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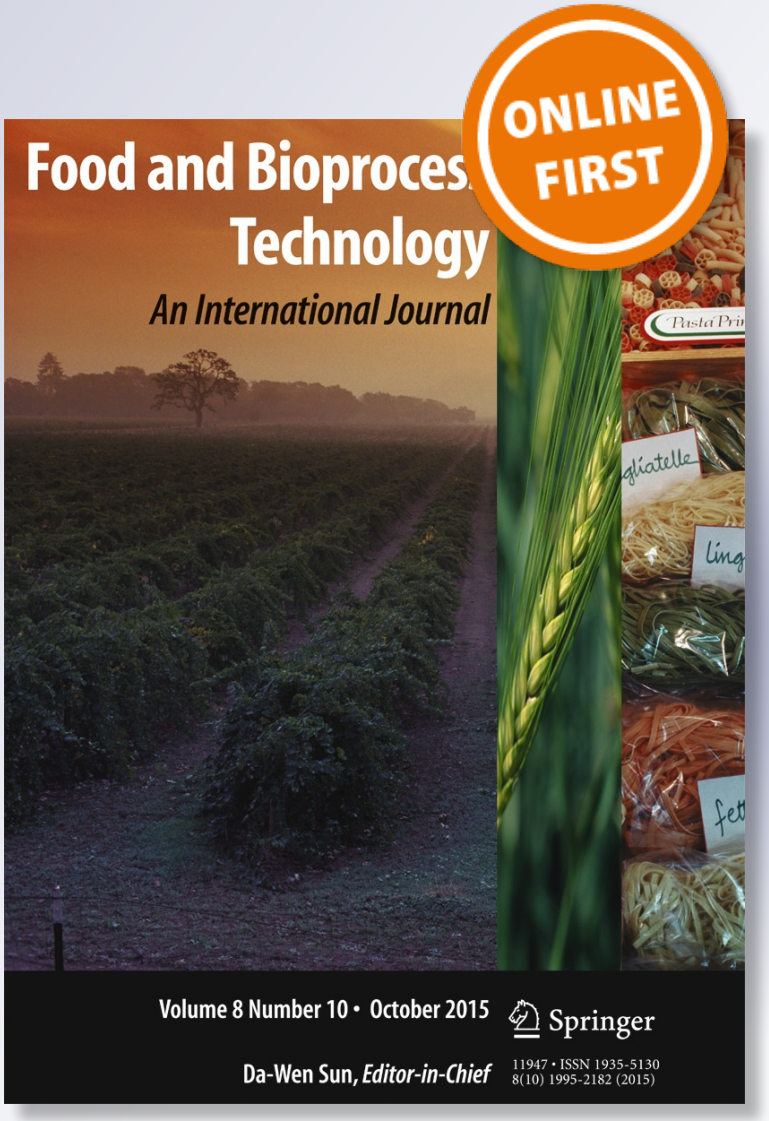

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ORIGINAL PAPER

Pea Protein Isolates: Novel Wall Materials for Microencapsulating Flaxseed Oil

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Abstract This study investigated the potential of three commercially available pea protein isolates (PPIs), Nutralys (Roquette, USA), PP (Znaturals, USA), and Pulseplus80 (AGT, Canada) as wall materials for microencapsulating flaxseed oil. Microencapsulation with spray drying was conducted with PPIs at 10 % concentration and varied flaxseed-oil-to-wall-material ratios (1:5, 1:3.3, and 1:2.5). All three PPIs emulsion prepared using 1:5 core-to-wall ratio were stable. Microencapsulation efficiencies (MEs) at 1:5 core-to-wall-material ratio were 90.46, 84.9, and 71.9 % for Nutralys, PP, and Pulseplus80, respectively. Results show that when the core-to-wall-material ratio increased to 1:2.5, the MEs decreased to 67.9, 75.6, and 44.6 % for Nutralys, PP, and Pulseplus80, respectively. Proximate composition of PPIs influenced the functional properties and emulsion stability and, ultimately, MEs. Electrophoresis and Fourier transform infrared spectroscopy (FTIR) analyses were conducted to determine differences in these three proteins. This study also evaluated microcapsules prepared with 1:5 ratio for water content, water activity, solubility, and morphological properties. Findings demonstrate that PPI, a natural, low-cost, allergen-free ingredient can be used effectively as a wall material for microencapsulation at a 10 % solid concentration.

Keywords Emulsion · FTIR · Microencapsulation efficiency · Physical properties · Spray drying

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Introduction

Peas (*Pisum sativum L.*) are an important part of the human diet due to their high protein, fiber, vitamin, and mineral contents. Pea proteins are gaining popularity as a non-allergenic source of vegetable protein which offers clean label to food product. These proteins can be used as functional ingredient, as they have been proven to reduce the risk of cardiovascular disease (Rigamonti et al. 2010; Kingman et al. 1993) and blood pressure (Li et al. 2011). Hydrolyzed pea protein contains bioactive peptides which act as an angiotensin I-converting enzyme inhibitor and antioxidant agent (Roy et al. 2010). Protein from peas can be easily incorporated into other food products due to their unique functional properties which include emulsifying, foaming, gelation, water holding, and fat absorption properties (Toews and Wang 2013). Polar and non-polar amino acids present on the surface of proteins are responsible for their functional properties (Yu et al. 2007). Based on functional and nutritional properties and current green trend, companies are using pea proteins in food products such as protein bars, pasta, gluten-free confectionery and bakery products, and as a replacer for egg proteins in mayonnaise (Boyle 2014).

Flaxseed oil is widely known for its health benefits due to its unsaturated fatty acid content, especially α-linolenic acid (ALA) which is an essential fatty acid. ALA has been shown to decrease the risk of coronary heart disease (Harper and Jacobson 2005), as well as breast and prostate cancer (Gerber 2012). However, these fatty acids are highly unsaturated and immiscible, limiting their use in food systems. Microencapsulation of oils using polymers is a common technique used to prolong shelf-life by protecting unsaturated fatty acids from oxidation (Ahn et al. 2008, Serfert et al. 2009). This also improves miscibility and the controlled release of the oil in the desired system (Drusch et al. 2007). Wall material used for

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microencapsulation influences properties of microcapsules; therefore, it is important to select suitable wall material. Carbohydrates are preferred wall materials due to its lower viscosity at high concentration and film-forming properties. Modified starches and gum arabic with and without maltodextrin were used to encapsulate flaxseed oil to protect it from lipid oxidation (Carneiro et al. 2013; Tonon et al. 2012). Carbohydrate-protein complexes such as gum arabic-soy protein complex (Dong et al. 2015), legume proteins-maltodextrin (Karaca et al. 2013), and xanthan gum-soy lecithin (Omar et al. 2009) were also used for microencapsulation of flaxseed oil. Combinations of wall materials such as fish gelatin, chitosan, microbial transglutaminase, and maltodextrin (Pourashouri et al. 2014); maltodextrin, gum arabic, and gelatin (Rajabi et al. 2015); and microcrystalline cellulose and sodium alginate (Bajaj et al. 2010) were also used to encapsulate bioactive agents.

Pea protein isolates are in demand due to its low cost and non-allergenic nature. However, few studies have explored the use of pea protein to encapsulate bioactive molecules. Gharsallaoui et al. (2010) used pea protein isolate (PPI) as an emulsifier, along with maltodextrin, to encapsulate miglyol oil. Little research has explored the possibility of using commercial PPIs as wall materials for microencapsulation. Commercial PPI was used alone and along with maltodextrin to encapsulate ascorbic acid (Pierucci et al. 2006; Pereira et al. 2009). Costa et al. (2015) used PPI and pea protein concentrate to encapsulate conjugated linoleic acid. However, in these studies, incorporation of pea proteins in emulsion was limited to less than 8 % of total solids.

This study evaluated the potential of commercially available PPIs alone as a wall material for encapsulation of highly unsaturated flaxseed oil at concentration more than 8 %. Commercial PPIs should possess certain properties to be used as a wall material. Efforts were made to study the difference in physicochemical properties of commercial PPIs. Influence of proximate composition of PPIs, functional properties of PPIs, and core-to-wall-material ratio on microencapsulation efficiency were also evaluated.

Materials and Methods

Commercial PPIs Nutralys (S85F), PP, and Pulseplus80 were procured from Roquette (Geneva, IL), Znatural Foods (West Palm Beach, FL) and Alliance Grain Traders (Regina, SK, Canada), respectively. The flaxseed oil was provided as a gift from Heartland Flax (Valley City, ND). Protease from *Aspergillus oryzae* (P6110-50ML) and all reagents were procured from Sigma-Aldrich, Inc., (St. Louis, MO) unless otherwise noted. All reagents were of an analytical grade.

Properties of Commercial PPI Powders

Proximate Composition

The proximate composition of the PPIs was determined according to AOAC official methods 925.10 (moisture), 923.03 (ash), and 920.85 (lipid). The protein and carbohydrate content of PPI was determined with the combustion method (Leco, model FP-428, St. Joseph, MI) and the difference method, respectively. All measurements were performed at least three replicates.

Fat Absorption Capacity and Approximate Water Hydration Capacity

Fat absorption capacity (FAC) was measured according to Wang et al. (2011), with few modifications. In this, 5 ml of flaxseed oil was added to 1 g of pea protein isolate (PPI) powder and vortexed for 15 s at 3000 rpm every 5 min for a total of 20 min. Samples were further centrifuged at 1600×*g* for 25 min at 20 °C. Supernatant oil was drained, and the oil absorbed was calculated using Eq. 2.1.

$$\mathrm{FAC} = \frac{\text{weight of PPI after removing oil} - \text{weight of PPI}}{\text{weight of PPI}} \tag{2.1}$$

The approximate water hydration capacity (WHC) was measured according to the AACC method 56–30.01 (AACC, 2012) and WHC was calculated using Eq. 2.2.

$$\mathrm{WHC} = \frac{\text{weight of PPI after removing supernatant} - \text{weight of PPI}}{\text{weight of PPI}} \tag{2.2}$$

Determination of Sodium Ion Concentrations

Different extraction processes used in the preparation of pea protein isolates affect the functional properties of PPIs. The addition of sodium salts is a common practice in the extraction and modification processes of proteins. Moreover, sodium ion (Na^+) concentration affects the viscosity of protein solution. Therefore, concentration of Na^+ was measured to determine ionic strength differences within these three proteins. In this study, Na^+ of the PPI solution was measured with a calibrated ROSS® Sure-Flow® Sodium Combination Ion Selective Electrode (ISE, 8611BNWP, Thermo Fisher Scientific, Beverly, MA) connected to an electrochemical analytical meter (SevenEasyTM S20, Mettler-Toledo AG, Schwerzenbach, Switzerland). Prior to measurement, 10 % ionic strength adjuster (Orion 841111, Thermo Scientific Inc., Newington, NH) was added to 10 % PPI solution to adjust and maintain constant ionic strength in solutions. ISE was put into the

solution to obtain electrical potential. Solutions were continuously agitated during measurement to avoid settling of particles. Sodium ISE was rinsed with 10 % (*v*/*v*) ISA in deionized water between measurements. A standard curve was used to relate the measured voltage difference between the sensing and reference electrodes to the Na^+ concentration. Na^+ is expressed as NaCl concentration in this study.

Electrophoresis

Protein fractions of Nutralys, PP, and Pulseplus80 were analyzed with sodiumdodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) described by Laemmli (1970) with slight modifications. PPI was diluted in a sample buffer to get 1.5 g/L concentration buffer solution, and 20 μL of the buffer solution was loaded into each well. A 4–15 % precast polyacrylamide gel was used (Bio-Rad, Hercules, CA). Electrophoresis was carried out with a Mini Protean Tetra system (Eppendorf AG, Germany) at ~25 °C. A constant voltage of 30 mV was used until the bromophenol blue reached the bottom. After electrophoresis, the gel was stained using Coomassie brilliant blue R-250 (Bio-Rad, Hercules, CA) for 2 h followed by destaining. For destaining, the gel was kept in the destaining solution for ~4 h, which consisted of water:methanol:acetic acid (5:4:1).

Fourier Transform Infrared Spectroscopy Analysis

Fourier transform infrared spectroscopy (FTIR) spectra measurements were collected using a Thermo Nicolet 360 FTIR spectrometer (Thermo Electron Inc., San Jose, CA). PPIs (1 g) were compressed using hydrolytic pressure machine to prepare tablets for FTIR analysis. Tablets were placed on an attenuated total reflectance (ATR) zinc selenide (ZnSe) crystal. Spectra were obtained for the desired wavelength (4002 to 399/cm). The resolution of the FTIR instrument was set at 4/cm, with spectra taken at a mean of 64 separate scans.

Preparation of PPI Emulsion

PPI solutions were prepared by dissolving pea protein isolate samples (at 10 % *w*/*v* concentration) in water at 4 °C for 18 h. The concentration of the wall material was optimized at 10 % *w*/*v*, based on a preliminary study conducted to determine the maximum concentration of PPIs that can be incorporated in the solution. Z-naturals produced a very viscous solution when more than 10 % solids was used, making it unable to pass through the inlet of the high-pressure homogenizer. Therefore, all solutions used in this study had 10 % total solids. The pH values of 10 % PPI solutions were 7.4, 7.5, and 6.8 for Nutralys, PP, and Pulseplus80, respectively. We did not adjust the solution pH as any change in the pH would result in modifying protein structure (Sathe et al. 1984) which may change the functional properties of the proteins. For this study, pH factor was not taken into consideration as the major objective was to investigate the potential of utilizing commercially available PPIs for microencapsulation. Coarse PPI emulsions were prepared by adding the desired quantity of oil to 10 % PPI solution (Table 1) and homogenizing them at 15,000 rpm for 5 min with a Polytron PT 4000 (Kinematica, USA). The coarse emulsion was further homogenized in a high-pressure homogenizer (M 110-P Microfluidizer, USA) at 30,000 psi (2 passes) and 10,000 psi (1 pass) to form a stable emulsion.

Emulsion Stability

Emulsions were stored at 25 °C for 24 h to measure the emulsion stability index (ESI) using Eq. 2.3 (Sarkar and Singhal 2011).

$$\mathrm{ESI} = 1 - \frac{\text{oil seperated in emulsion}}{\text{total oil in emulsion}} \tag{2.3}$$

Droplet Size Distribution

The droplet size distribution of the emulsion was checked with a ZetaSizer Nano ZS (Malvern Instruments, Inc., UK) at 25 °C using dynamic light scattering principle and Stokes-Einstein equation (n = 8) (Jiang et al. 2009). Emulsions were diluted (1:5 ratio) with water prior to measurement to avoid error due to the multiple scattering effect.

Viscosity

The steady shear viscosity was measured using an AR 2000 rheometer (TA Instruments, USA) over a shear rate range of 5–100/s with cone-plate geometry (CP 40/41). All measurements were performed at 25 °C within 2 h of emulsion preparation.

Preparation of Microcapsules

Spay drying was conducted following the procedure described by Kuang et al. (2015) with few modifications. Briefly, stable emulsions were fed into in a pilot-scale spray dryer (Model LAB-S1, Anhydro, Denmark). Inlet and outlet temperatures were maintained at 150 ± 2 °C and 80 ± 2 °C, respectively. Flow rates were maintained at 15–16 mL/min. Microcapsules prepared were kept in polyethylene pouches and stored in glass bottles at −20 °C for later analysis.

Table 1 Formulation of emulsions with different oil loadings

	Nutralys			Pulseplus80			PP		
Trial No	1	2	3	4	5	6	7	8	9
Pea protein isolate	10	10	10	10	10	10	10	10	10
Oil	2	3	4	2	3	4	2	3	4
Water	88	87	86	88	87	86	88	87	86

Physicochemical Properties of Microcapsules

The surface oil of the microcapsules was analyzed following the method by Liu et al. (2010) with few modifications. A sample of 0.2 g was mixed with 10 ml of hexane, stirred at 200 rpm for 30 min, and filtered through Whatman #41. The powder collected on the filter was washed twice with 5 ml of hexane to extract remaining surface oil. Hexane was allowed to evaporate at 60 °C in the fume hood. The surface oil was then determined gravimetrically by measuring the weight of the oil after heating the beaker at 105 °C for 30 min to remove any residual hexane. The total amount of oil was determined by enzymatic extraction method in two steps. In the first step, 0.2 g of powder was mixed with 10 ml of water and 0.05 % of Flavorzyme in a conical flask and kept at 50 °C under constant stirring at 50 rpm for 4 h. In the second step, 20 ml mixture of hexane:ethanol (1:1) was added to the conical flask and stirred at 3000 rpm for 2 min. This mixture was centrifuged at 15, 000 rpm for 10 min to separate the hexane. The second step was repeated twice to extract all of the oil from the powder. The total oil was determined gravimetrically, similar to surface oil.

The microencapsulation efficiency (ME) of spray drying can be determined with the methodology given by Aghbashlo et al. (2012) as follows:

$$\mathrm{EE} = \frac{\text{total oil} - \text{surface oil}}{\text{total oil}} \times 100 \qquad (2.4)$$

The water activity (a_w) of the flaxseed microcapsules was measured at 25 °C with a Model CX-2 vapor sorption analyzer (AquaLab, Decagon Devices, Pullman, WA). To determine the water content of the powder, about 0.250 g of the sample was dried at 105 °C for 24 h. The difference in weight of the empty plate and after drying was used to determine the water content (AOAC, 1995).

Scanning Electron Microscope

Environmental scanning electronic microscope (ESEM; Quanta 200 ESEM, FEI Co., Hillsboro, OR) was used to obtain micrographs of flaxseed oil microcapsules. For this analysis, microcapsules were spread on the sample holder and placed in the specimen chamber for measurement of their morphology.

Solubility of Powder

The solubility in water of microencapsulated flaxseed oil was determined as described by Costa et al. (2015) with few modifications. A 0.5 g of spray-dried sample (dry weight) was reconstituted in 5 mL of distilled water by stirring at 3000 rpm every 10 min for 30 min to form an emulsion. This emulsion was centrifuged 15,000 rpm for 20 min. The supernatant was separated and allowed to dry at 105 °C until it reached a constant weight. The solubility in water was calculated with Eq 2.5.

$$\text{solubility}\ (\%) = \frac{\text{wt of dried supernatant}}{\text{wt of sample}} \times 100 \qquad (2.5)$$

Statistical Analysis

All statistical comparisons were based on triplicate results unless otherwise noted. Measurement values are presented as means and standard deviations. Means were compared using a one-way ANOVA with Tukey's post test, and significance was set at $P < 0.05$.

Results and Discussion

Properties of PPI Powders

Proximate composition, FAC, WHC, Na^+, and color parameters were determined to evaluate difference between commercial PPIs and their influence on functional properties. As shown in Table 2, all three PPI powders had significantly different protein contents (on dry basis). The protein content of Pulseplus80 was greater than Nutralys and PP. Nutralys had a higher moisture content (7.64 %), followed by Pulseplus80

Table 2 Proximate composition of PPI powders (on dry basis)

	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Nutralys	83.24 ± 0.01^{a}	0.15 ± 0.03^{d}	5.67 ± 0.21^{f}	10.95 ± 0.22^{h}
PP	80.99 ± 0.37^{b}	0.28 ± 0.06^{d}	5.52 ± 0.34^{f}	13.21 ± 0.453^{i}
Pulseplus80	85.45 ± 0.45^{c}	1.70 ± 0.34^{e}	4.84 ± 0.06^{g}	8.02 ± 0.32^{j}

Means in each column followed by different letters were significantly different ($P < 0.05$)

Table 3 Physical properties of PPI powders

	FAC (g oil/g powder)	WHC (g H_2O/g powder)	NaCl concentration (%)	L^*	a^*	b^*
Nutralys	1.45 ± 0.27^{a}	2.78 ± 0.07^{c}	0.32^{l}	78.3 ± 0.05^{g}	4.9 ± 0.03^{e}	19.3 ± 0.20^{j}
PP	1.39 ± 0.14^{a}	3.84 ± 0.02^{d}	0.38^{l}	73.5 ± 0.18^{h}	2.8 ± 0.17^{f}	18.6 ± 1.07^{j}
Pulseplus80	0.70 ± 0.13^{b}	2.90 ± 0.08^{c}	0.19^{m}	76.7 ± 0.04^{i}	4.3 ± 0.08^{e}	27.7 ± 0.39^{k}

Means in each column followed by different letters were significantly different ($P < 0.05$)

(6.62 %) and PP (3.78 %). No significant ($P > 0.05$) difference was observed for the fat and ash contents of Nutralys and PP; however, the carbohydrate content differed significantly ($P < 0.05$) due to difference in moisture and protein contents. Pulseplus80 contained greater fat content and lower ash content than the Nutralys and PP.

The FAC of the Nutralys and PP samples were greater than Pulseplus80. These values accord with FAC values found by Butt and Batool (2010) (1.40 g oil/g of dry matter), as well as Tomoskozi et al. (2001) (0.5 g oil/g of dry matter). Differences in the functional properties or encapsulation efficiency of PPIs could be due to differences in their proximate composition (protein, fat, and ash), protein fractions, and processing steps involved in manufacturing the PPIs (Toews and Wang 2013). Karaca et al. (2013) found that PPI prepared by isoelectric precipitation has a higher protein content (88.8 %) and lower moisture content (5.08 %) than PPI prepared by salt extraction, with protein and moisture content of 81.1 and 9.55 %, respectively. Pulseplus80 contained significantly higher fat which might be the reason of lower FAC values, as more amount of fat was already attached to non-polar amino group present in protein thus limiting adsorption of externally added oil. WHC of PP was significantly greater than WHC of Nutralys and Pulseplus80 (Table 3) which was negatively correlated with the moisture content of PPIs. WHC values were in accordance with values reported by Toews and Wang (2013) and Boye et al. (2010). Toews and Wang (2013) found positive correlation of WHC with protein, ash content, and negative correlation with the fat content of PPIs. We didn't find any correlation between WHC, protein, fat and ash content of PPIs. However difference in WHC could be attributed to presence of polar amino acids on the surface of protein. Nutralys and PP contained significantly higher NaCl compared to Pulseplus80 which positively correlated with the ash content of PPIs ($P < 0.05$) (Table 3).

The brightness value L^* of Nutralys was highest followed by Pulseplus80 and PP (Table 3). Redness (a^*) values ranged from 2.75 to 4.88, while yellowness values (b^*) ranged from and 18.6 to 27.7. PPIs with greater L^* and lower a^* and b^* values are preferred to minimize any change in color after their incorporation in food product. With respect to color prospective, either Nutralys, because of its greater L^* value, or PP, because of its lower a^* and b^* values, could perform better than Pulseplus80. Differences in the color of the protein were likely due to different processing techniques and different types of pea seeds used to manufacture the isolates. The b^* values positively correlated with the fat content and negatively correlated to the ash content of PPIs; this observation was in accordance with Toews and Wang (2013).

Figure 1 displays the electrophoretic patterns of commercial PPIs. The SDS-PAGE technique was used to differentiate protein subunits based on their molecular weights, as it may affect the functionality of the proteins (Koyoro and Powers 1987). Pea protein consists of different types of protein fractions which include globulins, albumins, and glutelins. Globulin fraction in pea protein contains three subfractions: legumin, vicilin, and convicilin which affects emulsifying

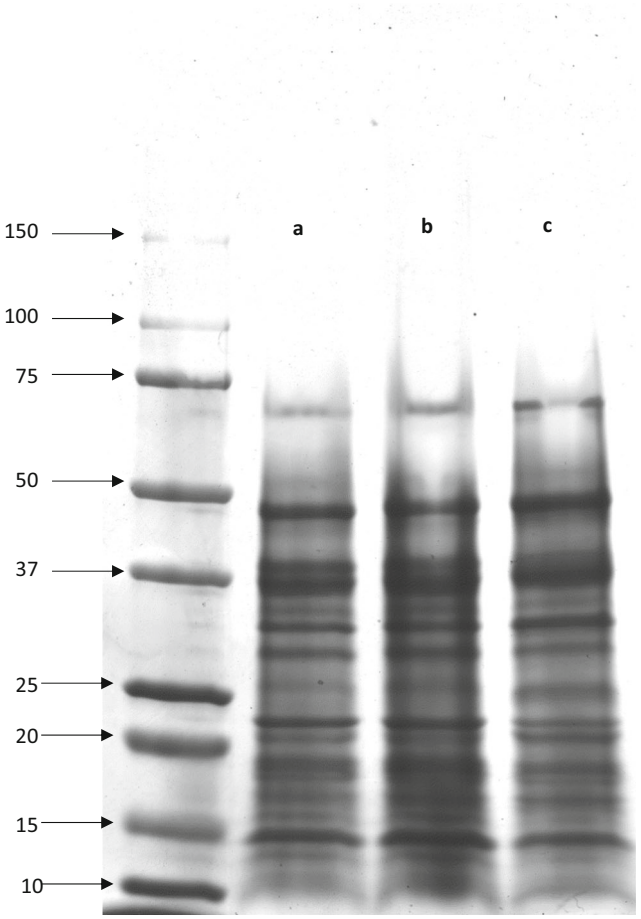

Fig. 1 SDS-PAGE patterns of commercial PPIs: (***a***) Nutralys, (***b***) PP, and (*c*) Pulseplus80

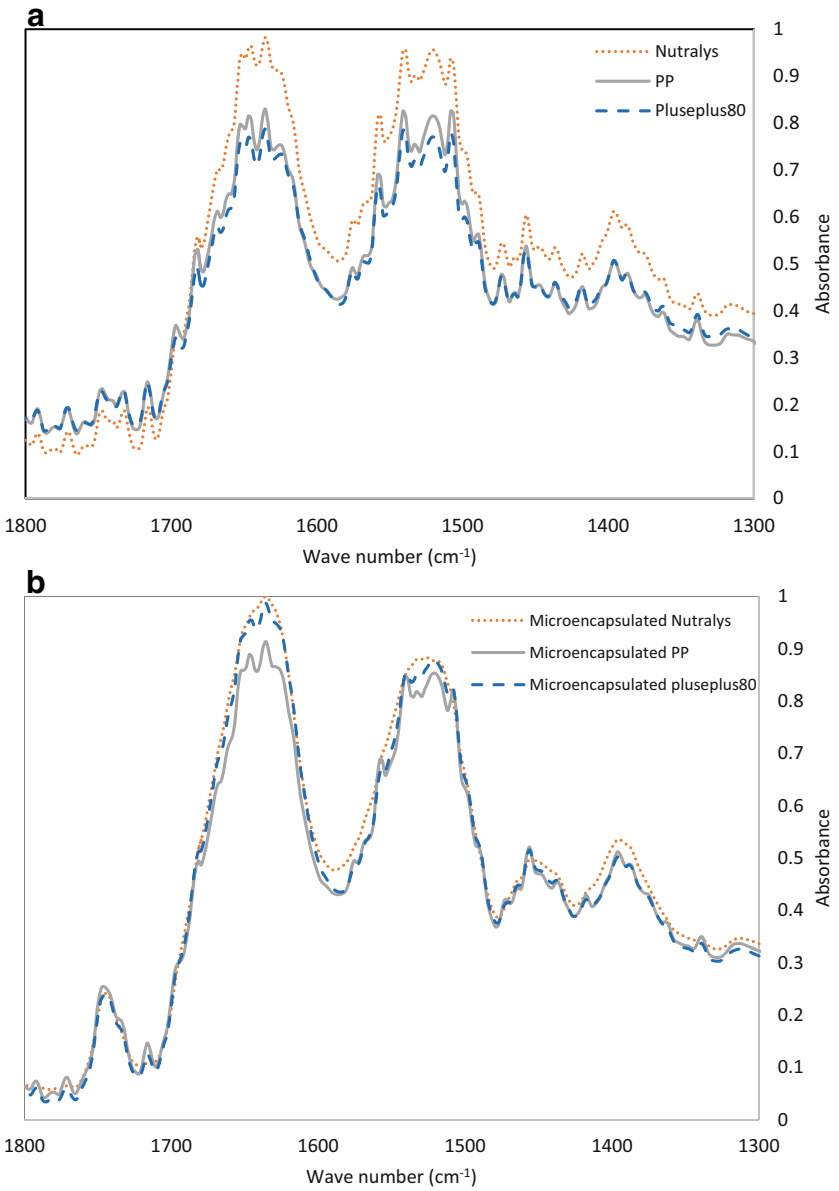

Fig. 2 FTIR spectra of **a** PPIs and **b** microencapsulated flaxseed oil

properties of proteins (Koyoro and Powers 1987). Three protein subfractions legumin, vicilin, and convicilin were present in all three PPIs and ranged from ~75 to 15 kDa (Fig. 1), but their concentration may be different (Dziuba et al. 2014). All PPIs showed the same bands, and no difference in the electrophoretic pattern was observed. Taherian et al. (2011) also observed similar electrophoretic pattern in PPIs extracted using different techniques and proposed that extraction procedures did not affect protein composition in terms of fractionation.

Table 4 Properties of PPI-flax seed oil emulsions for 1:5 core to wall material ratio

	Emulsion stability index	Droplet size distribution (nm)		Viscosity (at 40/s shear rate) (pa.s)
		0 h	24 h	
Nutralys	1.00	97.60 ± 3.20^{ab}	101.00 ± 2.49^{a}	0.012
PP	1.00	101.00 ± 4.51^{a}	104.00 ± 5.20^{c}	0.014
Pulseplus80	1.00	95.90 ± 4.23^{b}	102.00 ± 3.92^{a}	0.010

Means in each column followed by different letters were significantly different ($P < 0.05$)

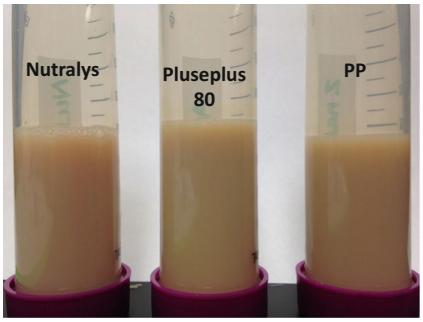

Fig. 3 Emulsion stability (1:5 core-to-wall-material ratio) test after 24 h at 20 °C

FTIR spectra of PPIs were obtained to check conformational differences in commercial PPIs (Fig. 2). All PPIs showed similar peaks at wavelength range from 1500 to 1650/cm which indicates presence of amide I and amide II bonds. Peaks obtained in the range of 1200 to 1300/cm indicate the presence of amide III bonds, which were also similar in all three proteins. Although peaks of all PPIs were similar, there was a difference in the intensity of absorbance. Peaks obtained from Nutralys indicated higher intensities, followed by PP and Pulseplus80. This means that the concentration of the amide bonds in Nutralys was greater than that in PP and Pulseplus80 which could be responsible for the difference in the functional properties in protein.

Properties of PPI Emulsions

ESI mainly depends on the emulsifying properties of the wall material and homogenization technique used to prepare the emulsion (Pinnamaneni et al. 2003). As discussed before, emulsifying properties (FAC and WHC) were also influenced by the proximate composition of the protein. The ESI of all emulsions prepared in this study was 1 (Table 4). No creaming or separation was observed when emulsions were stored at 25 °C for 24 h (Fig. 3). Emulsion prepared from Nutralys showed no significant increase in the droplet size, unlike PP and Pulseplus80 (Table 4) which indicate emulsion stability.

Higher emulsion activity index and surface hydrophobicity were often correlated with NaCl content/ionic strength of the emulsion (Zhang et al. 2009; Cheung et al. 2015). Though Nutralys and PP contained greater NaCl contents than Pulseplus80, no difference in ESI was observed. This could be due to similar homogenization conditions used in the preparation of the emulsions. All emulsions were prepared using a high-pressure homogenization process and therefore formed very fine particles. Fine particles create kinematically stable emulsions (Donsì et al. 2010). At 0 h, the mean droplet size of Nutralys emulsion was similar to droplet size of PP and Pulseplus80; however, droplet size of PP and Pulseplus80 emulsions was significantly ($P > 0.05$) different (Table 4). Mean droplet size of Nutralys emulsion after 24 h did not

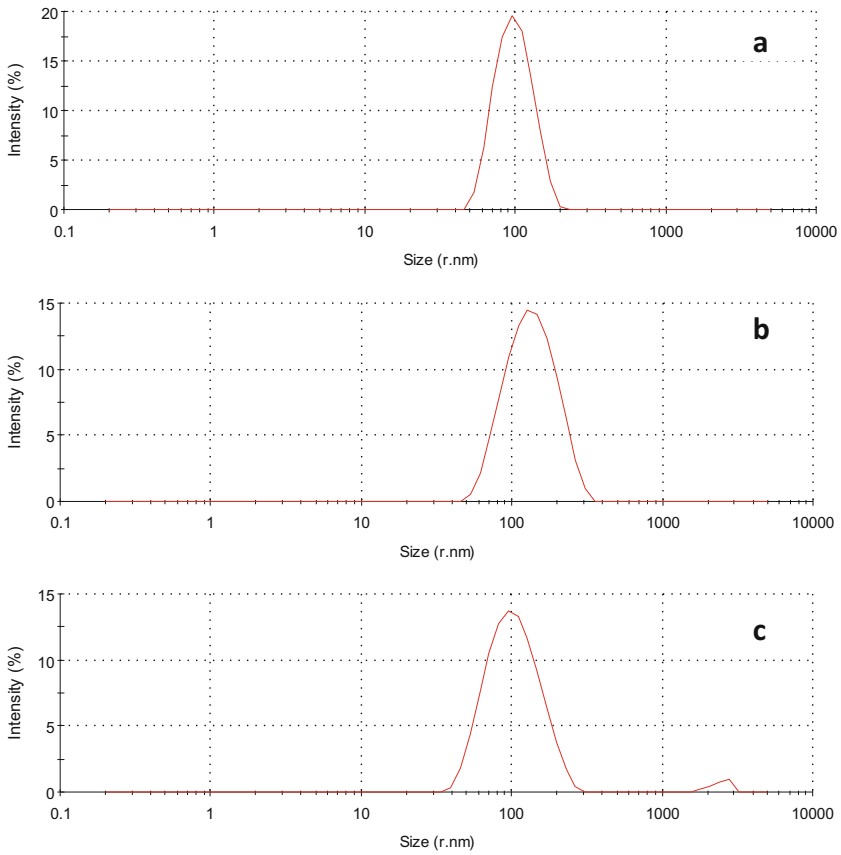

Fig. 4 Droplet size distribution of emulsion 0 h **a** Nutralys, **b** PP, and **c** Pulseplus80

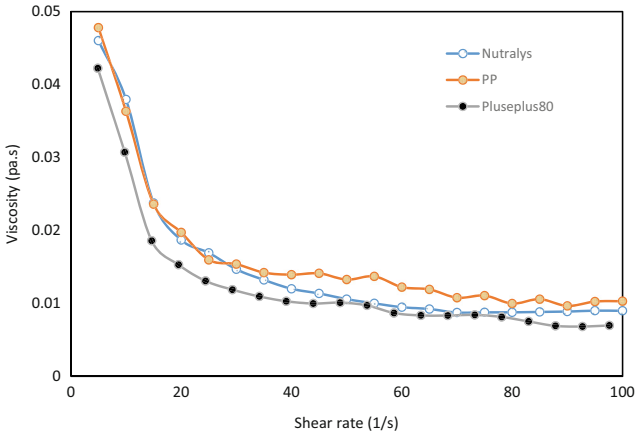

Fig. 5 Viscosity curves of pea protein emulsion (20 % oil loading) at 25 °C

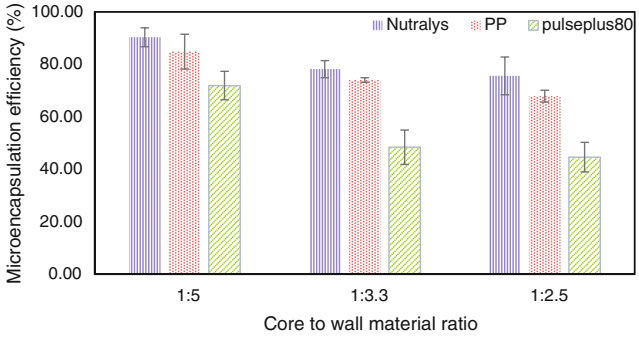

Fig. 6 Influence of oil loading on microencapsulation efficiency of powders

change significantly suggesting the greater emulsion stability. The PP and Pulseplus80 emulsions were also visually stable; however, the mean size oil droplets increased after 24 h (Table 4). Nutralys and PP emulsions showed monomodal distribution, and Pulseplus80 emulsion showed bimodal distribution (Fig. 4). Emulsion with monomodal distribution along with narrowest particle size distribution or smaller area under the peak denotes homogeneous and stable emulsion (Goyal et al. 2014). Nutralys emulsion showed narrower peak (45–220 nm) compared to PP (35–370 nm) and Pulseplus80 (35–200 and 1500–3250 nm). Second peak observed in Pulseplus80 suggested either coalescence of oil droplets or aggregation of proteins adsorbed on to different fat droplets, thus making the emulsion relatively less stable (Sourdet et al. 2003).

Figure 5 shows the viscosity curves for all three emulsions. All emulsions showed shear-thinning behaviors as their viscosities decreased with increasing shear rates. However, degree of decrease differed for all three emulsions. Taherian et al. (2007) reported that shear-thinning properties of emulsion indicate emulsion stability. It prevents droplets from creaming due to greater viscosity at zero shear rate, but food emulsions could flow easily when the shear is applied or decanted from a container. Higher viscosity limits the movement of the oil droplet in emulsion, thus reduces creaming or as coalescence phenomenon (Jafari et al. 2012). The viscosity of emulsions was positively correlated to the droplet size of the emulsion since Pulseplus80 showed the lowest viscosity, followed by Nutralys and PP. It was difficult to compare our viscosity results with literature values due to differences in wall material, type of oil, homogenization conditions used to prepare emulsions which significantly affect the ESI, droplet size, and viscosity of the emulsion.

Properties of Microcapsules

Nutralys and PP had higher microencapsulation efficiencies (ME) than Pulseplus80 (Table 5). No significant differences were observed in the MEs of PP and Nutralys ($P < 0.05$). Differences in encapsulation efficiency could be attributed to the stability and viscosity of the initial emulsion. Conditions used for the microencapsulation (e.g., preparation emulsions, homogenization, and spray drying) largely affect the ME; since we have used similar processing conditions, we did not consider these factors in present study. A viscous initial emulsion decreases droplet movement and speeds up semi-permeable membrane formation compared to a less viscous emulsion (Jafari et al. 2008). The ME values in this study cannot be compared with the results from other studies, since none have investigated PPIs alone at 10 % as a wall material for microencapsulation of flaxseed oil. Wang et al. (2011) used barley protein (15 % solid concentration) to encapsulate fish oil, resulting in encapsulation efficiencies ranging from 92.9 to 100 % for a core-to-wall-material ratio of 1:1. Fish oil encapsulated with mixture of gelatin and maltodextrin with microbial transglutaminase gave 88 % of encapsulation efficiency (Pourashouri et al. 2014). Tonon et al. (2011) reported that ME of encapsulated flaxseed oil prepared using gum arabic (10 % concentration) and 20 % of oil (on total solid basis)

Table 5 Properties of PPI-flax seed oil microcapsules for 1:5 core to wall material ratio

	Microencapsulation efficiency (ME, %)	Water content (%)	Water activity (a_w)	Solubility (%)
Nutralys	90.4 ± 3.6^{a}	3.90 ± 0.12^{d}	0.18 ± 0.01^{c}	10.9 ± 0.11
PP	84.9 ± 6.7^{a}	3.95 ± 0.44^{d}	0.18 ± 0.00^{c}	8.69 ± 0.10
Pulseplus80	71.9 ± 5.4^{b}	4.28 ± 0.76^{d}	0.18 ± 0.00^{c}	9.44 ± 0.94

Means in each column followed by different letters were significantly different ($P < 0.05$)

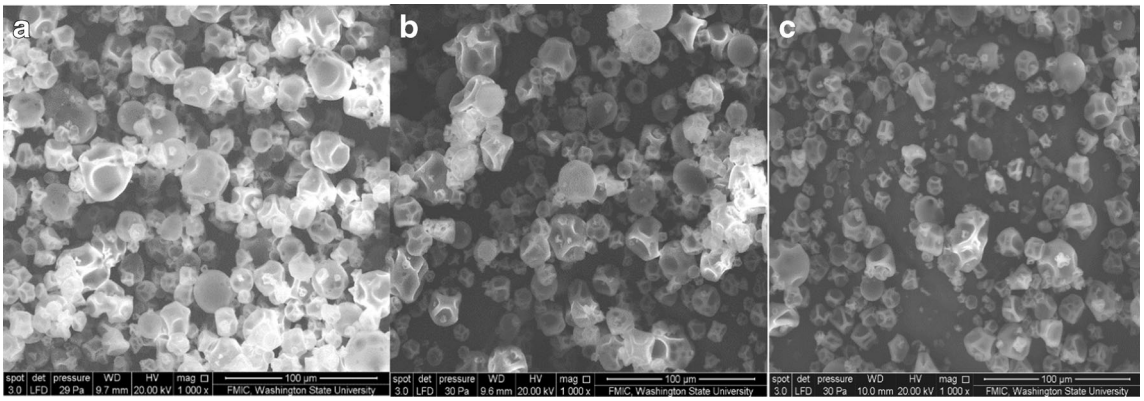

Fig. 7 SEM images of encapsulated powders: **a** Nutralys, **b** PP, and **c** Pulseplus80

was 51.5 %. Carneiro et al. (2013) obtained ME in the range of 62.3 to 95.7 % when flaxseed oil was encapsulated using a combination of modified starches (Hicap100 and Capsul TA) and maltodextrin, whey protein concentrate and maltodextrin, and gum arabic and maltodextrin. ME values obtained using PPIs (Table 5) were comparable with the literature values, hence denotes potential of PPIs to be used as a wall material for microencapsulation purpose. Figure 2b shows the FTIR spectra of microencapsulated flaxseed oil. Peak at 1743/cm in all three samples indicates presence of ester functional group from fatty acids or lipid. Although ME of Nutralys is greater than PP and Pulseplus80, no difference in peak intensities for all three powders was observed and this could be attributed to a higher fat content in Pulseplus80 and PP powders (Table 2).

Gharsallaoui et al. (2007) reported that ME of microcapsules was influenced by the ratio between the core and wall material. We observed that increase in the core-to-wall-material ratio in the emulsion reduced the encapsulation efficiency of microcapsules in all three samples (Fig. 6). These results are in agreement with the findings by Karaca et al. (2013), who found an increase in the surface oil of spray-dried chickpea and lentil protein isolates microcapsules from 5.3 to 21.1 % when the oil concentration in the emulsion increased from 1.0 to 16.9 %. The insufficiency of the wall material to cover the surface area of oil droplets could be the reason for this trend.

Water content and water activity of powder were determined to get indication of powder stability and flowability. Solubility of powders is another important parameter which needs to be considered before incorporation in food product. Water content of all three samples was similar, ranging from 3.9 to 4.25 %, and there was also no significant difference in water activity (Table 5). This was likely due to similar inlet temperature, feed rate, atomizer speed, and outlet temperature maintained during spray drying. The solubility of Nutralys powder was greater than that of Pulseplus80 and PP. The solubility of protein is influenced by the extraction procedures used in manufacturing isolates, the protein fractions present in the powder, and the pH (Koyoro and Powers 1987).

Figure 7 shows the surface morphology of PPI microcapsules containing flaxseed oil. All microcapsules using PPIs became shriveled, unlike microcapsules that are produced with carbohydrates. However, Nutralys forms more spherical and smoother microcapsules compared to PP and Pulseplus80, which might be the reason for its greater encapsulation efficiency. Shriveled structure could be due to spray drying conditions and the viscoelastic properties of the wall material. Xu et al. (2013) observed similar morphological characteristics in microcapsules containing sunflower oil as core materials and whey protein isolate and maltodextrin as wall materials. Although shriveled structure may affect the flowability of the powders, no major visible cracks or fissures were observed which is an indication of a better oxidative stability of encapsulated oil.

Conclusions

This study confirms that PPI can be used as a wall material for microencapsulation of flaxseed oil due to its functional properties. PPIs at 10 % concentration in emulsion form stable emulsions. Our results showed that microcapsules produced with 1:5 flaxseed-oil-to-wall-material ratios had a higher ME, and ME decreased with an increase in the oil loading since the wall material was not sufficient to form a continuous film around the oil droplets. Findings also showed that Nutralys had a higher ME, followed by PP and Pulseplus80. Proximate composition of protein, emulsifying properties (FAC and WHC), Na^+, and droplet size distribution affected ME and should be considered while selecting suitable PPIs for encapsulation purpose. Encapsulated flaxseed oil using PPI can be used as a functional ingredient in food products due to their health benefits. Further efforts need to be made to increase the PPI concentration in the emulsion to improve the profitability of microencapsulation and spray drying processes while maintaining lower viscosities.

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