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Encapsulation of Flaxseed Oil Using a Benchtop Spray Dryer for Legume Protein−Maltodextrin Microcapsule Preparation

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ABSTRACT: Flaxseed oil was microencapsulated employing a wall material matrix of either chickpea (CPI) or lentil protein isolate (LPI) and maltodextrin using a benchtop spray dryer. Effects of emulsion formulation (oil, protein and maltodextrin levels) and protein source (CPI vs LPI) on the physicochemical characteristics, oxidative stability, and release properties of the resulting capsules were investigated. Microcapsule formulations containing higher oil levels (20% oil, 20% protein, 60% maltodextrin) were found to have higher surface oil and lower encapsulation efficiencies. Overall, LPI−maltodextrin capsules gave higher flaxseed oil encapsulation efficiencies (~88.0%) relative to CPI−maltodextrin matrices (~86.3%). However, both designs were found to provide encapsulated flaxseed oil protection against oxidation over a 25 d room temperature storage study relative to free oil. Overall, ~37.6% of encapsulated flaxseed oil was released after 2 h under simulated gastric fluid, followed by the release of an additional ~46.6% over a 3 h period under simulated intestinal fluid conditions.

KEYWORDS: *chickpea, lentil protein, spray drying, flaxseed oil, oxidative stability, maltodextrin*

INTRODUCTION

Canada is the largest producer and exporter of flaxseed (*Linum usitatissimum*), with Saskatchewan accounting for approximately 70% of Canada's total production.[1] Flaxseed oil represents a rich source of polyunsaturated fatty acids (PUFAs) (e.g., α-linolenic acid), which have been positively correlated with a variety of human health benefits, such as reducing the risk of coronary heart diseases,[2] protection against inflammation,[3] and prevention of certain types of cancer.[4] However, its use in foods has been hindered due to its lack of miscibility in aqueous systems, susceptibility to oxidation, and distinct flavor. Microencapsulation technology offers a means to circumvent these problems by protecting flaxseed oil PUFAs against oxidation, improving their aqueous miscibility, and masking its taste. Similar technology has been examined for PUFAs protection in fish oils with some success.[5−13] Flaxseed oil has been previously encapsulated by spray drying within a variety of wall materials, such as whey protein isolate,[14] gum Arabic,[15] zein,[16] and sodium caseinate/lactose.[17] Spray drying involves the atomization of an emulsion into a wall material under a hot air current, resulting in rapid water evaporation and instantaneous entrapment of the core material.[18]

To the best of our knowledge, the microencapsulation of flaxseed oil using legume proteins as wall materials has not been reported in the literature. Chickpea and lentil proteins appear to be promising alternatives to animal proteins in encapsulation systems due to their nutritional value, low cost, and possible beneficial health effects (e.g., reducing the risk of cardiovascular diseases, diabetes, digestive tract diseases, and obesity).[19,20] The major storage proteins in legume seeds are globulins and albumins. Globulins represent ~70% of the protein found in legume seeds and are classified as either 11S (legumins; S - Svedberg Unit) or 7S (vicilins) based on their sedimentation coefficients.[21] Legumin is a hexameric protein with an overall molecular weight of 300−400 kDa, whereas vicilin is a trimeric protein with a molecular weight between 150 and 180 kDa.[22] Albumins constitute 10−20% of the protein in legume seeds and can have variable molecular weights (16−483 kDa).[23] In the present study, maltodextrin-DE 9 was used as a secondary wall material (i.e., filler) to improve microcapsule drying properties.[6] Maltodextrins are widely used as wall materials for capsule formation as they exhibit good solubility and low viscosities at high solids contents.[18,24] The objectives of this study were to investigate the effects of oil concentration and wall material type on the physicochemical properties of microcapsules containing flaxseed oil as produced by a benchtop spray dryer, and to evaluate their ability to protect against oxidation and release properties. Benchtop spray drying is typically used as an initial step in narrowing down formulation and processing conditions prior to scaling up to pilot plant testing or industrial scale production. Successful encapsulation of flaxseed oil using only plant-derived materials may open up new markets for encapsulated ingredients who restrict the use of animal-based ingredients (e.g., gelatin, as a wall material).

MATERIALS AND METHODS

Materials. Chickpea (CDC Frontier, Kabuli) and lentil (CDC Grandora) seeds were provided by the Crop Development Centre at the University of Saskatchewan (Saskatoon, SK, Canada). Maltodextrin (DE 9; Dry MD 01918) and flaxseed oil were kindly donated by Cargill Inc. (Cargill Texturizing Solutions, Cedar Rapids, IA) and Bioriginal Food & Science Corp. (Saskatoon, SK, Canada), respectively. All chemicals used were of reagent grade and purchased from Sigma-Aldrich (Oakville, ON, Canada). The water used in this research was produced from a Millipore Milli-Q water system (Millipore Corp., Milford, MA).

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Table 1. Formulations of CPI- and LPI-Stabilized Emulsions before and after Spray Drying

in initial emulsion					in spray-dried powder			
% oil	% protein	% maltodextrin	% total solids	core:wall	% oil	% protein	% maltodextrin	% total solids
2	4	14	20	1:9	10	20	70	100
3	4	13	20	1:5.7	15	20	65	100
4	4	12	20	1:4	20	20	60	100

Proximate Analysis. Proximate composition analyses for protein isolates and maltodextrin-DE 9 were 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).[25] Carbohydrate content was determined on the basis of percent differential from 100%.

Proximate Composition of Maltodextrin. The chemical composition of maltodextrin-DE 9 was determined to be 4.6% moisture, 0.0% protein, 0.0% lipid, 95.0% carbohydrate, and 0.4% ash.

Protein Isolate Preparation. Whole chickpea and lentil seeds were ground into fine flour using an IKA A11 basic analytical mill (IKA Works, Inc., Wilmington, NC) for 1 min and then defatted using hexane (1:3 w/v flour/hexane ratio) for 40 min. The mixture was then filtered through a 110 mm Whatman Gr. One filter (Whatman International Ltd., Maidstone, U.K.) and air-dried in a fume hood. This defatting procedure was repeated twice for each flour.

Chickpea protein isolate (CPI) was prepared according to the method of Papalamprou et al.[23] In brief, defatted flour (100 g) was mixed with water at 1:10 ratio (w/v), adjusted to pH 9.0 using 1.0 M NaOH, and stirred at 500 rpm for 45 min at room temperature (21−23 °C). The suspension was then centrifuged at 4500*g* for 20 min at 4 °C using a Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC) to collect the supernatant. The resulting pellet was resuspended in water at a ratio of 1:5 (w/v), adjusted to pH 9.0, stirred for an additional 45 min, followed by centrifugation (4500*g*, 20 min, 4 °C). Supernatants were pooled and adjusted to pH 4.6 using 0.1 M HCl to precipitate the protein. The protein was recovered by centrifugation as above, collected and stored at −30 °C until freeze-drying which was performed using a Labconco FreeZone 6 freeze drier (Labconco Corp., Kansas City, MO) to yield a free-flowing powder. Proximate analysis of CPI showed a composition of, 85.40% protein, 6.52% moisture, 3.05% ash, 4.11% carbohydrate, and 0.92% lipid.

Lentil protein isolate (LPI) was produced with a combined method of Bamdad et al.[26] and Lee et al.[27] In brief, defatted flour (100 g) was mixed with water at 1:10 ratio (w/v), adjusted to pH 9.5 with 1.0 M NaOH, and stirred at 500 rpm for 1 h at room temperature. The mixture was kept static at 4 °C overnight to allow for the sedimentation of nonprotein constituents. After centrifugation at 1600*g* for 30 min at 4 °C, the supernatant was collected and pH adjusted to 4.5 with 0.1 M HCl. The precipitated protein was collected by centrifugation (1600*g*, 30 min, 4 °C) and stored at −30 °C until freeze-drying. Proximate analysis of LPI showed a composition of 81.90% protein, 5.04% moisture, 3.63% ash, 9.00% carbohydrate, and 0.43% lipid.

Emulsion Preparation. Protein solutions were prepared by dispersing the isolates (corrected on a weight basis for protein content) in water followed by adjustment to pH 3.0 with 0.1 M HCl. The resulting mixtures were stirred at 500 rpm overnight at 4 °C to ensure complete dispersion. Maltodextrin solutions were prepared by dispersing the samples in water followed by stirring at 300 rpm overnight at 4 °C. Prior to the homogenization, pH of the protein solutions was readjusted to 3.0. Oil-in-water emulsions were prepared by homogenizing varying amounts of protein solutions, maltodextrin solution and flaxseed oil (Table 1) in a 500 mL container by using Omni Macro Homogenizer (Omni International, Marietta, GA) with a 20 mm sawtooth generating probe at speed 4 (∼7200 rpm) for 10 min. Results from the corresponding formulations will be denoted by their oil content in the final powder (10, 15, and 20%) for discussion purposes. Protein solutions were adjusted to pH 3.0 based on an earlier emulsion optimization study for CPI and LPI by our research group.[28]

Droplet Size Measurements. Droplet size distributions of initial and reconstituted emulsions were measured using a Mastersizer 2000 laser light scattering instrument (Malvern Instruments Ltd., Worcestershire, U.K.) equipped with a Hydro 2000S sample handling unit (containing water). Emulsion samples were taken from the bottom of the container immediately after homogenization for analysis. The sample was stirred continuously within the sample cell to ensure homogeneity at room temperature. Obscuration in all the measurements was kept at ∼14% by adding distilled water. Droplet size distributions were calculated by the instrument according to the Mie Theory which uses the refractive index difference between the droplets and the dispersing medium to predict the intensity of the scattered light. The ratio of refractive index of flaxseed oil (1.479) to that of the dispersion medium (1.330) was 1.112. Droplet size measurements were reported as volume-length mean diameters ($d_{4,3}$), which is expressed as:

$$d_{4,3} = \frac{\sum_{i=1} n_i \cdot d_i^4}{\sum_{i=1} n_i \cdot d_i^3} \quad (1)$$

where n_i is the number of droplets of diameter (d_i).[31]

Emulsion Reconstitution. Spray-dried microcapsule samples of 0.5 g were dispersed in 4 mL of water and stirred at 500 rpm for 5 min. Samples were withdrawn for particle size distribution measurements performed as described above.

Bench-top Spray Drying. The emulsion samples were spray-dried by a mini spray drier B-290 (Büchi Labortechnik AG, Flawil, Switzerland) with an atomizer nozzle of 700 μm diameter. The dryer had an evaporation rate of 1 L/h and a chamber with diameter of 70 cm. The inlet air temperature was adjusted to 180 °C, and the outlet temperature was kept at 90 ± 3 °C by controlling the flow rate. In order to maintain homogeneity and to prevent coalescence of oil droplets, the emulsions were gently stirred using a magnetic stirrer while fed into the spray dryer. The spray-dried microcapsules were collected in the cyclone collection vessel.

Moisture Content and Water Activity. Moisture content of spray-dried microcapsules was determined gravimetrically, after drying the capsules in a forced-air oven at 105 °C for ∼12 h, whereas the water activity was determined using an AquaLab CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA).

Color Measurements. The color values of spray-dried microcapsules were measured using a Hunter colorimeter (ColorFlex EZ 45/0, Hunter Associates Laboratory, Inc., Reston, VA), which was standardized using a reference white tile. The results were expresses as *L* (lightness), *a* (redness), and *b* (yellowness) tristimulus values.

Microcapsule Surface and Total Oil Content. Surface oil of the microcapsules was determined according to the method of Liu et al.[29] Briefly, 2 g of microcapsules was dispersed in 30 mL of hexane followed by vigorous shaking for 30 s. The solvent was filtered through a Whatman Gr. One paper into a 40 mL beaker, and the beaker plus solvent was placed in a fume hood overnight to afford solvent evaporation. Microcapsule surface oil was then determined gravimetrically, after heating the beaker at 105 °C for 30 min to remove any residual solvent. Total oil content of the microcapsules was determined using a method described by Klinkesorn et al.[30] with some modifications. Briefly, 8 mL of water was added to 2 g of microcapsules followed by mixing at 300 rpm for 2 min. The resulting solution was then mixed with 40 mL hexane/2-propanol (3:1 v/v), stirred at 300 rpm for 15 min, and centrifuged at 1500*g* for 2 min. The clear organic phase was collected, and the aqueous phase was re-extracted with the solvent mixture. The organic phases were pooled

and filtered through anhydrous Na_2SO_4, and then the solvent was allowed to evaporate overnight in a fume hood. The amount of total oil was determined gravimetrically, after heating the beaker at 105 °C for 30 min. The flaxseed oil encapsulation efficiency (EE) was calculated from the quantitative determinations as follows:[11]

$$\text{EE}\ (\%) = \frac{\text{total oil} - \text{surface oil}}{\text{total oil}} \times 100 \quad (2)$$

Oxidative Stability. Oxidative stability of free (i.e., control) and encapsulated flaxseed oil was characterized during storage at room temperature over a 25 d period using the peroxide value and 2-thiobarbituric acid reactive substances tests. Microcapsules (~5 g/bottle) or bulk oil (~3 mL) were stored within individually sealed nitrogen-flushed 10 mL amber glass bottles for storage stability studies. Oxidative testing was carried out every 5 d over the 25 d testing period, using a separate unopened bottle of microcapsules and oil. Extraction of flaxseed oil from the microcapsules was performed according to the same procedure described previously for total oil determination, except the solvent was dried under a stream of nitrogen.

Peroxide Value (PV). In brief, ~0.2 g of sample oil was weighed into a 250 mL Erlenmeyer flask, followed by the addition of 30 mL of 3:2 acetic acid/chloroform (v/v) solution and 0.5 mL of saturated potassium iodide (KI). After vigorous shaking for exactly 1 min, 30 mL of water was added to this mixture. Half a milliliter aliquot of 1% (w/v) starch indicator was then added to the mixture, and the resulting solution was titrated using 0.001 N sodium thiosulfate ($Na_2S_2O_3$) until the purple color disappeared. PV was calculated as

$$\text{PV} = \frac{(S - B) \times N \times 1000}{W} \quad ([3])$$

where S is the volume of $Na_2S_2O_3$ added to the sample (mL), B is the volume of $Na_2S_2O_3$ of the blank (mL), N is the normality of $Na_2S_2O_3$ solution, and W is the sample weight (g).[34]

2-Thiobarbituric Acid Reactive Substances (TBARS). In brief, ~40 mg of sample oil was weighed into a 10 mL volumetric flask, dissolved, and brought to volume with *n*-butanol. To 2.0 mL Eppendorf tubes were added 50 μL of 8.1% (w/v) sodium dodecyl sulfate (SDS), 375 μL of 20% acetic acid, 375 μL of 0.8% (w/v) thiobarbituric acid (TBA), 8.25 μL of 0.02% (w/v) butylated hydroxytoluene (BHT) (in dimethyl sulfoxide (DMSO)), and 200 μL of the oil−butanol mixture. Samples were then heated at 95 °C for 1 h. After cooling in cold water, 0.9 mL of *n*-butanol/pyridine (15:1, v/v) was added, followed by vigorous shaking for 30 s. Samples were centrifuged at 4000*g* for 10 min, and the upper organic layer was transferred to a 1.5 mL cuvette and the absorbance at 532 nm was measured against a butanol blank. A standard curve was prepared using malondialdehyde (MDA) (1.25−50 μM) under the same experimental conditions. TBA values were expressed as mg MDA eq/mg oil, which equates to the reactive aldehyde content (nmol)/sample oil weight (mg) (modified from Pegg[31] and Akhlaghi and Bandy[32]).

Release Characteristics. Release behavior of the flaxseed oil from the microcapsules triggered by pH and ionic strength was determined by a combined method of Zhong and Jin[33] and Choi et al.[37] In brief, microcapsule samples of 5 g were dispersed in 50 mL of aqueous NaCl solutions (0, 50, 100, 150, and 200 mM) or water (pH adjusted to 3.0, 5.0, 7.0, or 9.0 with 0.1 M HCl or NaOH) followed by stirring at 500 rpm for 1 h. The amount of released oil was determined by gravimetric analysis after two 30 mL hexane extractions.

In vitro release behavior of microencapsulated flaxseed oil was also investigated by using a simulated gastrointestinal model according to the method of Burgar et al.[34] Simulated gastric fluid (SGF) was prepared by dissolving 2.0 g of NaCl and 7.0 mL of 11.6 M HCl in 900 mL of water. After the addition of 3.2 g of pepsin to this solution, pH was adjusted to 1.2 with 0.1 M HCl and the final volume was made up to 1000 mL with water. Simulated intestinal fluid (SIF) was prepared by dissolving 6.8 g of K_2HPO_4 in 800 mL of water. After addition of 77 mL of 0.2 M NaOH and 100.0 g of pancreatin, the solution was allowed to stir overnight at 4 °C. The pH was adjusted to 6.8 with 1 M NaOH or 1 M HCl, and the final volume was made up to 1000 mL with water.

A microencapsulated flaxseed oil sample of 5 g was mixed with 50 mL of SGF and incubated for 2 h at 37 °C and 100 rpm in a water bath. Released oil was extracted using hexane and then determined gravimetrically. For exposure to SGF and SIF in sequence, 5 g of microcapsule sample was mixed with 50 mL of SGF and incubated under same conditions for 2 h. The pH was adjusted to 6.8 using 1 M NaOH, followed by addition of 50 mL of SIF, and the sample was incubated under the same conditions for another 3 h. The amount of flaxseed oil released from the microcapsules was determined at the end of exposure to SGF and SIF. The amount of released oil was determined by gravimetric analysis as outlined above.

Statistical Analyses. Three replicates were measured on duplicate batches of capsules. All experiments were reported as the mean ± one standard deviation. A two-way analysis of variance (ANOVA) with a Scheffe posthoc test was used to measure statistical differences in microcapsule characteristics and oxidative stability as a function of protein source and oil concentration. A general linear model was employed to determine statistical differences in release profile of the microcapsules as a function of protein source, oil concentration, pH or ionic strength of the release medium. All statistical analyses were performed with SPSS version 17.0 software (SPSS, Inc., 2008, Chicago, IL).

RESULTS AND DISCUSSION

Physicochemical Characteristics of Microcapsules. The moisture contents and water activities of spray dried produced CPI and LPI microcapsules containing flaxseed oil are shown in Table 2. The moisture content of the microcapsules ranged between 3.65 and 4.12% ($p > 0.05$), and their water activity varied from 0.05 to 0.08 ($p > 0.05$). These results meet both the maximum moisture and water activity specifications for dried powders in the food industry which are ~3−4% and ~0.3, respectively.[35] The Hunter *L* (lightness), *a* (redness), and *b* (yellowness) tristimulus color values of spray-dried microcapsules containing flaxseed oil differed significantly for CPI and LPI as shown in Table 3 ($p < 0.05$). CPI-microcapsules were creamy in surface color, which was demonstrated by a mean *L*, *a*, and *b* values of 91.3, −0.3, and 9.1, respectively, whereas LPI−microcapsules were darker (beige) in color with mean *L*, *a*, and *b* values of 87.2, 1.6, and 11.4, respectively. The observed darker color of the LPI−microcapsules containing may be explained by the presence of pigments (e.g., proanthocyanidins, chlorophyll) within the hull of the lentil proteins used in isolate production.[26]

Table 2. Moisture Content (%) and Water Activity of Spray-Dried Flaxseed Oil Microcapsules (Data Represent the Mean ± One Standard Deviation ($n = 6$)[a])

	moisture content (%)		water activity	
oil (%)	CPI	LPI	CPI	LPI
10	3.66 ± 0.32[a]	4.12 ± 0.31[a]	0.08 ± 0.01[a]	0.08 ± 0.01[a]
15	4.07 ± 0.31[a]	3.89 ± 0.23[a]	0.08 ± 0.00[a]	0.05 ± 0.01[a]
20	3.71 ± 0.46[a]	3.65 ± 0.10[a]	0.06 ± 0.00[a]	0.06 ± 0.01[a]

[a]Means in each row followed by different letters were significantly different ($p < 0.05$).

In general, the presence of oil on the microcapsule surface has been shown to have an adverse effect on several characteristics of spray-dried powders such as flow, dispersion, and oxidative stability.[24] For the present study, the effect of emulsion formulation on both surface oil and encapsulation efficiency is presented in Table 4. The lowest surface oil and

Table 3. Hunter Color Values of Spray-Dried Microcapsules (Data Represent the Mean ± One Standard Deviation (n = 6)[a])

	CPI microcapsules			LPI microcapsules		
oil (%)	L	a	b	L	a	b
10	89.2 ± 0.0[a]	−0.5 ± 0.0[c]	10.4 ± 0.0[e]	87.5 ± 0.0[b]	1.6 ± 0.0[d]	11.4 ± 0.0[f]
15	92.9 ± 0.0[a]	−0.1 ± 0.0[c]	8.5 ± 0.0[e]	87.8 ± 0.0[b]	1.4 ± 0.0[d]	11.0 ± 0.0[f]
20	91.8 ± 0.0[a]	−0.4 ± 0.0[c]	8.4 ± 0.0[e]	86.3 ± 0.0[b]	1.6 ± 0.0[d]	11.8 ± 0.0[f]

[a]Means in each row followed by different letters were significantly different (p < 0.05).

Table 4. Changes in Surface Oil and Encapsulation Efficiency as a Function of Emulsion Formulation (Data Represent the Mean ± One Standard Deviation (n = 6)[a])

	surface oil (%)		encapsulation efficiency	
oil (%)	CPI	LPI	CPI	LPI
10	1.13 ± 0.07[a]	1.05 ± 0.08[a]	88.72 ± 0.69[a]	90.42 ± 0.64[a]
15	1.49 ± 0.11[b]	1.45 ± 0.12[b]	86.69 ± 0.95[b]	87.89 ± 0.96[b]
20	2.64 ± 0.04[c]	2.49 ± 0.07[c]	83.62 ± 0.24[c]	85.61 ± 0.40[c]

[a]Means in each column followed by different letters were significantly different (p < 0.05).

highest encapsulation efficiency for flaxseed oil with either plant protein source was observed at an initial oil concentration of 10%; with values of 1.13 and 1.05% for surface oil and 88.72 and 90.42% for encapsulation efficiency for CPI and LPI, respectively. An analysis of variance indicated that as the amount of flaxseed oil used in the emulsion formulation increased (from 10 to 20%), surface oil increased whereas the encapsulation efficiency decreased for both CPI and LPI (p < 0.05). The observed increase in surface oil as a function of oil content in the emulsion formulation was in accordance with the findings of Rusli et al.[36] and Polavarapu et al.,[37] both of whom reported lower encapsulation efficiencies at higher oil concentrations. The authors postulated that this was a result of having an insufficient amount of wall material for complete coverage of the emulsified oil droplets. Findings from this study indicate the potential of the wall material for entrapping sensitive oil, especially for the feed industry. However, further refinement may be needed to meet food industry targets of <2% total surface oil and >98% entrapment efficiencies.

The mean droplet diameter of CPI- and LPI-stabilized emulsions before spray-drying and after reconstitution is shown in Figure 1. The volume-weighted mean oil droplet diameters ($d_{4,3}$) of flaxseed oil-in-water emulsions stabilized by CPI and LPI ranged between 16.3 and 24.0 and 21.0−26.1 μm, respectively. An analysis of variance of droplet size indicated that the main effects of total oil concentration (p < 0.001) and sample conditions (i.e., those found in fresh vs reconstituted emulsions) (p < 0.01) were found to be significant, whereas protein-type (CPI vs LPI) (p > 0.05) was not. Furthermore, all 2-way interaction terms were found to be significant (p < 0.001). As the total oil content increased within the sample, size of the droplets increased significantly from ∼15.8 μm at the 2% level within the emulsion (10% in the reconstituted capsules) to 24.6 μm at the 3−4% level within the emulsion (15−20% in the reconstituted capsules) (p < 0.001). Droplet size was similar at the two higher oil concentrations (p > 0.05). Overall, droplet size was found to be reduced from ∼22.4 μm in the fresh emulsion to ∼21.0 μm in the reconstituted emulsion (p > 0.01).

Oxidative Stability of Encapsulated Flaxseed Oil. The peroxide value (PV) and TBARS results for free and CPI and

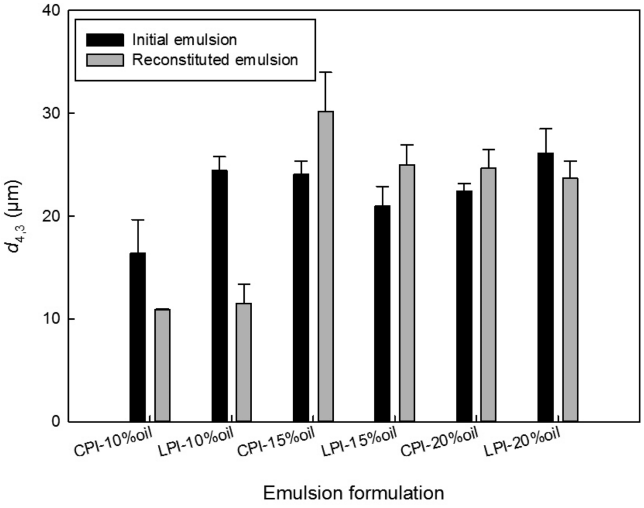

Figure 1. Effect of spray drying on mean droplet diameter for CPI- and LPI-stabilized emulsions. Data represent the mean ± one standard deviation (n = 6).

LPI encapsulated flaxseed oil maintained at room temperature over a 25 d period are presented in Table 5. Primary oxidation products, mainly peroxides, are highly reactive and readily break down to free radicals, which propagate oxidation reactions. They also participate in the autoxidation process producing a variety of secondary oxidation products, such as aldehydes and ketones.[31] The PV of flaxseed oil before microencapsulation was 5.73 ± 0.30 mequiv of active O_2/kg while that of the microencapsulated oil immediately after spray drying (Day 0) ranged from 6.31 to 6.80 mequiv of active O_2/kg. This increase in PV value for flaxseed oil during the microencapsulation process can be attributed to oxygen contact with oil during the emulsification spray drying processes. The PV results for both CPI and LPI microencapsulated flaxseed oil versus that of free oil were significantly different at storage days 15 to 25 (p < 0.05) (Table 5). This is illustrated by the PV values for free, CPI and LPI microencapsulated flaxseed oil values at storage day 25 of, 12.91 ± 0.40, 7.31 ± 0.56, and 6.86 ± 0.46 mequiv of active O_2/kg, respectively. These results clearly show that the plant protein microencapsulation process employed in this study provides significant protection to flaxseed oil oxidation during a 25 d storage period at room temperature. Modest increases in PV values for microencapsulated flaxseed oil were observed for both CPI and LPI, however these changes were not found to be significant. In addition, no significant differences in PV values in microencapsulated flaxseed oil was found between the two plant protein sources. On the other hand, the PV of bulk oil started to increase from 6.12 to 9.38 meq active O_2/kg at day 15 and kept increasing to 11.28 meq active O_2/kg at day 20 and finally to 12.91 meq active O_2/kg at day 25 (p < 0.05, Table 5). This finding could be attributed to the higher amount of surface oil in the microcapsules

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Table 5. Changes in (a) Peroxide Value (PV) and (b) Thiobarbituric Acid-Reactive Substances (TBARS) for Free and Microencapsulated Flaxseed Oil (Data Represent the Mean ± One Standard Deviation (n = 6)[a])

	protein source in the microcapsule	oil[b] (%)	day 0	day 5	day 10	day 15	day 20	day 25
PV (mequiv of active O_2/kg)	CPI	10	6.33 ± 0.10[a]	6.23 ± 0.14[a]	6.45 ± 0.23[a]	6.62 ± 0.21[a]	6.48 ± 0.32[a]	6.68 ± 0.36[a]
		15	6.43 ± 0.22[a]	6.34 ± 0.37[a]	6.51 ± 0.33[a]	6.80 ± 0.36[a]	6.69 ± 0.35[a]	6.71 ± 0.55[a]
		20	6.80 ± 0.21[a]	7.08 ± 0.23[a]	7.18 ± 0.26[a]	7.36 ± 0.31[a]	7.27 ± 0.26[a]	7.31 ± 0.56[a]
	LPI	10	6.31 ± 0.23[a]	6.29 ± 0.21[a]	6.42 ± 0.33[a]	6.57 ± 0.24[a]	6.54 ± 0.21[a]	6.62 ± 0.40[a]
		15	6.47 ± 0.25[a]	6.34 ± 0.22[a]	6.52 ± 0.30[a]	6.75 ± 0.38[a]	6.62 ± 0.26[a]	6.82 ± 0.31[a]
		20	6.73 ± 0.24[a]	6.84 ± 0.21[a]	6.74 ± 0.24[a]	6.89 ± 0.32[a]	6.99 ± 0.35[a]	6.86 ± 0.46[a]
	free oil		5.73 ± 0.30[a]	6.25 ± 0.16[a]	6.37 ± 0.14[a]	9.38 ± 0.75[b]	11.28 ± 0.36[c]	12.91 ± 0.40[d]
TBARS (nmol of MDA equiv/mg oil)	CPI	10	2.04 ± 0.27[a]	1.90 ± 0.22[a]	2.20 ± 0.15[a]	2.24 ± 0.18[a]	1.92 ± 0.25[a]	2.13 ± 0.24[a]
		15	2.00 ± 0.27[a]	2.07 ± 0.23[a]	2.29 ± 0.18[a]	2.18 ± 0.24[a]	2.10 ± 0.26[a]	2.22 ± 0.24[a]
		20	2.14 ± 0.21[a]	2.22 ± 0.18[a]	2.34 ± 0.14[a]	2.33 ± 0.16[a]	2.24 ± 0.24[a]	2.47 ± 0.18[a]
	LPI	10	2.03 ± 0.15[a]	2.03 ± 0.19[a]	2.12 ± 0.26[a]	1.91 ± 0.24[a]	2.21 ± 0.19[a]	2.13 ± 0.25[a]
		15	1.99 ± 0.18[a]	1.99 ± 0.20[a]	2.16 ± 0.35[a]	2.09 ± 0.27[a]	2.14 ± 0.22[a]	2.22 ± 0.24[a]
		20	2.10 ± 0.22[a]	2.02 ± 0.26[a]	2.37 ± 0.24[a]	2.22 ± 0.17[a]	2.31 ± 0.21[a]	2.40 ± 0.27[a]
	free oil		2.21 ± 0.15[a]	2.13 ± 0.20[a]	2.42 ± 0.16[a]	2.47 ± 0.09[a]	3.15 ± 0.27[b]	3.94 ± 0.30[c]

[a]Means in each row followed by different letters were significantly different (p < 0.05). [b]Concentration of oil in the microcapsule.

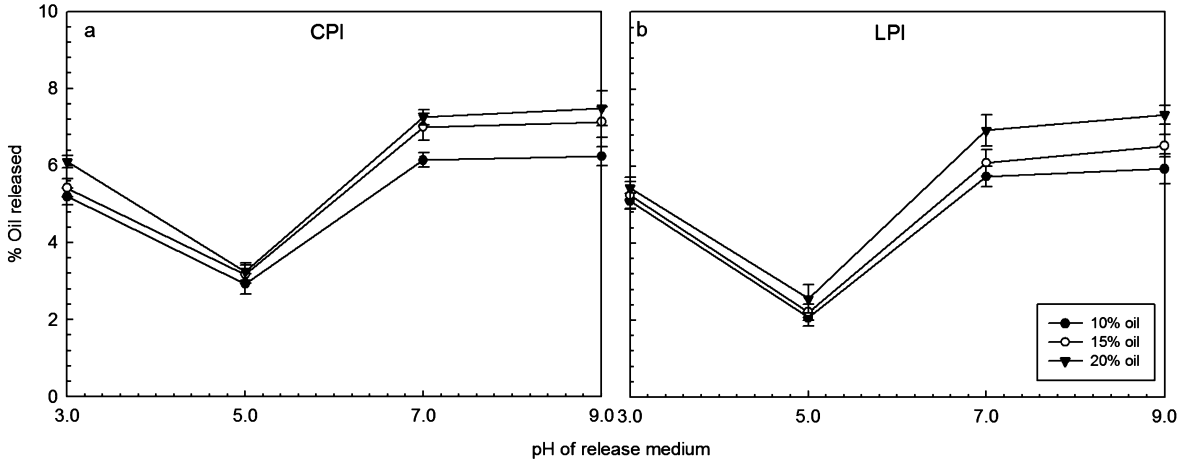

Figure 2. Release behavior of flaxseed oil microcapsules containing (a) CPI and (b) LPI, triggered by pH. Data represent the mean ± one standard deviation (n = 6).

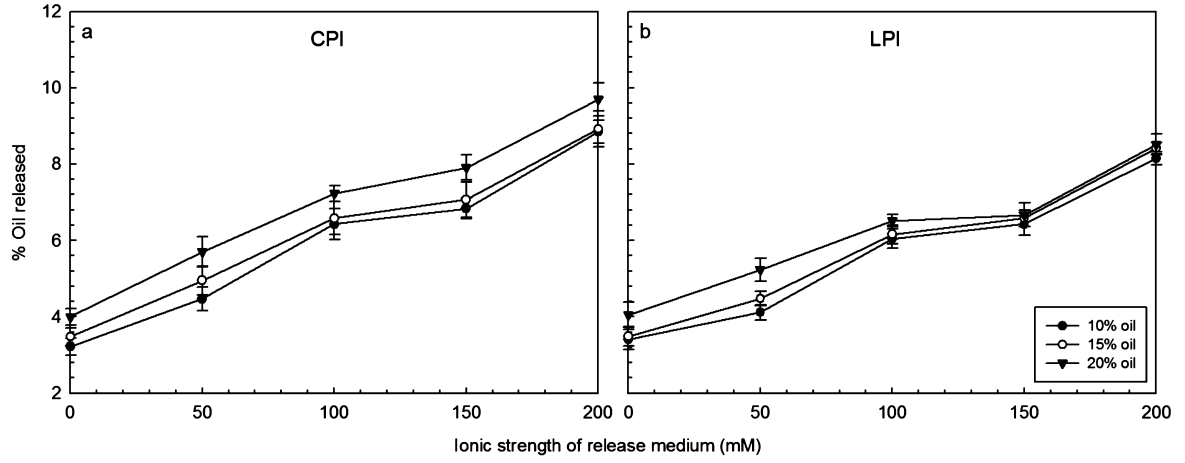

Figure 3. Release behavior of flaxseed oil microcapsules containing (a) CPI and (b) LPI, triggered by ionic strength. Data represent the mean ± one standard deviation (n = 6).

containing higher amounts of oil. Rusli et al.[36] also reported an increase in the amount of primary oxidation products in tuna oil microcapsules with increasing oil load. Tonon et al.[15] spray-dried flaxseed oil in a gum Arabic matrix and reported that lower solid content and higher oil concentration led to higher peroxide values.

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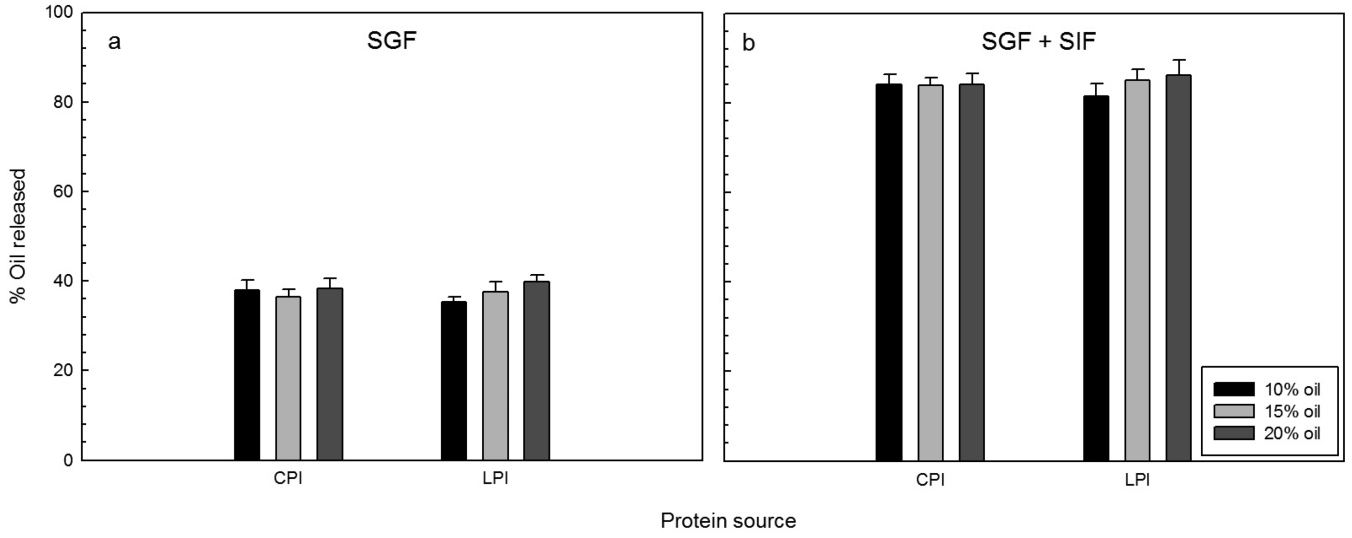

Figure 4. Release behavior of flaxseed oil microcapsules containing CPI and LPI in a) simulated gastric fluid (SGF); b) sequential exposure to simulated gastric and intestinal fluids (SGF + SIF). Data represent the mean ± one standard deviation (n = 6).

In this study, the TBARS test was employed to measure the secondary oxidation products of free and CPI and LPI microencapsulated flaxseed oil. The TBARS value of flaxseed oil before microencapsulation was 2.21 ± 0.15 nmol MDA eq/mg oil while that of the microencapsulated oil immediately following spray drying (Day 0) ranged from 1.99 to 2.14 nmol MDA eq/mg oil. No significant difference was observed in the TBARS values of flaxseed oil before and after microencapsulation indicating that the microencapsulation process had no effect on the formation of secondary oxidation products in flaxseed oil. The TBARS value of microencapsulated flaxseed oil in both CPI- and LPI-containing microcapsules was between 1.90 and 2.47 nmol MDA eq/mg oil and did not change over the 25 day storage period (p > 0.05, Table 5). In contrast, TBARS value of bulk oil started to increase from 2.21 to 3.15 nmol MDA eq/mg oil at day 20 and kept increasing to 3.94 nmol MDA eq/mg oil at day 25 (p < 0.05, Table 5); indicating an increase in secondary oxidative products such as aliphatic aldehydes, alcohols, ketones, cyclic compounds, and hydrocarbons (Table 5). These results clearly show that the legume protein-maltodextrin matrices tested improved the oxidative stability of flaxseed oil when compared to bulk oil as indicated by both primary and secondary oxidative products during a 25 d storage period at room temperature. To our knowledge, spray drying of flaxseed oil using legume proteins as wall materials has not been reported yet. Partanen et al.[14] spray dried flaxseed oil using whey protein isolate matrix and found that oxidation of microencapsulated flaxseed oil was retarded compared to that of bulk oil. Pu et al.[17] reported that oxidative stability of flaxseed oil microencapsulated in a sodium caseinate and lactose matrix was further improved by addition of shrimp astaxanthin into flaxseed oil prior to microencapsulation.

Release Characteristics of Spray-Dried Flaxseed Oil. The relationships between flaxseed oil release from CPI- and LPI-microcapsules as a function of pH, ionic strength and simulated gastrointestinal environments are shown in Figures 2, 3 and 4, respectively. For pH-triggered release, an analysis of variance showed that protein source, oil concentration, pH of the release medium and interactions between these factors were highly significant (p < 0.001). Percentage of oil released was found to be the lowest for pH 5.0 at ∼2.7%, and increased in conjunction with pH to 6.8% at pH 9.0. Lowest amount of oil released at pH 5.0 could be arising from the low solubility of CPI and LPI at pH values close to their isoelectric point whereas increased amounts of released oil from the microcapsules at lower (pH 3.0) and higher pH values (pH 7.0−9.0) could be attributed to increased solubility of CPI and LPI at these regions. In case of salt-triggered release, an analysis of variance indicated that protein source, oil concentration, ionic strength of the release medium plus the interactions between protein source-oil concentration and protein source-ionic strength were significant (p < 0.05). Percentage of oil released was found to be the lowest at 0 mM NaCl and increased with increasing ionic strength (p < 0.05), as it was assumed the addition of NaCl promoted protein solubility through increasing ordering of water molecules around the capsule's surface (i.e., salting-in effect).[38] Increased amounts of oil in the microcapsules resulted in increased amounts of released oil (p < 0.05) regardless of the protein source used in the microcapsules and ionic strength of the release medium.

The in vitro release behavior of CPI- and LPI-microencapsulated flaxseed oil was investigated employing both simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) models. Overall, CPI and LPI microcapsules released ∼37% of encapsulated flaxseed oil after 2 h in pepsin-containing simulated gastric fluid (pH 1.2), with a further ∼47% release after 3 h in pancreatin-containing simulated intestinal fluid (pH 6.8). The differences in the amount of oil released are thought to the different susceptibilities of the matrix components to digestion by different enzymes. For the CPI and LPI microcapsules, no significant differences (p > 0.05) in the amount of oil released under these experimental condition was observed with respect to the oil concentration (10−20%) of the microcapsules.

In conclusion, findings of the present study indicated that CPI- and LPI-based microcapsules have the potential for the entrapment and gastrointestinal delivery of flaxseed oil. Oil concentration and protein source had significant effects on physicochemical characteristics, encapsulation efficiency and release characteristics of microcapsules prepared by spray drying flaxseed oil using CPI or LPI and maltodextrin as wall materials. Encapsulation matrices tested showed a protective

effect against oxidation over a 25 d period of room temperature storage. Microcapsules were able to deliver 84.2% of the encapsulated oil within the gastrointestinal environments. Although this study was performed using a benchtop spray dryer, processing and formulation conditions could serve as a starting point for scaling up the process to the pilot scale. However further refinement to both parameters would be need for larger scale processing to obtain >98% entrapment efficiencies and <2% surface oil content. This study also identified opportunities for increasing the utilization of flaxseed oil and legume proteins in feed and food applications.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ANOVA, analysis of variance; CPI, chickpea protein isolate; EE, encapsulation efficiency; LPI, lentil protein isolate; SPI, soy protein isolate; PPI, pea protein isolate; FBI, fava bean protein isolate; MDA, malondialdehyde; PUFA, polyunsaturated fatty acid; PV, peroxide value; SEM, scanning electron microscopy; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; TBARS, 2-thiobarbituric acid reactive substances

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