperature. The suspension was then centrifuged at 10,000 × g for about 30 min at 5–10 °C using a temperature controlled laboratory centrifuge (Sorvall RC5C, Thermo Scientific, USA). The supernatant was collected and filtered out through a 0.45 μm Whatman #1 filter paper (Whatman International Ltd., Maidstone, UK). The pH of the filtrate was adjusted to 4.0 (pI of CPI) with 0.5 M citric acid to facilitate protein precipitation. The precipitate was recovered by centrifuging at 10,000g for 30 min. The protein precipitate was washed three times with Milli-Q water (3 × 1 L) and freeze-dried. The freeze-dried protein isolate was then powdered finely and sieved through a 125 μm sieve. The extraction method is presented schematically in Fig. 1.