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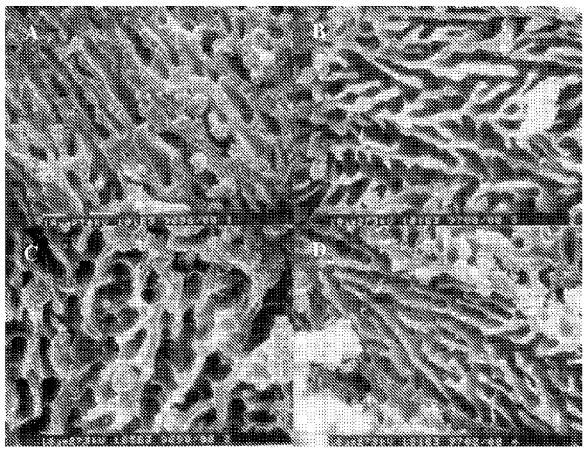

FIGURE 4

(57) Abstract: Microcapsules having a core comprising a material to be encapsulated and a shell comprising a legume protein and a low molecular weight carbohydrate are provided. Methods of producing the microcapsules are provided.

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MICROENCAPSULATION USING LEGUME PROTEINS

Technical Field

[0001] Some embodiments of the present invention relate to methods and compositions for the preservation and/or improvement of materials by microencapsulation. Some embodiments of the present invention relate to methods and compositions for the preservation and/or improvement of oils by microencapsulation. Some embodiments of the present invention relate to methods and compositions for the preservation and/or improvement of oils rich in polyunsaturated fatty acids by microencapsulation.

Background

[0002] Flaxseed oil is rich in essential fatty acids including polyunsaturated fatty acids (PUFA) such as α-linolenic acid, which are purported to induce a variety of health benefits upon consumption. These health benefits include: reducing the risk of coronary heart diseases (Li, Attar-Bashi & Sinclair, 2003), protection against inflammation (Bloedon, L. T. et al., 2008), and the prevention of breast and prostate cancers (Bougnoux & Chajès, 2003). Despite these purported health promoting properties, flaxseed oil remains underutilized by the food industry due to its susceptibility to oxidation because of its high PUFA content, distinct taste, and lack of miscibility in aqueous food systems (Łukaszewicz, Szopa & Krasowska, 2004; Bozan & Temelli, 2008). However, through the use of encapsulation technologies these limitations can potentially be circumvented so as to offer PUFA protection to the harsh environmental conditions experienced during food processing and storage, and to improve flaxseed oil miscibility in foods and mask its distinct taste.

[0003] As flaxseed oil is rich in polyunsaturated fatty acids (PUFAs), it is highly susceptible to oxidation resulting in the onset of rancidity. Łukaszewicz, Szopa & Krasowska (2004) investigated the oxidative stability of flaxseed oil produced from nine different cultivars by measuring both conjugated dienes and 2-thiobarbituric acid-reactive substances (TBARS) formation following sample storage in air at 140°C for 40 min. Results from these experiments showed that the concentration of conjugated dienes reached 50-200 mol/kg, whereas TBARS reached 0.1-0.5 mol/kg. Based on these results,

the authors concluded that flaxseed oil isolated from all nine cultivars was easily oxidized.

[0004] Encapsulation is defined as a process whereby an active ingredient becomes enclosed or packaged within micron-sized carrier matrices, which in turn segregates and protects the inner core from the surrounding environment (Gibbs, Kermasha, Alli & Mulligan, 1999). The particle size of the active ingredient (e.g. oil) dispersed within the aqueous phase of the emulsion has been shown to be a significant factor for retention within microcapsules, where the smaller the particle size the greater the retention (Rish and Reineccius, 1988). Smaller oil droplet sizes have also been shown to minimize microcapsule surface oil, increase oil encapsulation efficiency and decrease lipid oxidation rates (Lee & Ying, 2008).

[0005] Although gelatin is one of the most widely used encapsulating materials it suffers from a number of perceived safety concerns (e.g., prion disease), and religious and dietary restrictions. Therefore, the development of plant protein based encapsulation systems as an alternative to animal proteins is of considerable interest and importance.

[0006] Legume proteins can serve as a potential source for this purpose because of their high nutritional value, low cost and purported beneficial health benefits including but not limited to, reducing the risk of cardiovascular disease, as an aid in glycemic control in diabetic individuals, and in the prevention of digestive tract diseases (Boye et al., 2010; Duranti, 2006). 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 (Roy et al., 2010). 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 150-180 kDa (Derbyshire et al., 1976). Albumins constitute 10–20% of the protein in legume seeds and can have variable molecular weights (16-483 kDa) (Papalamprou et al., 2010).

[0007] Maltodextrins are widely used as wall materials for capsule formation as they exhibit good solubility and low viscosities at high solids contents (Gharsallaoui et al.,

2007). Maltodextrin can be used as a secondary wall material (i.e., filler) to improve microcapsule drying properties (Kagami et al., 2003; Bae and Lee, 2008).

[0008] A large number of different encapsulation methodologies are known. For example, freeze drying (or lyophilization) is a dehydration process whereby an emulsion is initially frozen, then put on under pressure in a vacuum to allow the ice to sublimate from a solid to a gas. Fluidized bed coating is a process whereby a pressurized emulsion is introduced to a solid secondary wall material (e.g., maltodextrin). The solid/fluid mixture behaves as a fluid within the drying bed to ensure excellent heat transfer.
Spray chilling can also be used to produce microcapsules. Spray drying is a common step used in the production of microencapsulated food ingredients (Pegg & Shahidi, 2007). Spray-drying involves the atomization of an emulsion into a wall material (e.g., whey protein isolate, gum Arabic, maltodextrin, etc.) under a hot air current, resulting in rapid water evaporation and instantaneous entrapment of the core material (Gharsallaoui et al., 2007).

[0009] The foregoing examples of the related art and limitations related thereto are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification and a study of the drawings.

Summary

[0010] In some embodiments, a microcapsule has a core of a material to be encapsulated and a shell comprising a legume protein and a low molecular weight carbohydrate. In some embodiments, the material to be encapsulated is an oil. In some embodiments, the oil is an oil rich in polyunsaturated fatty acids. In some embodiments, the oil is rich in omega-3 fatty acids, α-linolenic acid, eicosapentaenoic acid (EPA), and/or docosahexaenoic acid (DHA). In some embodiments, the material to be encapsulated is flaxseed oil, fish oil, flavour oil, fragrance oil, and/or an oil-soluble vitamin.

[0011] In some embodiments, the legume protein is chickpea protein, lentil protein, faba bean protein, or soybean protein. In some embodiments, the low molecular weight carbohydrate is dextrin or glycose syrup solids. In some embodiments, the dextrin is maltodextrin.

[0012] In some embodiments, the amount of legume protein in an emulsion from which the microcapsule is prepared is between about 3% and about 10% w/v. In some embodiments, the amount of low molecular weight carbohydrate in an emulsion from which the microcapsule is prepared is between about 20% w/v to about 50% w/v. In some embodiments, the amount of material to be encapsulated in an emulsion from which the microcapsule is prepared is in the range of about 5% w/v to about 25% w/v.

[0013] In some embodiments, the microcapsules are incorporated into a food product. In some embodiments, the food product is a dairy product or a dry food product. In some embodiments, the food product has a relatively neutral pH, for example, between about 6.5 and 7.0.

[0014] In some embodiments, a method for producing microcapsules includes combining a material to be encapsulated, a legume protein and a low molecular weight carbohydrate, mixing the resultant solution to form an emulsion, and forming microcapsules. In some embodiments, forming microcapsules comprises spray drying, freeze drying, spray chilling, or fluidized bed coating.

[0015] In some embodiments, the microcapsules are incorporated in a food product to provide delayed release of the encapsulated material. In some embodiments, the microcapsules are incorporated in a food product to provide delivery of the encapsulated material to the gastrointestinal tract of an animal, including a human. In some embodiments, the microcapsules are incorporated in a food product to hide the taste of the encapsulated material. In some embodiments, the microcapsules are formed to enhance the miscibility of the material encapsulated in the microcapsules in a food product.

Brief Description of Drawings

[0016] Exemplary embodiments are illustrated in referenced figures of the drawings. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[0017] Figure 1 shows changes in surface oil content as a function of emulsion formulation. Data represent the mean ± one standard deviation (n = 3).

[0018] Figure 2 shows changes in flaxseed oil encapsulation efficiency as a function of emulsion formulation. Data represent the mean ± one standard deviation (n = 3).

[0019] Figure 3 shows the effect of freeze-drying on mean droplet diameter for a) CPI- and b) LPI-stabilized emulsions. Data represent the mean ± one standard deviation (n = 3).

[0020] Figure 4 shows SEM images of freeze-dried CPI- and LPI-based microcapsules produced at conditions of 10.5% flaxseed oil and 35.5% maltodextrin-DE 9: a) CPI at pH 3.0; b) LPI at pH 3.0; c) CPI at pH 7.0; d) LPI at pH 7.0.

[0021] Figure 5 shows 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 = 3). CPI- and LPI-based microcapsules were produced at optimum conditions of 10.5% flaxseed oil and 35.5% maltodextrin-DE 9 for both CPI and LPI at pH's 3.0 and 7.0.

[0022] Figure 6 shows the effect of spray drying on mean droplet diameter for CPI- and LPI-stabilized emulsions. Data represent the mean ± one standard deviation (n = 6).

[0023] Figure 7 shows SEM images of spray-dried flaxseed oil microcapsules containing: a) CPI and 10% oil; b) CPI and 15% oil; c) CPI and 20% oil; d) LPI and 10% oil; e) LPI and 15% oil; f) LPI and 20% oil.

[0024] Figure 8 shows release behavior of flaxseed oil microcapsules containing a) CPI; b) LPI, triggered by pH. Data represent the mean ± one standard deviation (n = 6).

[0025] Figure 9 shows release behavior of flaxseed oil microcapsules containing a) CPI; b) LPI, triggered by ionic strength. Data represent the mean ± one standard deviation (n = 6).

[0026] Figure 10 shows 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).

Description

[0027] Throughout the following description specific details are set forth in order to provide a more thorough understanding to persons skilled in the art. However, well known elements may not have been shown or described in detail to avoid unnecessarily obscuring the disclosure. Accordingly, the description and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

[0028] Some embodiments of the present invention provide microcapsules having a core of material to be encapsulated and a shell comprising a legume protein and a low molecular weight carbohydrate. In some embodiments, such microcapsules are used to protect the material to be encapsulated, e.g. from oxidation or degradation, and/or to improve the properties of the material to be encapsulated, e.g. to facilitate delayed or gradual release of the material to be encapsulated, e.g. for use in a product consumed orally by a human or other animal, or to hide the taste of the material to be encapsulated from the human or other animal.

[0029] In some embodiments, the material to be encapsulated is an oil. In some embodiments, the material to be encapsulated is an oil rich in polyunsaturated fatty acids. In some embodiments, the material to be encapsulated is an oil rich in omega-3 fatty acids, such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), such as a fish oil. In some embodiments, the material to be encapsulated is an oil rich in α-linolenic acid, such as flaxseed oil. In some embodiments, the material to be encapsulated is a flavour oil, fragrance oil, oil-soluble vitamin, or the like.

[0030] In some embodiments, the legume protein is chickpea protein or lentil protein. In some embodiments, the legume protein is faba bean protein or soybean protein. The preparation of stable emulsions of chickpea, faba bean, lentil and soy protein isolates has been previously described (Can Karaca et al., 2011). In some embodiments, the legume protein is chickpea protein isolate or lentil protein isolate.

[0031] In some embodiments, the low molecular weight carbohydrate is dextrin, i.e. a low molecular weight carbohydrate produced by the hydrolysis of starch or glycogen. In some embodiments, the low molecular weight carbohydrate is maltodextrin. In some embodiments, the low molecular weight carbohydrate is maltodextrin-DE 9 or maltodextrin-DE 18. In some embodiments, the low molecular weight carbohydrate is maltodextrin-DE 3, maltodextrin-DE 4, maltodextrin-DE 5, maltodextrin-DE 6, maltodextrin-DE 7, maltodextrin-DE 8, maltodextrin-DE 9, maltodextrin-DE 10, maltodextrin-DE 11, maltodextrin-DE 12, maltodextrin-DE 13, maltodextrin-DE 14, maltodextrin-DE 15, maltodextrin-DE 16, maltodextrin-DE 17, maltodextrin-DE 18, maltodextrin-DE 19, or maltodextrin-DE 20. In some embodiments, the low molecular weight carbohydrate is a combination of two or more of the foregoing maltodextrins. In some embodiments, the low molecular weight carbohydrate is glycose syrup solids.

[0032] In some embodiments, methods for preparing microcapsules having a core of material to be encapsulated and a shell comprising a legume protein and a low molecular weight carbohydrate are provided. In some embodiments, legume protein isolates are dissolved in aqueous solution, the low molecular weight carbohydrate is dissolved in aqueous solution, and the dissolved low molecular weight carbohydrate and legume protein isolates are combined with the material to be encapsulated. In some embodiments, the pH of any of the solutions, including the legume protein isolate solution, is optionally adjusted to any desired pH. In some embodiments, the pH of the lentil protein isolate solution is adjusted to 3.0 or 7.0 prior to its addition to the low molecular weight carbohydrate and the material to be encapsulated.

[0033] The resulting solution is mixed to form an emulsion solution. In some embodiments, mixing the resulting solution includes homogenizing the solution. Any suitable equipment may be used to homogenize the solution, for example, homogenizers, including e.g. high pressure valve homogenizers.

[0034] The emulsion solution is then processed in any suitable manner to produce microcapsules, for example by freeze drying, spray drying, fluidized bed coating, spray chilling, or the like.

[0035] In some embodiments, the amount of legume protein in the emulsion solution is in the range of about 3% to about 10% w/v or any value therebetween, e.g. 4%, 5%, 6%, 7%, 8% or 9%. In some embodiments, the amount of legume protein in the emulsion solution is about 4% w/v.

[0036] In some embodiments, the amount of low molecular weight carbohydrate in the emulsion solution is in the range of about 20% w/v to about 50% w/v, or any value therebetween, e.g. 22%, 24%, 26%, 28%, 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46% or 48% w/v. In some embodiments, the amount of low molecular weight carbohydrate is in the range of about 30% to about 40% w/v.

[0037] In some embodiments, the amount of material to be encapsulated in the emulsion solution is in the range of about 5% w/v to about 25% w/v, or any value therebetween, e.g. 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23% or 24% w/v. In some embodiments, the amount of material to be encapsulated in the emulsion solution is in the range of about 10% to about 20% w/v. Decreasing the amount of material to be encapsulated in the emulsion solution can increase the encapsulation efficiency and result in lower surface oil content in the resultant microcapsules.

[0038] In some embodiments, after the emulsion solution has been processed to form microcapsules, the amount of material to be encapsulated in the microcapsules is in the range of about 10% w/w to about 20% w/w or any value therebetween, e.g. 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18% or 19% w/w.

[0039] In some embodiments, microcapsules are used to provide the material to be encapsulated in a form suitable for use in food products. Microencapsulation can be used for many purposes; for example, to protect the material to be encapsulated from oxidation, to hide the taste of the material to be encapsulated from a person or other organism consuming the food product, to allow for delayed release of the material to be encapsulated after consumption of the food product, and/or to render the material to be encapsulated miscible in the food product. In some embodiments, the food product has a relatively neutral pH. In some embodiments, the food product is a dairy product, for example, milk, cheese, yogurt, sour cream, ice cream or the like. In some embodiments,

the food product is a dry product such as cereals, granola bars, or the like. In some embodiments, the dry food product has a relatively neutral pH, e.g. a pH of approximately 6.5 to 7.0.

[0040] In some embodiments, the food product is animal feed. In some embodiments, microcapsules are added to feed to potentially increase the transfer of oils (containing essential fatty acids including omega-3 fatty acids) through the animal (for example, dairy cows, beef cows, chickens or other livestock) to give omega-3 fatty acid-rich products (for example, milk, meat and eggs that are higher in omega-3 fatty acid content than equivalent product obtained from animals not fed animal feed including microcapsules containing oils high in desirable fatty acids such as omega-3 fatty acids). The microcapsules would in principle protect the oil within the feed and within the gastrointestinal tract of the animal from oxidation and/or degradation.

[0041] In some embodiments, the amount of material to be encapsulated that is released from the microcapsules at a relatively neutral pH, for example as would be found in certain food products such as some dairy products or some dry products, is relatively low, e.g. less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6% or less than about 5% of the encapsulated material is released at a relatively neutral pH of between about 6.0 and about 8.0 or any value therebetween, e.g. a pH of 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6 or 7.8. In some such embodiments, the amount of material to be encapsulated that is released from the microcapsules under gastric conditions, e.g. as would be experienced in the stomach of an animal that has consumed the microcapsules (e.g. a pH of 1.2 with pepsin present in the human stomach), and/or the amount of material released from the microcapsules under intestinal conditions, e.g. as would be experienced in the intestine of an animal that has consumed the microcapsules (e.g. a pH of 6.8 in the presence of pancreatin in the human intestine) is relatively high, e.g. greater than about 30%, greater than about 40%, greater than about 50%, greater than about 60%, greater than about 70%, greater than about 80%, greater than about 90% or greater than about 95%.

[0042] In some embodiments, microcapsules are used to encapsulate flaxseed oil so that the flaxseed oil can be incorporated into a food product while being protected from oxidation or degradation, to hide the taste of the flaxseed oil, and/or to render the flaxseed

oil miscible in the food product. In some embodiments, the microcapsules containing the flaxseed oil are incorporated into a dairy product, for example, milk, cheese, yogurt, sour cream, ice cream or the like. In some embodiments, the microcapsules containing the flaxseed oil are incorporated into a dry food product, for example a cereal or granola bar.

[0043] In some embodiments, microcapsules are used to encapsulate an oil rich in omega-3 fatty acids, e.g. fish oil, so that the oil can be incorporated into a food product while being protected from oxidation or degradation, to hide the taste of the oil, and/or to render the oil miscible in the food product. In some embodiments, the microcapsules containing the oil are incorporated into a dairy product, for example, milk, cheese, yogurt, sour cream, ice cream or the like. In some embodiments, the microcapsules containing the oil are incorporated into a dry food product, for example a cereal or granola bar.

[0044] In some embodiments, the microcapsules produced by the methods described above have a final moisture content of between about 2% and about 4%, or any value therebetween, e.g. 2.2%, 2.4%, 2.6%, 2.8%, 3.0%, 3.2%, 3.4%, 3.6% or 3.8%.

[0045] In some embodiments, the microcapsules have a water activity of between about 0.05 and about 0.2 or any value therebetween, e.g. 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18 or 0.19.

[0046] In some embodiments where the legume protein is chickpea protein isolate, the microcapsules have *L* (lightness), *a* (redness), and *b* (yellowness) tristimulus colour values including *L* values ranging from about 87 to about 93 or any value therebetween, e.g. 88, 89, 90, 91, or 92; *a* values ranging from about -0.5 to about 0.5 or any value there between, e.g. -0.4, -0.3, -0.2, -0.1, 0, 0.1, 0.2, 0.3 or 0.4; and *b* values ranging from about 8 to about 21 or any value therebetween, e.g. 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

[0047] In some embodiments where the legume protein is lentil protein isolate, the microcapsules have *L* (lightness), *a* (redness), and *b* (yellowness) tristimulus colour values including *L* values ranging from about 77 to 89 or any value therebetween, e.g. 78, 79, 80, 81, 82, 83, 84, 85, 86, 87 or 88; *a* values ranging from about 1.4 to about 2.9 or any value therebetween, e.g. 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7 or

2.8; and *b* values ranging from about 11 to about 21 or any value therebetween, e.g. 12, 13, 14, 15, 16, 17, 18, 19 or 20.

[0048] In some embodiments, the surface oil content of the microcapsules ranges from about 0.5% to about 20% or any value therebetween, e.g. 1%, 2%, 3%, 4%, 5%, 6%, 8%, 10%, 12%, 14%, 16% or 18%.

[0049] In some embodiments, the encapsulation efficiency (or ratio of the total oil minus the surface oil to the total oil in the microcapsules) is in the range of about 45% to about 95% or any value therebetween, e.g. 50%, 55%, 60%, 65%, 70%, 75%, 80%, 82%, 84%, 86%, 88%, 90%, 91%, 92%, 93% or 94%.

[0050] In some embodiments in which the microcapsules are produced by freeze drying, the volume-weighted mean droplet diameter range of the emulsion solution is between about 2 and about 5 μm or any value therebetween, e.g. 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6 or 4.8 μm.

[0051] In some embodiments in which the microcapsules are produced by freeze drying, the volume-weighted mean droplet diameter range of the microcapsules when redispersed as an emulsion in aqueous solution is between about 1.8 and 12 μm or any value therebetween, e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 μm.

[0052] In some embodiments in which the microcapsules are produced by spray drying, the volume-weighted mean oil droplet diameter range of the emulsion solution is between about 16 and 24 μm or any value therebetween, e.g. 17, 18, 19, 20, 21, 22 or 23 μm.

[0053] In some embodiments in which the microcapsules are produced by spray drying, the volume-weighted mean droplet diameter range of the microcapsules when redispersed as an emulsion in aqueous solution is between about 10 and about 30 μm or any value therebetween, e.g. 12, 14, 16, 18, 20, 22, 24, 26 or 28 μm.

[0054] In some embodiments, the peroxide value of the microcapsules is between about 5 and about 7 meq active O_2/kg or any value therebetween, e.g. 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.4, 6.6 or 6.8 meq active O_2/kg. In some embodiments, the peroxide value of the

microcapsules is between about 5 and about 8 meq active O_2/kg or any value therebetween over a 25-day storage period.

[0055] In some embodiments, the production of secondary oxidation products of the microcapsules measured using a 2-thiobarbituric acid reactive substances (TBARS) test is between about 1.5 and about 2.5 MDA eq nmol/mg or any value therebetween, e.g. 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3 or 2.4 MDA eq nmol/mg. In some embodiments, the production of secondary oxidation products as measured using a TBARS test over a 25-day storage period is within the aforementioned range, i.e. between about 1.5 and about 2.5 MDA eq nmol/mg or any value therebetween.

[0056] In some embodiments, the material to be encapsulated can be released from the microcapsules by the pH or ionic strength of a solution in which the microcapsules are dispersed. In some embodiments in which the material to be encapsulated is flaxseed oil, the microcapsules release between about 5% and about 6% of the encapsulated flaxseed oil after one hour at pH 3.0; between about 2.5% and about 5% of the encapsulated flaxseed oil after one hour at pH 5.0; between about 6% and 10% of the encapsulated flaxseed oil after one hour at pH 7.0; and between about 6% and 10% of the encapsulated flaxseed oil after one hour at pH 9.0.

[0057] In some embodiments in which the material to be encapsulated is flaxseed oil, at a pH of 7.0 the microcapsules release between about 3% and about 6% of the encapsulated flaxseed oil after one hour at 0 mM NaCl; between about 4% and 8% of the encapsulated flaxseed oil after one hour at 50 mM NaCl; between about 6% and 9% of the encapsulated flaxseed oil after one hour at 100 mM NaCl; between about 6% and 10% of the encapsulated flaxseed oil after one hour at 150 mM NaCl; and between about 8% and 11% of the encapsulated flaxseed oil after one hour at 200 mM NaCl.

[0058] In some embodiments in which the material to be encapsulated is flaxseed oil, under a simulated gastrointestinal model, the microcapsules release between about 35% and 45% of the encapsulated flaxseed oil after two hours in simulated gastric fluid, and release between about 80% and 95% of the encapsulated flaxseed oil after two hours in simulated gastric fluid followed by three hours in simulated intestinal fluid.

Examples

[0059] Some exemplary embodiments of the present invention are further described with reference to the following examples, which are intended to be illustrative and not limiting in nature.

Example 1.0 – Materials and Methods

1.1 Materials

[0060] 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 samples (DE 9, Dry MD™ 01918 and DE 18, Dry MD™ 01909-Z) were donated by Cargill Inc. (Cargill Texturizing Solutions, Cedar Rapids, IA, USA). Flaxseed oil was donated by Bioriginal Food & Science Corp. (Saskatoon, SK, Canada). 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, USA).

1.2. Proximate Analysis of Proteins and Maltodextrin

[0061] Proximate composition analyses for protein isolates and maltodextrin-DE samples 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) (*AOAC*, 2003; Drusch, S. et al., 2006). Carbohydrate content was determined on the basis of percent differential from 100%.

[0062] 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. For maltodextrin-DE 18 the results were: 4.7% moisture, 0.0% protein, 0.0% lipid, 95.0% carbohydrate and 0.3% ash.

1.3. Protein Isolate Preparation

[0063] Whole chickpea and lentil seeds were ground into a fine flour using an IKA A11 basic analytical mill (IKA Works Inc., Wilmington, NC, USA) for 1 minute, and then defatted using hexane (1:3 [w/v] flour:hexane ratio) for 40 minutes. The mixture was then filtered employing Whatman Gr. 1 paper (110 mm; Whatman International Ltd.,

Maidstone, United Kingdom), and air-dried in a fume hood. This defatting procedure was repeated twice for each flour.

[0064] Chickpea protein isolate (CPI) was prepared according to the method of Papalamprou et al. (2010). In brief, defatted flour (100 g) was mixed with water at a 1:10 ratio (w/v), adjusted to pH 9.0 using 1.0 M NaOH and stirred at 500 rpm for 45 minutes at room temperature (21-23°C). The suspension was then centrifuged at 4,500 × g for 20 minutes at 4°C 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 9.0, stirred for an additional 45 minutes, followed by centrifugation (4,500 × 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, USA) to yield a free flowing powder. Proximate analysis of CPI showed a chemical composition of, 85.40% protein, 6.52% moisture, 3.05% ash, 4.11% carbohydrate and 0.92% lipid.

[0065] Lentil protein isolate (LPI) was produced employing the combined methods of Bamdad, Goli, & Kadivar (2006) and Lee, Htoon, & Paterson (2007). Defatted flour (100 g) was mixed with water at a 1:10 ratio (w/v), adjusted to pH 9.5 with 1.0 M NaOH, and stirred at 500 rpm for 1 hour at room temperature. The mixture was kept static at 4°C overnight to allow for non-protein sedimentation. After centrifugation at 1,600 × g for 30 minutes at 4°C, the supernatant was collected, and pH was adjusted to 4.5 with 0.1 M HCl. The precipitated protein was collected by centrifugation (1,600 × g, 30 min, 4°C) and stored at −30°C until freeze-drying. Proximate analysis of LPI showed a chemical composition of, 81.90% protein, 5.04% moisture, 3.63% ash, 9.00% carbohydrate and 0.43% lipid.

1.4. Emulsion Preparation

[0066] For preparation of freeze-dried microcapsules, 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 or 7.0 with either 0.1 M NaOH or 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 either DE 9 or 18 in water followed

by stirring at 300 rpm overnight at 4°C. Prior to sample homogenization, the pH of the protein solutions was re-adjusted to 3.0 or 7.0 as described above. Twenty-eight oil-in-water emulsions were prepared (Table 1) by homogenizing (Polyton PT2100, Kinematica AG, Lucerne, Switzerland) varying amounts of protein isolate, maltodextrin solutions and flaxseed oil in 15 mL plastic centrifuge tubes employing a 12 mm PT-DA 2112/2EC generating probe at 13,000 rpm for 3 minutes with a sample volume of approximately 10 mL.

Table 1. Formulations of CPI- and LPI-stabilized emulsions prior to freeze drying.

Protein (%)	Maltodextrin (%)	Oil (%)	Water (%)
4.0	40.7	5.3	50.0
4.0	38.1	7.9	50.0
4.0	35.5	10.5	50.0
4.0	32.8	13.2	50.0
4.0	30.2	15.8	50.0
4.0	27.6	18.4	50.0
4.0	25.0	21.0	50.0

[0067] For preparation of spray dried microcapsules, 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 re-adjusted to 3.0. Oil-in-water emulsions were prepared by homogenizing varying amounts of protein solutions, maltodextrin solution and flaxseed oil (Table 2) in a 500 mL container by using Omni Macro Homogenizer (Omni International, Marietta, GA, USA) with a 20 mm saw tooth generating probe at speed 4 (~7,200 rpm) for 10 minutes with a sample volume of approximately 400 mL. Results from the corresponding formulations will be denoted by their oil content in the final powder (10, 15 and 20%) for discussion purposes.

Table 2. Formulations of CPI- and LPI- stabilized emulsions prior to and following 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

1.5 Droplet Size Measurements

[0068] Droplet size distributions of initial and reconstituted emulsions were measured using a Mastersizer 2000 laser light scattering instrument (Malvern Instruments Ltd., Worcestershire, United Kingdom) equipped with a Hydro 2000S sample handling unit (containing water). Emulsion samples were taken from the bottom of the tube immediately after homogenization for analysis. This sample was stirred continuously within the sample cell to ensure homogeneity at room temperature. Obscuration in all the measurements was kept at ~14% by water addition. 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 the 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-surface mean diameters ($d_{4,3}$) which is expressed as:

$$d_{4,3} = \frac{\sum_{i=1} n_i \cdot d_i^4}{\sum_{i=1} n_i \cdot d_i^3} \qquad \text{[eq. 1]}$$

where n_i is the number of droplets of diameter (d_i) (McClements, 2005).

1.6 Emulsion Reconstitution

[0069] Freeze-dried or spray-dried microcapsule samples of 0.5 g were dispersed in 4 mL of water and stirred at 500 rpm for 5 minutes. Samples were withdrawn for particle size distribution with measurements performed as described above.

1.7 Freeze-Drying

[0070] The emulsion preparation samples were placed in aluminum pans (diameter = 70 mm; approximate layer thickness = 5 mm) and frozen at –40°C for 24 h. Freeze dried emulsions were prepared as previously described with the ice condenser set at –50°C, and the vacuum pressure was approximately 0.120 mbar; the freeze drying time was 72 h. Following freeze drying the samples were manually ground to obtain a fine powder.

1.8 Spray Drying

[0071] 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.

1.9 Microcapsule Characterization

1.9.1 Moisture Content and Water Activity

[0072] The moisture content of microcapsules was determined gravimetrically, following drying in a forced-air oven at 105°C for ~12 h. Microcapsule water activity was determined using an AquaLab CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA, USA).

1.9.2 Colour Measurements

[0073] The tristimulus colour values of microcapsules were measured using a Hunter colourimeter (ColorFlex EZ 45/0, Hunter Associates Laboratory, Inc., Reston, VA, USA), which was standardized using a white reference tile. The results were expresses as *L* (lightness), *a* (redness), and *b* (yellowness) tristimulus values.

1.9.3 Microcapsule Surface and Total Oil Content

[0074] Microcapsule surface oil was determined according to the method of Liu et al. (2010). Briefly, 1 g of microcapsules (freeze-dried) or 2 g of microcapsules (spray-dried)

was dispersed in 30 mL of hexane followed by vigorous shaking for 30 seconds. The solvent was filtered (Whatman Gr. 1 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 minutes to remove any residual solvent.

[0075] Total oil content of the microcapsules was determined using the method described by Klinkesorn, Sophanodora, Chinachoti, Decker & McClements (2006) with some modifications. Briefly, 4 mL (freeze-dried) or 8 mL (spray-dried) of water was added to 1 g (freeze-dried) or 2 g (spray-dried) of microcapsules followed by mixing at 300 rpm for 2 min. The resulting solution was then mixed with 25 mL (freeze-dried) or 40 mL (spray-dried) hexane/isopropanol (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 aforementioned solvent mixture. The organic phases were pooled and filtered through anhydrous Na_2SO_4, and the solvent was allowed to evaporate overnight in a fume hood. Total oil content was determined gravimetrically, after heating at 105°C for 30 min.

1.9.4 Flaxseed Oil Encapsulation Efficiency

[0076] The flaxseed oil encapsulation efficiency (EE) was calculated from the quantitative determinations as follows (Anwar & Kunz, 2011):

$$EE = (\text{Total Oil} - \text{Surface Oil}) / \text{Total Oil} \times 100\% \quad \text{[eq. 2]}$$

1.9.5 Scanning electron microscopy (SEM)

[0077] Microcapsule samples were mounted onto aluminum stubs with double-sided tape and gold coated with a sputter coater. The coated samples were then viewed with a Philips SEM 505 (Eindhoven, The Netherlands) operating at an accelerating voltage of 27 kV with 6× and 1000× magnification.

1.10 Oxidative stability

[0078] Oxidative stability of free (i.e. control) and encapsulated flaxseed oil was characterized during storage at room temperature over a 25 day period employing both the peroxide value and 2-thiobarbituric acid reactive substances tests. Microcapsules (3-4

g/bottle freeze-dried or 5 g/bottle spray-dried) or free oil (~2 mL freeze-dried or ~3 mL spray-dried) were stored in individually sealed nitrogen-flushed 10 mL amber glass bottles for storage stability studies. Oxidative testing was carried out every 5 days over the 25 day testing period, using a new set of unopened samples (i.e. an unopened bottle of microcapsules and oil). Flaxseed oil extraction from the microcapsules followed the same procedure as that outlined previously for total oil determination, except the extraction solvent was dried under a stream of nitrogen.

1.10.1 Peroxide Value (PV)

[0079] In brief, ~0.2 g of extracted flaxseed 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. A 0.5 mL aliquot of 1% (w/v) starch indicator was then added to the mixture, and the resulting solution was titrated using 0.001N sodium thiosulfate ($Na_2S_2O_3$) until the purple colour disappeared. Sample PV was calculated as:

$$PV = (S - B) \times N \times 1000 / W \qquad \text{[eq. 3]}$$

where S is the volume of $Na_2S_2O_3$ added to the sample, B is the volume of $Na_2S_2O_3$ of the blank, N is the normality of $Na_2S_2O_3$ solution, and W is the sample weight (g) (Pegg, 2005).

1.10.2 2-Thiobarbituric Acid Reactive Substances (TBARS)

[0080] In brief, ~40 mg of extracted flaxseed oil was weighed into a 10 mL volumetric flask and was dissolved and brought to volume with n-butanol. To a 2.0 mL Eppendorf tube was added 50 µL of 8.1% (w/v) sodium dodecyl sulphate (SDS), 375 µL of 20% acetic acid, 375 µL of 0.8% (w/v) 2-thiobarbituric acid (TBA), 8.25 µL of 0.02% (w/v) butylated hydroxytoluene (BHT) (in dimethylsulfoxide (DMSO)) and 200 µL of the oil-butanol mixture. A standard curve was prepared using malondialdehyde (MDA) (1.25-50 µM) under the same experimental conditions. Samples and standards 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 and standards 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. TBA values were expressed as mg MDA eq/mg oil, which equates to the MDA content (nmol)/sample oil weight (mg) (modified from Pegg, 2005 and Akhlaghi & Bandy, 2010).

1.11 Release Characteristics

[0081] Release behaviour of flaxseed oil from the microcapsules triggered by pH and ionic strength (at pH 7) was determined by the combined methods of Zhong & Jin (2009) and Choi, Ruktanonchai, Min, Chun & Soottitantawat (2010). In brief, microcapsule samples of 1 g (freeze-dried) or 5 g (spray-dried) were individually dispersed in 10 mL (freeze-dried) or 50 mL (spray-dried) aqueous NaCl solutions (0, 50, 100, 150 and 200 mM) that were pH adjusted (0.1 M HCl or NaOH) to produce values of 3.0, 5.0, 7.0 or 9.0 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.

[0082] *In-vitro* release behaviour of microencapsulated flaxseed oil was also investigated using a simulated gastrointestinal model according to the method of Burgar, Hoobin, Weerakkody, Sanguansri, & Augustin (2009). Simulated gastric fluid (SGF) was prepared by dissolving 2.0 g of NaCl and 7.0 mL 36% HCl in 900 mL of water. After the addition of 3.2 g pepsin, the 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 K_2HPO_4 in 800 mL of water. To this solution was added 77 mL of 0.2 M NaOH and 100.0 g of pancreatin and the solution was stirred overnight at 4°C. Solution 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.

[0083] A 2 g (freeze-dried) or 5 g (spray-dried) microcapsule sample was mixed with 20 mL or 50 mL of SGF, respectively, 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, a 2 g (freeze-dried) or 5 g (spray-dried) microcapsule sample was mixed with 20 mL or 50 mL of SGF, respectively, and incubated under the same conditions for 2 h. Sample pH was adjusted to 6.8 using 1 M NaOH, followed by addition of 20 mL or 50 mL, respectively, of SIF and the sample was incubated under the same conditions for 3 h. The amount of flaxseed oil released from the microcapsules was determined by gravimetric analysis as outlined above.

1.12 Statistical Analyses

[0084] All experiments were performed in triplicate and reported as the mean ± one standard deviation. A one-way analysis of variance (ANOVA) with a Scheffe post-hoc test was used to measure statistical differences in microcapsule characteristics and oxidative stability among different formulations. For freeze-dried microcapsules, a general linear model was employed to measure statistical differences in: (1) the physicochemical characteristics of the microcapsules as a function of protein source (CPI vs. LPI), maltodextrin type (DE 9 vs. DE 18) and oil concentration; and (2) microencapsulated flaxseed oil release properties as a function of protein source, pH of the protein solution used for preparing the microcapsules, and pH or ionic strength of the release medium. All statistical analyses were performed with SPSS version 17.0 software (SPSS Inc., 2008, Chicago, IL, USA).

Example 2.0 – Analysis of Freeze-Dried Microcapsules

2.1 Physicochemical characteristics of CPI- and LPI-based microcapsules produced at pH 3.0 and 7.0

[0085] The moisture content and water activity of freeze dried flaxseed oil microcapsules containing CPI and LPI produced at pH 3.0 are shown in Tables 3 and 4, respectively. The moisture content of the microcapsules varied from 2.26 to 4.18% while their water activities ranged between 0.07-0.19. The majority of these results were within the maximum moisture specification for dried powders in the food industry which is between 3-4% (Klinkesorn et al., 2006). Changes in oil concentration, protein source (CPI vs. LPI) and maltodextrin type (DE 9 vs. DE 18) did not have a significant effect on microcapsule moisture content and water activity ($p>0.05$).

Table 3. Moisture content (%) of freeze-dried CPI- and LPI-based microcapsules produced at pH 3.0. Data represent the mean ± one standard deviation (n = 3).

Oil (%)	CPI		LPI	
	DE 9	*DE 18*	*DE 9*	*DE 18*
5.3	2.44 ± 0.45^{a}	2.77 ± 0.66^{a}	2.76 ± 0.13^{a}	3.40 ± 0.11^{a}
7.9	$3.25 \pm 0.20^{a,b}$	$3.10 \pm 0.18^{a,b}$	2.84 ± 0.13^{a}	$3.24 \pm 0.19^{a,b}$
10.5	3.89 ± 0.06^{b}	3.95 ± 0.14^{b}	4.18 ± 0.57^{b}	$2.48 \pm 0.10^{c,d}$
13.2	$3.28 \pm 0.28^{a,b}$	$3.54 \pm 0.14^{a,b}$	3.25 ± 0.18^{a}	$2.90 \pm 0.12^{b,c}$
15.8	2.77 ± 0.03^{a}	2.70 ± 0.10^{a}	2.44 ± 0.15^{a}	$2.63 \pm 0.17^{c,d}$
18.4	2.68 ± 0.32^{a}	$3.20 \pm 0.19^{a,b}$	3.13 ± 0.24^{a}	$3.17 \pm 0.14^{a,b}$
21.0	2.94 ± 0.12^{a}	2.63 ± 0.24^{a}	2.78 ± 0.05^{a}	2.26 ± 0.06^{d}

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

Table 4. Water activity of freeze-dried CPI- and LPI-based microcapsules produced at pH 3.0. Data represent the mean ± one standard deviation (n = 3).

Oil (%)	CPI		LPI	
	DE 9	*DE 18*	*DE 9*	*DE 18*
5.3	0.12 ± 0.02^{a}	0.17 ± 0.01^{a}	0.19 ± 0.01^{a}	0.17 ± 0.00^{a}
7.9	0.14 ± 0.01^{a}	0.12 ± 0.01^{a}	$0.16 \pm 0.01^{a,b}$	0.16 ± 0.01^{a}
10.5	0.15 ± 0.00^{a}	0.16 ± 0.01^{a}	0.15 ± 0.01^{b}	0.07 ± 0.00^{b}
13.2	0.14 ± 0.01^{a}	0.16 ± 0.00^{a}	0.14 ± 0.00^{b}	0.15 ± 0.01^{a}
15.8	0.15 ± 0.00^{a}	0.14 ± 0.02^{a}	0.13 ± 0.01^{b}	0.14 ± 0.00^{a}
18.4	0.13 ± 0.02^{a}	0.14 ± 0.00^{a}	0.13 ± 0.01^{b}	0.15 ± 0.02^{a}
21.0	0.13 ± 0.01^{a}	0.14 ± 0.02^{a}	0.14 ± 0.01^{b}	0.09 ± 0.02^{b}

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

[0086] The *L* (lightness), *a* (redness), and *b* (yellowness) tristimulus colour values of freeze dried microcapsules containing flaxseed oil produced at pH 3.0 are presented in Table 5. Microcapsules containing CPI were slightly yellow in colour, which was illustrated by *L* values ranging from 87.3 to 90.6, *a* values from –0.5 to 0.3, and *b* values from 11.2 to 20.3. On the other hand, microcapsules containing LPI were beige in colour with *L* values ranging from 77.9 to 84.4, *a* values from 2.1 to 2.9, and *b* values from 14.1 to 20.1. As microcapsule oil content increased, the *L* value decreased and the *b* value increased for both CPI- and LPI-containing microcapsules ($p<0.05$) which indicated that the microcapsules became more yellow as the amount of flaxseed oil increased. Maltodextrin type (DE 9 vs. DE 18) did not have a significant effect on microcapsule colour ($p>0.05$). Overall, the CPI-microcapsules had significantly higher *L* values (~88.9) compared to LPI-microcapsules (~82.5, $p<0.05$), whereas *a* values of LPI-microcapsules (~2.3) were significantly higher than those of CPI-microcapsules (~0.0). The darker

colour of the LPI-microcapsules is most likely due to the presence of hull pigments which were extracted by the alkaline solvents used in isolate preparation (Bamdad et al., 2006).

Table 5. The Hunter colour values of freeze dried CPI- and LPI-based microcapsules produced at pH 3.0. Data represent the mean ± one standard deviation (n = 3).

Oil (%)	*L*				*a*				*b*			
	CPI		LPI		CPI		LPI		CPI		LPI	
	DE 9	DE 18	DE 9	DE 18	DE 9	DE 18	DE 9	DE 18	DE 9	DE 18	DE 9	DE 18
5.3	90.5± 0.4^{a}	90.6± 0.5^{a}	83.1± 0.4^{a,b}	84.0± 0.9^{a,b}	-0.3± 0.1^{a}	-0.5± 0.2^{a}	2.3± 0.1^{a,b}	2.1± 0.1^{a}	11.9± 0.2^{a}	11.2± 0.1^{a}	14.3± 0.1^{a}	14.1± 0.4^{a}
7.9	90.3± 0.3^{a,b}	90.3± 0.3^{a,b}	83.5± 0.9^{a}	84.4± 0.6^{a}	-0.3± 0.1^{a}	-0.5± 0.3^{a}	2.3± 0.2^{a,b}	2.1± 0.1^{a}	13.7± 0.2^{b}	12.7± 0.9^{a}	14.8± 0.5^{a}	14.1± 0.5^{a}
10.5	89.1± 0.5^{a,b,c}	88.7± 0.9^{b,c}	83.5± 0.7^{a}	82.2± 0.5^{b,c}	0.1± 0.1^{b}	0.0± 0.1^{b,c}	2.1± 0.2^{a}	2.3± 0.1^{a}	14.6± 0.4^{b}	14.9± 0.6^{b}	14.8± 0.4^{a}	15.5± 0.2^{b}
13.2	89.7± 0.1^{b,c,d}	88.9± 0.6^{a,b,c}	83.7± 0.4^{a}	83.3± 0.6^{a,b,c}	0.1± 0.1^{b}	0.0± 0.1^{a,b,c}	2.0± 0.1^{a}	2.1± 0.1^{a}	14.5± 0.3^{b}	15.5± 0.4^{b,c}	14.7± 0.4^{a}	15.2± 0.4^{b}
15.8	88.6± 0.6^{c,d,e}	88.0± 0.2^{c}	82.1± 0.3^{a,b}	82.2± 0.1^{b,c}	0.1± 0.1^{b}	0.1± 0.1^{c}	2.3± 0.1^{a,b}	2.1± 0.0^{a}	16.2± 0.7^{c}	17.0± 0.1^{c,d}	16.3± 0.4^{b}	16.8± 0.1^{c}
18.4	88.1± 0.3^{d,e}	87.5± 0.3^{c}	82.2± 1.0^{a,b}	81.6± 0.4^{c}	0.1± 0.1^{b}	0.3± 0.1^{c}	2.4± 0.2^{a,b}	2.4± 0.1^{a}	17.6± 0.3^{c,d}	18.6± 0.5^{d}	17.6± 0.5^{c}	17.7± 0.4^{c}
21.0	87.7± 0.4^{e}	87.3± 0.4^{c}	81.3± 0.2^{b}	77.9± 0.5^{a}	0.1± 0.3^{a,b}	0.3± 0.2^{c}	2.7± 0.1^{b}	2.9± 0.1^{b}	19.0± 0.6^{d}	20.3± 0.6^{e}	18.3± 0.2^{c}	20.1± 0.1^{d}

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

[0087] Surface oil represents the portion of oil present on the surface of the microcapsule (Bao, Hu, Zhang, Xu, Zhang & Huang, 2011). Minimizing the amount of surface oil is important in lipid microencapsulation as this material can potentially oxidize at more rapid rates than the encapsulated oil, causing rancidity and reducing the shelf life of the finished product (Pegg & Shahidi, 2007). The effect of emulsion formulation on surface oil content is presented in Figure 1. It was noted that the surface oil content of the microcapsules produced at pH 3.0 ranged from 0.7-19.8% depending on the formulation. An analysis of variance revealed the following as significant factors: oil concentration ($p<0.001$), maltodextrin type ($p<0.001$) and interactions between protein source × maltodextrin type ($p<0.05$), protein source × oil concentration ($p<0.001$) and maltodextrin type × oil concentration ($p<0.001$). Overall, the surface oil in the microcapsules increased

from 1.0 to 16.9% as the oil concentration in the initial emulsions increased from 5.3 to 21.1%.

[0088] In the present study, two types of maltodextrin (DE 9 and 18) were used as a secondary wall material (i.e., filler) to improve microcapsule drying properties (Kagami et al., 2003). Maltodextrins are widely used as wall materials for capsule formation as they exhibit good solubility and low viscosities at high solids contents (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Overall, microcapsules prepared with maltodextrin-DE 9 had lower surface oil contents (6.5%) when compared to microcapsules prepared with maltodextrin-DE 18 (8.3%). Maltodextrin-DE 18 is a more hydrolyzed starch product with a higher concentration of lower molecular weight glucose polymers, which are responsible for its higher water solubility compared to maltodextrin-DE 9. Without being bound by theory, it is believed that the lower surface oil content observed in microcapsules prepared with DE 9 is most likely due to its higher hydrophobicity when compared to DE 18 due to the presence of higher molecular weight glucose polymers in this material.

[0089] The effect of emulsion formulation on flaxseed oil encapsulation efficiency is shown in Figure 2. The encapsulation efficiency of flaxseed oil ranged from 46.2 to 92.1% in the microcapsules depending on the formulation. An analysis of variance showed that oil concentration ($p<0.001$), maltodextrin type ($p<0.001$) and interactions between protein source × maltodextrin type ($p<0.05$), protein source × oil concentration ($p<0.001$) and maltodextrin type × oil concentration ($p<0.001$) were significant. Overall, as the concentration of flaxseed oil increased in the emulsion from 5.3 to 21.0%, encapsulation efficiency values decreased from ~89 to ~53%, respectively, which agreed with the trend observed in surface oil content.

[0090] In this study, higher flaxseed oil encapsulation efficiencies coupled with lower surface oil contents were achieved in CPI- and LPI-based microcapsules containing maltodextrin-DE 9 (Figure 2). Experimental results also showed that flaxseed oil encapsulation efficiencies in CPI- and LPI-maltodextrin systems were dependent upon multiple factors.

[0091] The particle size of the active ingredient (e.g. flaxseed oil) dispersed within the aqueous phase of the emulsion has been shown to be a significant factor for retention within microcapsules, where the smaller the particle size the greater the retention (Rish and Reineccius, 1988). Smaller oil droplet sizes have also be shown to minimize microcapsule surface oil, increase oil encapsulation efficiency and decrease lipid oxidation rates (Lee & Ying, 2008). The mean droplet diameter of CPI- and LPI-stabilized emulsions before freeze-drying and after reconstitution are shown in Figure 3. Panel (a) shows results for CPI-stabilized emulsions. Panel (b) shows results for LPI-stabilized emulsions.

[0092] The volume-weighted mean droplet diameter range ($d_{4,3}$) of flaxseed oil-in-water emulsions stabilized by CPI and LPI were 2.4-4.8 μm and 2.7-4.5 μm, respectively, depending on the formulation. An analysis of variance revealed that protein source ($p<0.01$), maltodextrin-type ($p<0.001$), flaxseed oil concentration ($p<0.001$), the interactions between protein source × maltodextrin-type ($p<0.05$), protein source × oil concentration ($p<0.001$) and maltodextrin-type × oil concentration ($p<0.01$) were all significant. Overall, despite having a significant protein-type main effect, the $d_{4,3}$ values of 3.7 and 3.9 μm for LPI and CPI stabilized emulsions, respectively, were similar. Whereas emulsions containing maltodextrin DE 9 and DE 18 showed quite different overall $d_{4,3}$ values of 3.6 and 4.0 μm, respectively. Without being bound by theory, the lower droplet diameters observed for emulsions prepared with the less hydrolyzed maltodextrin (DE 9) may be due to a higher continuous phase viscosity imparted by the larger concentration of high molecular weight glucose polymers in this material when compared to DE 18. Also, as the concentration of flaxseed oil in the emulsion increased from 7.9% to >10%, the $d_{4,3}$ values increased from ~3.1 μm to ~4.1 μm. Again without being bound by theory, the larger oil droplets observed at oil concentrations >10% could be attributed to the limited availability of protein to cover the oil surface to sufficiently form a dense adsorption layer so as to prevent coalescence.

[0093] The droplet size of water-redispersed freeze dried microcapsules containing flaxseed oil showed that this drying process resulted in a significant increase in mean droplet diameter ($p<0.05$) at oil concentrations of 18.4% and 21.0% regardless of the protein source and maltodextrin type. Without being bound by theory, the observed increase in droplet size in reconstituted emulsions with higher amounts of oil could be

attributed to coalescence of the surface oil after drying and/or upon reconstitution (Polavarapu et al., 2011; Shaw, McClements & Decker, 2007).

2.2 Application of Microencapsulated Oil in Food Production

[0094] The goal of this portion of this study was to produce microcapsules containing flaxseed oil that would be suitable for use in food commodities with a more neutral pH such as dairy products. The physicochemical characteristics of microcapsules produced at pH 7.0 are presented in Table 6. Microcapsules produced with CPI at pH 7.0 had lower moisture content and water activity compared to pH 3.0 ($p<0.05$). In addition, lower *L* (lightness) and higher *a* (redness) and *b* (yellowness) values were observed in microcapsules produced with CPI at pH 7.0 compared to pH 3.0 ($p<0.05$), resulting in a slightly darker yellowish colour. No significant differences in surface oil content and flaxseed oil encapsulation efficiency were found between microcapsules produced at pH 7.0 and 3.0 ($p>0.05$). Microcapsules produced with LPI at pH 7.0 maintained their original droplet diameter after water-redispsersion of freeze-dried material while this process resulted in an increase in oil droplet diameter in microcapsules produced with CPI at pH 7.0. Microcapsules produced with LPI at pH 7.0 had similar moisture content ($p>0.05$) but lower water activity ($p<0.05$) than those produced at pH 3.0. A lower *L* (lightness) value was found in microcapsules produced with LPI at pH 7.0 compared to pH 3.0 ($p<0.05$). Microcapsules produced with LPI at pH 7.0 and 3.0 had similar surface oil content, encapsulation efficiency and maintained their original droplet diameter after water-redispersion following freeze-drying ($p>0.05$).

Table 6. Physicochemical characteristics of freeze dried CPI- and LPI-based microcapsules produced at pH 7.0. Data represent the mean ± one standard deviation (n = 3).

Sample	Moisture (%)	A_w	Colour			Surface oil (%)	Encapsulation efficiency (%)	Droplet diameter $d_{4,3}$ (µm)	
			L	*a*	*b*			*Before drying*	*After drying*
CPI-based microcapsules	2.80 ± 0.17[a]	0.14 ± 0.01[a]	88.3 ± 0.2[a]	0.5 ± 0.1[a]	15.7 ± 0.5[a]	2.68 ± 0.36[a]	83.40 ± 2.65[a]	2.15 ± 0.03[a]	4.33 ± 0.70[a]
LPI-based microcapsules	3.23 ± 0.20[b]	0.13 ± 0.00[a]	78.4 ± 0.4[b]	2.2 ± 0.1[b]	13.2 ± 0.2[b]	2.66 ± 0.41[a]	83.17 ± 1.75[a]	2.45 ± 0.05[b]	2.36 ± 0.08[b]

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

[0095] When comparing microcapsule formation at pH 7.0; LPI-based materials had a lower moisture content and darker colour than CPI-based ones ($p<0.05$), and no significant difference was observed in surface oil content and encapsulation efficiency between these protein isolates ($p>0.05$). Water-redispersion of the freeze-dried microcapsules resulted in an increase in droplet diameter for CPI-based materials while droplet diameter remained unchanged in LPI-based ones (Table 6).

2.3 Surface morphology of CPI- and LPI-based microcapsules containing flaxseed oil

[0096] SEM images of freeze-dried CPI- and LPI-based microcapsules containing 10.5% flaxseed oil are shown in Figure 4. All four samples (microcapsules produced at both pH 3.0 and 7.0) had similar surface morphology which were highly porous. A porous microcapsule morphology has also been observed by other research groups for freeze-dried oil-in water emulsions (Anwar & Kunz 2011; Heinzelmann, Franke, Jensen & Haahr 2000). Panel A shows CPI at pH 3.0. Panel B shows LPI at pH 3.0. Panel C shows CPI at pH 7.0. Panel D shows LPI at pH 7.0.

2.4 Oxidative stability of microencapsulated flaxseed oil

[0097] For these experiments, a CPI- and LPI-based microcapsule formulation was chosen having 4.0% protein, 35.5% maltodextrin-DE 9, and 10.5% oil. The reasons for this choice included high flaxseed oil encapsulation efficiency (~83.5%), minimum surface oil content (~2.8%) and acceptable mean droplet diameter (3.0 μm). The oxidative stability of free and the CPI- and LPI-based microencapsulated flaxseed oil stored under nitrogen and held at room temperature was monitored over a 25 day period, with sample peroxide value (PV) and TBARS results determined at five-day intervals for all samples (Figure 5). The PV of free flaxseed oil at time zero was 5.88 ± 010 meq active O_2/kg while that of CPI- or LPI-based microencapsulated flaxseed oil at immediately following freeze drying and extraction (time zero) ranged from 5.76-6.40 meq active O_2/kg; with no significant difference observed between protein source (CPI vs. LPI) and pH (3.0 vs. 7.0). Because the PV of time zero microencapsulated flaxseed oil was found to be similar to that of the free oil, the emulsification and encapsulation processes did not negatively impact oil stability. The PV results for CPI and LPI-based microencapsulated flaxseed oil remained unchanged over the 25 day storage period ($p>0.05$), whereas that of the free oil

steadily increased to 9.08 meq active O_2/kg at day 15, and to 11.43 meq active O_2/kg at day 20, and to 13.57 meq active O_2/kg at day 25 ($p<0.05$).

[0098] The primary oxidative products (hydroperoxides) measured by the PV test are odourless and colourless but can readily participate in the autoxidation process producing a variety of secondary oxidation products, such as aliphatic aldehydes, alcohols, ketones, cyclic compounds, and hydrocarbons which can have an adverse effect on the sensory attributes of the oil/product (Pegg, 2005). In the present study, secondary oxidation products were measured using the TBARS test. The TBARS value of CPI- and LPI-based microencapsulated flaxseed oil remained unchanged (~1.85 nmol/mg oil at time zero and ~2.15 nmol/mg oil at day 25) over the 25 d storage period ($p>0.05$), with no significant differences observed between protein isolate and pH. The TBARS values for free oil remained unchanged during the first 15 days (2.12-2.51 nmol/mg oil) but increased to 3.22 nmol/mg oil at day 20 and to 3.98 nmol/mg oil at day 25 ($p<0.05$). These results indicate that the increased formation of secondary oxidation products over time was significant when compared to the microencapsulated flaxseed oil ($p<0.05$). Experimental results clearly show that the CPI- and LPI-based microcapsules provided oxidative protection to the encapsulated flaxseed oil over the 25 d storage period.

2.5 Release characteristics of microencapsulated flaxseed oil

[0099] The relationships between flaxseed oil release from CPI- and LPI-based microcapsules as a function of a selection of NaCl (i.e. ionic strength) concentrations, and the pH of the microcapsule preparation protocol (3.0 and 7.0) and release medium (3.0, 5.0, 7.0 and 9.0) are shown in Table 7. With respect to pH-triggered release, an analysis of variance showed that the protein source, pH of the protein solution used for microcapsule preparation, pH of the release medium, and the interaction of the last two factors were significant ($p<0.001$). In general, the amount of flaxseed oil released from the microcapsules was found to be the lowest (2.6-4.5%) for pH 5.0 and increased in conjunction with pH to 7.2-9.2% at pH 9.0. Without being bound by theory, the observed increase in released flaxseed oil from the protein-based microcapsules at acidic (pH 3.0) and basic pH values (pH 9.0) is most likely due to the increased solubility of CPI and LPI, resulting in the loss of capsule integrity/structure with concomitant flaxseed oil release.

Table 7. Release behavior of freeze dried CPI- and LPI-based microcapsules triggered by pH, ionic strength and gastrointestinal environments. Data represent the mean ± one standard deviation (n = 3).

	pH				**Ionic Strength (mM NaCl)**					**SGF**	**SGF & SIF**
	3.0	*5.0*	*7.0*	*9.0*	*0*	*50*	*100*	*150*	*200*		
CPI-pH 3.0	6.2 ± 0.1^{a,b}	3.4 ± 0.3^{a,b}	7.4 ± 0.4^{a}	7.6 ± 0.3^{a}	4.2 ± 0.1^{a}	6.9 ± 0.5^{a}	7.8 ± 0.3^{a,b}	8.7 ± 0.5^{a}	9.2 ± 0.4^{a}	37.5 ± 0.5^{a,b}	85.6 ± 3.8^{a}
LPI-pH 3.0	5.6 ± 0.2^{a}	2.6 ± 0.6^{a}	7.6 ± 0.5^{a}	7.2 ± 0.2^{a}	4.1 ± 0.4^{a}	6.2 ± 0.8^{a}	6.9 ± 0.5^{a}	9.3 ± 0.4^{a}	9.1 ± 0.4^{a}	38.7 ± 2.4^{a,b}	92.6 ± 4.1^{a}
CPI-pH 7.0	6.4 ± 0.2^{b}	4.5 ± 0.5^{b}	9.2 ± 0.2^{b}	9.2 ± 0.6^{b}	5.1 ± 0.5^{a}	7.4 ± 0.2^{a}	8.6 ± 0.5^{b}	9.4 ± 0.2^{a}	10.0 ± 0.8^{a}	43.4 ± 3.3^{b}	90.0 ± 4.7^{a}
LPI-pH 7.0	5.6 ± 0.4^{a}	3.6 ± 0.5^{a,b}	8.5 ± 0.6^{a,b}	8.6 ± 0.8^{a,b}	4.6 ± 0.6^{a}	7.2 ± 0.7^{a}	8.2 ± 0.8^{a,b}	9.1 ± 0.1^{a}	9.2 ± 0.3^{a}	36.6 ± 2.2^{a}	84.5 ± 3.0^{a}

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

[0100] For salt-triggered release, an analysis of variance of the results showed that the protein source, ionic strength (50, 100, 150 and 200 mM NaCl), and pH of the protein solution used for microcapsule preparation were significant ($p<0.001$). The amount of flaxseed oil released from the microcapsules was found to be the lowest (4.1-5.1%) at 0 mM NaCl and steadily increased to 9.1-10.0% at 200 mM NaCl. Without being bound by theory, the observed increase in flaxseed oil release from the protein-based microcapsules as a function of ionic strength may be explained by the increased solubility of CPI and LPI at elevated NaCl concentrations (i.e. 200 mM) resulting in the loss of capsule integrity/structure with concomitant flaxseed oil release.

[0101] The *in-vitro* release behaviour of CPI- and LPI-based microencapsulated flaxseed oil was investigated employing both simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) systems. In general, microencapsulated flaxseed oil release under SGF conditions ranged from 36.6-43.4% with the highest value observed for the CPI capsules prepared at pH 7.0, whereas the lowest value was observed for the LPI capsules prepared at pH 7.0. Microencapsulated flaxseed oil release under the combined SGF-SIF conditions was significantly higher (84.5-92.6%) than that observed for SGF only. Without being bound by theory, this result is most likely explained by the presence of the

proteolytic enzyme pepsin in SGF which catalyses protein hydrolysis resulting in a change in capsule structure (e.g. large pore formation) with concomitant oil release.

Example 3.0 – Preparation of Spray-Dried Microcapsules

3.1 Physicochemical Characteristics of Microcapsules

[0102] The moisture contents and water activities of spray dried produced CPI and LPI microcapsules containing flaxseed oil are shown in Table 8. The moisture content of the microcapsules ranged between 3.65-4.12% and their water activity varied from 0.05 to 0.08. 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 (Klaypradit and Huang, 2008).

Table 8. Moisture content (%) and water activity of spray-dried flaxseed oil microcapsules. Data represent the mean ± one standard deviation (n = 6).

Oil (%)	Moisture Content (%)		Water Activity	
	CPI	*LPI*	*CPI*	*LPI*
10	3.66 ± 0.32	4.12 ± 0.31	0.08 ± 0.01	0.08 ± 0.01
15	4.07 ± 0.31	3.89 ± 0.23	0.08 ± 0.00	0.05 ± 0.01
20	3.71 ± 0.46	3.65 ± 0.10	0.06 ± 0.00	0.06 ± 0.01

[0103] The Hunter *L* (lightness), *a* (redness), and *b* (yellowness) tristimulus colour values of spray-dried microcapsules containing flaxseed oil differed significantly for CPI and LPI as shown in Table 9 ($p<0.05$). CPI-microcapsules were creamy in surface colour, which was demonstrated by a mean *L, a, b* values of 91.3, -0.3, and 9.1, respectively. Whereas, LPI-microcapsules were darker (beige) in colour with mean *L, a, b* values of 87.2, 1.6, and 11.4, respectively. The observed darker colour of the LPI- containing microcapsules may be explained by the hull colour of the lentil proteins used in isolate production (Bamdad et al., 2006).

Table 9. The Hunter colour values of spray-dried microcapsules. Data represent the mean ± one standard deviation (n = 6).

Oil (%)	CPI Microcapsules			LPI Microcapsules		
	L	*a*	*b*	*L*	*a*	*b*
10	89.2 ± 0.0	-0.5 ± 0.0	10.4 ± 0.0	87.5 ± 0.0	1.6 ± 0.0	11.4 ± 0.0
15	92.9 ± 0.0	-0.1 ± 0.0	8.5 ± 0.0	87.8 ± 0.0	1.4 ± 0.0	11.0 ± 0.0
20	91.8 ±0.0	-0.4 ± 0.0	8.4 ± 0.0	86.3 ± 0.0	1.6 ± 0.0	11.8 ± 0.0

[0104] 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 (Bae and Lee, 2008). The effect of emulsion formulation on both surface oil content and encapsulation efficiency is presented in Table 10. The lowest surface oil and highest encapsulation efficiency for flaxseed oil with either plant protein wall material was observed at an initial oil concentration of 10%; with values of 1.13 and 1.08% for surface oil and 88.72 and 90.42% for encapsulation efficiency for CPI and LPI, respectively. An analysis of variance ($p<0.05$) 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. 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. (2006) and Polavarapu et al. (2011), 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. Overall, CPI- and LPI-containing microcapsules had similar amounts of surface oil (~1.75% and ~1.66%, respectively, $p>0.05$). However, LPI resulted in significantly higher encapsulation efficiency (~88.0%) compared to CPI (~86.3%, $p<0.05$).

Table 10. Changes in surface oil and encapsulation efficiency as a function of emulsion formulation. Data represent the mean ± one standard deviation (n = 6).

Oil (%)	Surface Oil (%)		Encapsulation Efficiency	
	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}

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

[0105] The mean droplet diameter of CPI- and LPI-stabilized emulsions before spray-drying and after reconstitution are shown in Figure 6. Experimental results showed that the *volume*-weighted mean oil droplet diameters ($d_{4,3}$) of flaxseed oil-in-water emulsions stabilized by CPI and LPI ranged between 16.3-24.0 and 21.0-26.1 μm, respectively. Under these experimental conditions, the smallest plant protein based microcapsule size was observed at an emulsion oil concentration of 10%. An analysis of variance showed that microcapsule size increased concomitantly with emulsion oil concentration ($p<0.001$). Overall, CPI-stabilized emulsions had significantly smaller oil droplets (~20.9 μm) compared to LPI-stabilized emulsions (~23.9 μm, $p<0.05$). However, effect of oil concentration followed a different trend depending on the protein source. In case of CPI-stabilized emulsions, mean oil droplet diameter increased from ~16.3 μm to ~23.2 μm when the oil concentration increased from 2% to 3-4% in the initial emulsion. For LPI-stabilized emulsions, mean oil droplet diameter of emulsions containing 3% oil (~21.0 μm) was significantly lower than that of emulsions containing 2 and 4% oil (~25.3 μm, $p<0.05$). Effect of spray-drying process on mean oil droplet diameter depended on the protein source used to prepare the microcapsules. For CPI-containing microcapsules, mean oil droplet diameter significantly increased upon reconstitution in microcapsules containing 15% and 20% oil ($p<0.05$). In case of LPI-containing microcapsules, increase in mean oil droplet diameter upon reconstitution was only observed in microcapsules containing 15% oil ($p<0.05$).

3.2 Microcapsule Morphology

[0106] SEM images of spray-dried microcapsules are shown in Figure 7. Panel A shows CPI and 10% oil. Panel B shows CPI and 15% oil. Panel C shows CPI and 20% oil. Panel D shows LPI and 10% oil. Panel E shows LPI and 15% oil. Panel F shows LPI and 20% oil. Changes in protein source and oil concentration did not affect the morphology of the microcapsules which appeared to be composed of heterogeneous spheres of various sizes. All samples exhibited a rounded external surface and similar surface morphology, which was smooth and free of visible pores or cracks. Surface depression was evident and was also reported in other spray dried particles containing maltodextrin as part of their wall materials (Bae and Lee, 2008; Soottitantawat et al., 2005).

3.3 Oxidative Stability of Encapsulated Flaxseed Oil

[0107] The peroxide value (PV) and TBARS results for free and CPI and LPI encapsulated flaxseed oil maintained at room temperature over a 25 d period are presented in Table 11. The PV of flaxseed oil before microencapsulation was 5.73 ± 0.30 meq active O_2/kg while that of the microencapsulated oil immediately after spray drying (Day 0) ranged from 6.31-6.80 meq active O_2/kg. Without being bound by theory, 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.

[0108] An analysis of variance showed that oil concentration was the only factor affecting PV of the microencapsulated flaxseed oil ($p<0.001$) throughout the whole storage period. Overall, PV of microcapsules containing 20% oil (~7.00 meq active O_2/kg) was significantly higher than that of microcapsules containing 10-15% oil (~6.52 meq active O_2/kg, $p<0.05$). This finding could be attributed to the higher amount of surface oil in the microcapsules containing higher amounts of oil. In the present study, 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 ($p>0.05$, Table 11). In addition, no significant differences in PV values in microencapsulated flaxseed oil were found between the two plant protein wall materials. 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 11).

Table 11. 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).

	Protein source	Oil (%)[1]	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25
PV (meq 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 MDA eq./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

[1] Concentration of oil in the initial emulsion.
Means in each row followed by different letters were significantly different ($p<0.05$).

[0109] 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 MDA eq/mg oil while that of the microencapsulated oil immediately following spray drying (Day 0) ranged from 1.99-2.14 MDA eq/mg oil. These results were not significantly different showing 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 1.90-2.47 MDA eq/mg oil and did not change over the 25 day storage period ($p>0.05$, Table 11) showing no significant difference between protein source and oil concentration. In contrast, TBARS value of bulk oil started to increase from 2.29 to 3.15 nmol MDA eq/mg oil at day 20 and kept increasing to 3.95 MDA eq/mg oil at day 25 ($p<0.05$, Table 11); indicating an increase in secondary oxidative products. These results clearly show that the legume protein-

maltodextrin matrices tested dramatically improved the oxidative stability of flaxseed oil when compared to bulk oil as indicated by both primary and secondary oxidative products during a 25 day storage period at room temperature.

3.4 Release Characteristics of Spray-Dried Flaxseed Oil

[0110] The relationship between flaxseed oil release from CPI- and LPI-microcapsules as a function of pH, ionic strength and simulated gastrointestinal environments are shown in Figures 8-10, respectively. For pH-triggered release, an analysis of variance showed that protein wall material, 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 acidic (pH 3.0) and basic 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 improved solubility of protein isolates. 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.

[0111] The *in vitro* release behaviour 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). For the CPI microcapsules, no significant difference ($p>0.05$) in the amount of oil released under these experimental condition was observed with respect to the oil concentration (10-20%) of the microcapsules. For the LPI microcapsules, the ~40% flaxseed oil release in simulated gastric fluid from the 20% oil microcapsules was significantly higher than the ~37% observed for the 10 and 15% oil microcapsules. In addition, flaxseed oil

released from LPI microcapsules increased from 81.5 to 86.2% as the oil content in the microcapsules increased from 10 to 20%.

[0112] In conclusion, findings of the present study demonstrate that CPI- and LPI-based microcapsule formation was efficient for the entrapment and gastrointestinal delivery of flaxseed oil, based on the in vitro release of encapsulated flaxseed oil under simulated gastric and intestinal conditions. 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. The findings show that the legume protein-based microcapsule systems tested were capable of carrying, protecting and delivering flaxseed oil, including in food and bioproduct applications.

[0113] Based on the above experimental examples using microcapsules prepared from two exemplary legume proteins, chickpea protein isolate and lentil protein isolate, prepared in two exemplary methods (freeze drying and spray drying), it can be soundly predicted that legume proteins, such as chickpea, lentil, faba bean and soy, can be used to as an encapsulation material to form microcapsules. It has been previously demonstrated that chickpea, faba bean, lentil and soy protein isolates can form stable emulsions (Can Karaca et al., 2011). Thus, it can be soundly predicted that other legume proteins such as faba bean and soy protein can be used in the same manner as the lentil and chickpea proteins tested in the above examples as an encapsulation material to form microcapsules.

[0114] Based on the above experimental examples using two exemplary legume proteins, chickpea protein isolate and lentil protein isolate and an exemplary oil, flaxseed oil, it can be soundly predicted that microcapsules formed using legume proteins can encapsulate an oil or an oil-soluble vitamin that can form an emulsion with water. It can be soundly predicted that such microcapsules can be used to protect the encapsulated oil (for example, by preventing oxidative degradation as demonstrated in Examples 2.4 and 3.3). In some embodiments, it can be soundly predicted that such microcapsules can be used to provide delayed release of the oil in the gastrointestinal tract of an animal based on the

results showing that only a small portion of the encapsulated oil is released under pH conditions ranging from 3.0 to 9.0 and an ionic strength ranging from 0 to 200 mM NaCl, while a significant portion of the encapsulated oil is released under simulated gastric and intestinal conditions (Examples 2.5 and 3.4). In some embodiments, it can be soundly predicted that such microcapsules are suitable for addition to a food product, including based on results showing a moisture content and water activity within the maximum moisture specification for dried powders in the food industry (Examples 2.1 and 3.1). In some embodiments, it can be soundly predicted that such microcapsules are suitable for addition to a food product having a relatively neutral pH, including based on results showing that only a small portion of the encapsulated oil is released under pH conditions ranging from 3.0 to 9.0 (Examples 2.5 and 3.4).

[0115] While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions and sub-combinations thereof. It is therefore intended embodiments of the present invention encompass a number of aspects that are not to be limited by the preferred embodiments and examples described herein, but should be given the broadest interpretation consistent with the description as a whole.

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WHAT IS CLAIMED IS:

1. A microcapsule comprising:

a core comprising a material to be encapsulated; and

a shell comprising a legume protein and a low molecular weight carbohydrate.

2. A microcapsule as defined in claim 1, wherein the material to be encapsulated comprises an oil.

3. A microcapsule as defined in any one of claims 1 to 2, wherein the material to be encapsulated comprises an oil rich in polyunsaturated fatty acids, and wherein the polyunsaturated fatty acids optionally comprise omega-3 fatty acids.

4. A microcapsule as defined in any one of claims 1 to 3, wherein the material to be encapsulated comprises an oil rich in α-linolenic acid, eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA).

5. A microcapsule as defined in any one of claims 1 to 4, wherein the material to be encapsulated comprises flaxseed oil, fish oil, flavour oil, fragrance oil, and/or an oil-soluble vitamin.

6. A microcapsule as defined in any one of claims 1 to 5, wherein the legume protein is selected from the group comprising chickpea protein, lentil protein, faba bean protein, and soybean protein.

7. A microcapsule as defined in any one of claims 1 to 6, wherein the legume protein comprises chickpea protein isolate.

8. A microcapsule as defined in any one of claims 1 to 6, wherein the legume protein comprises lentil protein isolate.

9. A microcapsule as defined in any one of claims 1 to 8, wherein the low molecular weight carbohydrate comprises dextrin and/or glycose syrup solids, and wherein the dextrin optionally comprises maltodextrin.

10. A microcapsule as defined in any one of claims 1 to 9, wherein the low molecular weight carbohydrate comprises maltodextrin-DE 3, maltodextrin-DE 4, maltodextrin-DE 5, maltodextrin-DE 6, maltodextrin-DE 7, maltodextrin-DE 8, maltodextrin-DE 9, maltodextrin-DE 10, maltodextrin-DE 11, maltodextrin-DE 12, maltodextrin-DE 13, maltodextrin-DE 14, maltodextrin-DE 15, maltodextrin-DE 16, maltodextrin-DE 17, maltodextrin-DE 18, maltodextrin-DE 19, maltodextrin-DE 20, and/or a combination thereof.

11. A microcapsule as defined in any one of claims 1 to 10, wherein the low molecular weight carbohydrate comprises maltodextrin-DE 9 or maltodextrin-DE 18.

12. A microcapsule as defined in any one of claims 1 to 11, wherein the amount of legume protein in an emulsion from which the microcapsule is prepared is between about 3% and about 10% w/v, or any value therebetween including 4%, 5%, 6%, 7%, 8% or 9% w/v, optionally about 4% w/v.

13. A microcapsule as defined in any one of claims 1 to 12, wherein the amount of low molecular weight carbohydrate in an emulsion from which the microcapsule is prepared is between about 20% w/v to about 50% w/v or any value therebetween.

14. A microcapsule as defined in any one of claims 1 to 13, wherein the amount of material to be encapsulated in an emulsion from which the microcapsule is prepared is in the range of about 5% w/v to about 25% w/v or any value therebetween.

15. A microcapsule as defined in any one of claims 1 to 14, wherein the amount of material to be encapsulated in an emulsion from which the microcapsule is

prepared is in the range of about 10% w/w to about 20% w/w or any value therebetween.

16. A microcapsule as defined in any one of claims 1 to 15, wherein the amount of material to be encapsulated in an emulsion from which the microcapsule is prepared is approximately 10.5% w/v, the amount of low molecular weight carbohydrate in the emulsion is approximately 35.5% w/v, and the amount of legume protein in the emulsion is approximately 4.0% w/v.

17. A microcapsule as defined in claim 16, wherein the material to be encapsulated comprises flaxseed oil, the low molecular weight carbohydrate comprises maltodextrin-DE 9, and the legume protein comprises chickpea protein isolate or lentil protein isolate, and wherein the microcapsules are optionally formed by freeze drying or spray drying.

18. A food product comprising a microcapsule as defined in any one of claims 1 to 17.

19. The food product as defined in claim 18, wherein the food product comprises a dairy product, and wherein the dairy product optionally comprises milk, cheese, yogurt, sour cream or ice cream, or wherein the food product comprises a dry food product and the dry food product optionally comprises cereal or granola bars.

20. The food product as defined in any one of claims 18 to 19, wherein the material to be encapsulated in the microcapsule comprises flaxseed oil, the legume protein comprises chickpea protein isolate or lentil protein isolate, and the low molecular weight carbohydrate comprises maltodextrin.

21. The food product as defined in any one of claims 18 to 20, wherein the food product has a relatively neutral pH, optionally in the range of approximately 6.5 to 7.0.

22. A method for producing microcapsules comprising:

combining a material to be encapsulated, a legume protein, and a low molecular weight carbohydrate;

mixing the resultant solution to form an emulsion; and

forming microcapsules.

23. A method as defined in claim 22, wherein mixing the resultant solution comprises homogenizing the resultant solution.

24. A method as defined in any one of claims 22 to 23, wherein forming microcapsules comprises spray drying, freeze drying, spray chilling or fluidized bed coating.

25. A method as defined in any one of claims 22 to 24, wherein forming microcapsules comprises freeze drying the resultant solution, and optionally crushing the freeze dried material.

26. A method as defined in any one of claims 22 to 24, wherein forming microcapsules comprises spray drying the resultant solution.

27. A method as defined in any one of claims 22 to 26, comprising adjusting the pH of the resultant solution to a pre-selected pH.

28. A method as defined in claim 27, wherein adjusting the pH of the resultant solution comprises adjusting the pH of a solution containing the legume protein to a pH of 3.0 or 7.0.

29. A method as defined in any one of claims 22 to 28, wherein the method is used to produce microcapsules as defined in any one of claims 1 to 21.

30. A method of protecting a material from oxidation after incorporation into a food product comprising incorporating the material into microcapsules according to the method defined in any one of claims 22 to 29 and then incorporating the microcapsules into the food product.

31. A method of encapsulating a material for delayed release in a food product comprising incorporating the material into microcapsules according to the method

defined in any one of claims 22 to 30 and then incorporating the microcapsules into the food product.

32. A method of hiding the taste of a material in a food product comprising incorporating the material into microcapsules according to the method defined in any one of claims 22 to 31 and then incorporating the microcapsules into the food product.

33. A method of enhancing the miscibility of a material in a food product comprising incorporating the material into microcapsules according to the method defined in any one of claims 22 to 32 and then incorporating the microcapsules into the food product.

34. A microcapsule as defined in any one of claims 1 to 21 or produced by a method as defined in any one of claims 22 to 33, wherein the moisture content of the microcapsules is between about 2 to about 4%.

35. A microcapsule as defined in any one of claims 1 to 21 or 34 or produced by a method as defined in any one of claims 22 to 33, wherein the water activity of the microcapsules is between about 0.05 and about 0.2.

36. A microcapsule as defined in any one of claims 1 to 21, 34 or 35 or produced by a method as defined in any one of claims 22 to 33, wherein the lightness, redness and yellowness tristimulus colour values are between about 87 to about 93, about -0.5 to about 0.5 and about 8 to about 21, respectively, and wherein the legume protein optionally comprises chickpea protein isolate.

37. A microcapsule as defined in any one of claims 1 to 21 or 34 or 35 or produced by a method as defined in any one of claims 22 to 33, wherein the lightness, redness and yellowness tristimulus colour values are between about 77 to about 89, about 1.4 to about 2.9, and about 11 to about 21, respectively, and wherein the legume protein optionally comprises lentil protein isolate.

38. A microcapsule as defined in any one of claims 1 to 21 or 34 to 37 or produced by a method as defined in any one of claims 22 to 33, wherein the surface oil content of the microcapsules is between about 0.5% and about 20%.

39. A microcapsule as defined in any one of claims 1 to 21 or 34 to 38 or produced by a method as defined in any one of claims 22 to 33, wherein the surface oil content of the microcapsules is between about 1% and about 3%.

40. A microcapsule as defined in any one of claims 1 to 21 or 34 to 39 or produced by a method as defined in any one of claims 22 to 33, wherein the encapsulation efficiency is between about 45% and about 95%.

41. A microcapsule as defined in any one of claims 1 to 21 or 34 to 40 or produced by a method as defined in any one of claims 22 to 33, wherein the encapsulation efficiency is between about 80% and 95%.

42. A microcapsule as defined in any one of claims 1 to 21 or 34 to 41 or produced by a method as defined in any one of claims 22 to 33, wherein the volume-weighted mean droplet diameter range of the emulsion is between about 2 and about 5 µm, and wherein the microcapsules are optionally produced by freeze drying.

43. A microcapsule as defined in any one of claims 1 to 21 or 34 to 41 or produced by a method as defined in any one of claims 22 to 33, wherein the volume-weighted mean oil droplet diameter range of the emulsion is between about 16 and about 24 µm, and wherein the microcapsules are optionally produced by spray drying.

44. A microcapsule as defined in any one of claims 1 to 21 or 34 to 43 or produced by a method as defined in any one of claims 22 to 33, wherein the peroxide value of the microcapsules is between about 5 to about 7 meq active O_2/kg.

45. A microcapsule as defined in any one of claims 1 to 21 or 34 to 44 or produced by a method as defined in any one of claims 22 to 33, wherein the peroxide value of

the microcapsules is between about 5 to about 8 meq active O_2/kg over a 25-day storage period.

46. A microcapsule as defined in any one of claims 1 to 21 or 34 to 45 or produced by a method as defined in any one of claims 22 to 33, wherein the level of 2-thiobarbituric acid-reactive substances in the microcapsules is between about 1.5 and about 2.5 MDA eq nmol/mg oil.

47. A microcapsule as defined in any one of claims 1 to 21 or 34 to 46 or produced by a method as defined in any one of claims 22 to 33, wherein the level of 2-thiobarbituric acid-reactive substances in the microcapsules is between about 1.5 and about 2.5 MDA eq nmol/mg oil over a 25-day storage period.

48. A microcapsule as defined in any one of claims 1 to 21 or 34 to 47 or produced by a method as defined in any one of claims 22 to 33, wherein the amount of material to be encapsulated that is released from the microcapsules after one hour is between about 5% and about 6% at pH 3.0; between about 2.5% and about 5% at pH 5.0; between about 6% and about 10% at pH 7.0; or between about 6% and about 10% at pH 9.0.

49. A microcapsule as defined in any one of claims 1 to 21 or 34 to 48 or produced by a method as defined in any one of claims 22 to 33, wherein the amount of material to be encapsulated that is released from the microcapsules after one hour at a pH of approximately 7 is between about 3% and about 5% at 0 mM NaCl; between about 4% and about 8% at 50 mM NaCl; between about 6% and about 9% at 100 mM NaCl; between about 6% and about 10% at 150 mM NaCl; or between about 8% and about 11% at 200 mM NaCl.

50. A microcapsule as defined in any one of claims 1 to 21 or 34 to 49 or produced by a method as defined in any one of claims 22 to 33, wherein the amount of material to be encapsulated that is released from the microcapsules under simulated gastric fluid conditions is between about 35 % and about 45% after two hours.

51. A microcapsule as defined in any one of claims 1 to 21 or 34 to 50 or produced by a method as defined in any one of claims 22 to 33, wherein the amount of material to be encapsulated that is released from the microcapsules under simulated gastric fluid conditions for two hours followed by simulated intestinal fluid conditions for three hours is between about 80% and about 95%.

52. A microcapsule as defined in any one of claims 1 to 21 or 34 to 51, wherein the resultant mixture comprises 4.0% chickpea protein isolate or lentil protein isolate, 35.5% maltodextrin-DE 9, and 10% of the material to be encapsulated.

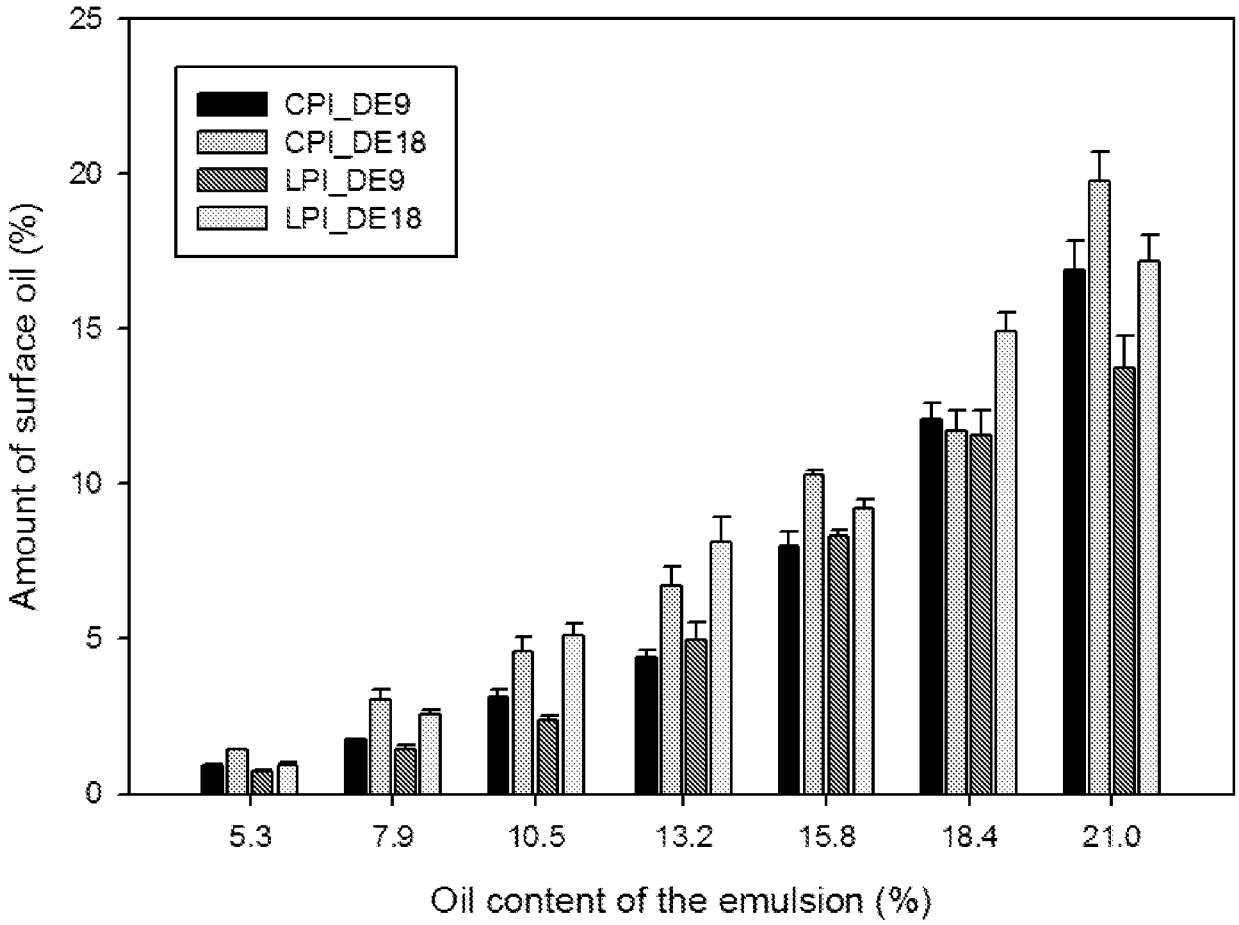
CPI_DE9
CPI_DE18
LPI_DE9
LPI_DE18
Amount of surface oil (%)
0
5
10
15
20
25
5.3
7.9
10.5
13.2
15.8
18.4
21.0
Oil content of the emulsion (%)

FIGURE 1

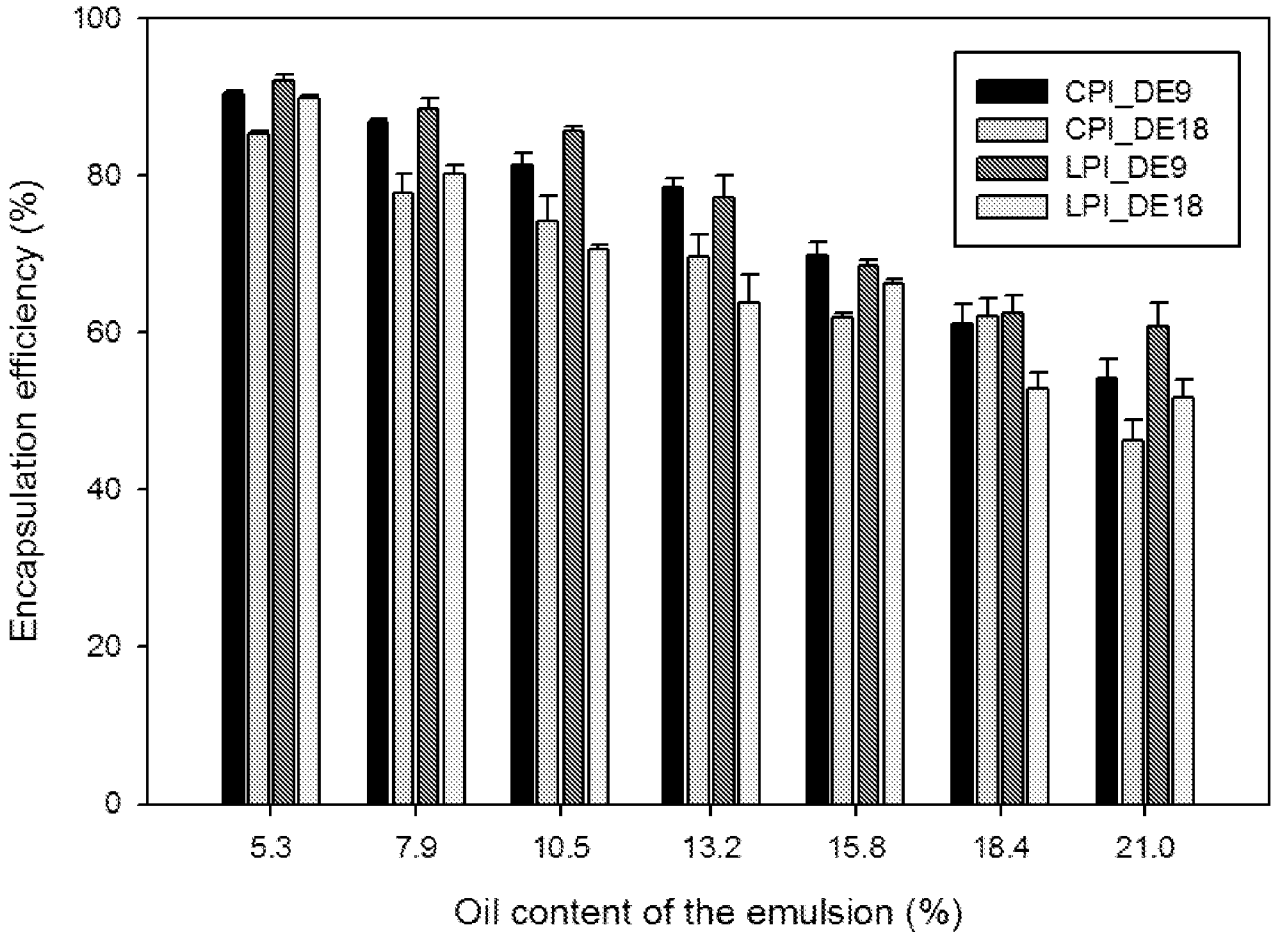
100
80
60
40
20
0
Encapsulation efficiency (%)
CPI_DE9
CPI_DE18
LPI_DE9
LPI_DE18
5.3
7.9
10.5
13.2
15.8
18.4
21.0
Oil content of the emulsion (%)

FIGURE 2

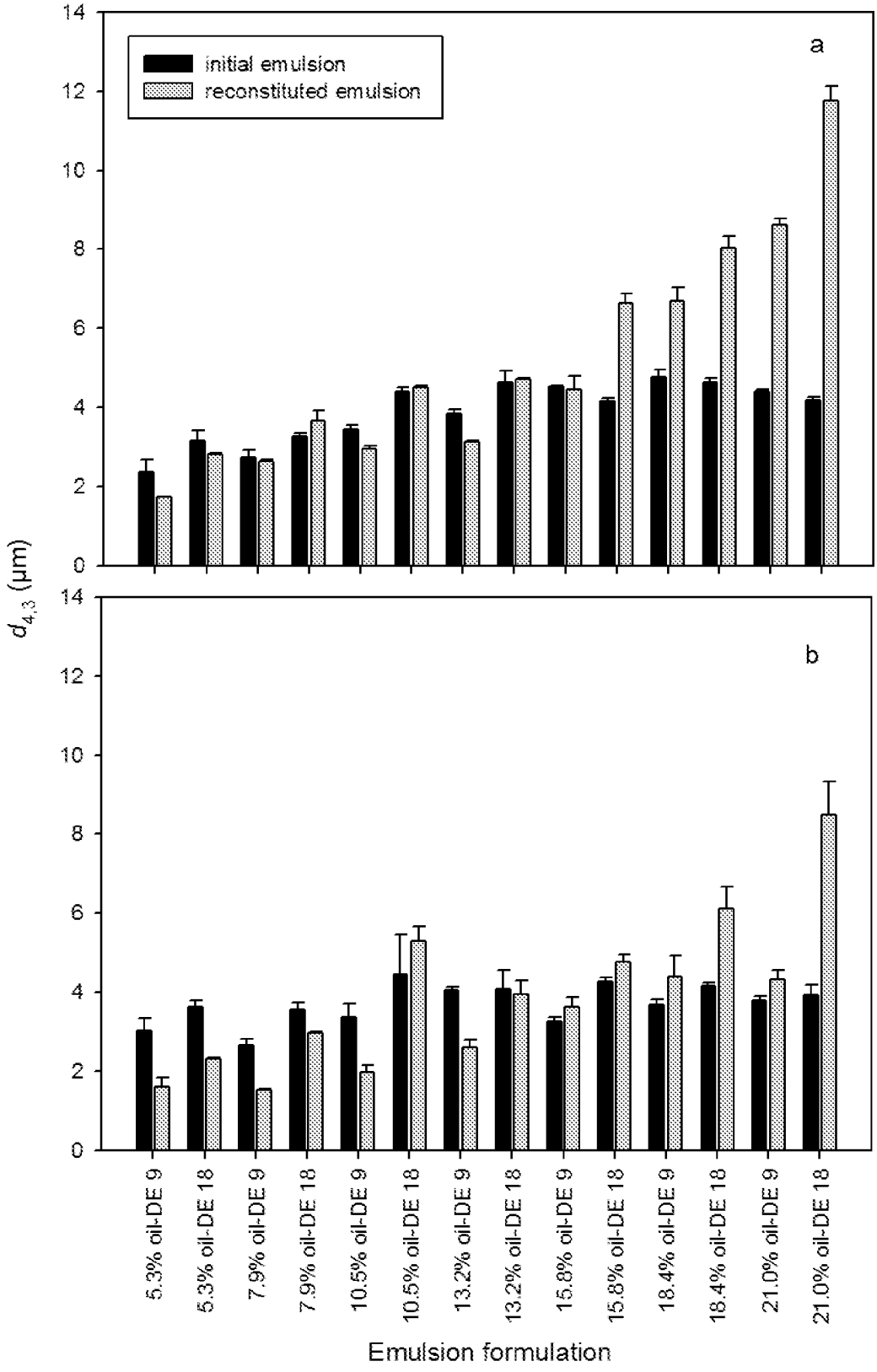
initial emulsion
reconstituted emulsion
a
b
$d_{4,3}$ (μm)
0
2
4
6
8
10
12
14
5.3% oil-DE 9
5.3% oil-DE 18
7.9% oil-DE 9
7.9% oil-DE 18
10.5% oil-DE 9
10.5% oil-DE 18
13.2% oil-DE 9
13.2% oil-DE 18
15.8% oil-DE 9
15.8% oil-DE 18
18.4% oil-DE 9
18.4% oil-DE 18
21.0% oil-DE 9
21.0% oil-DE 18
Emulsion formulation

FIGURE 3

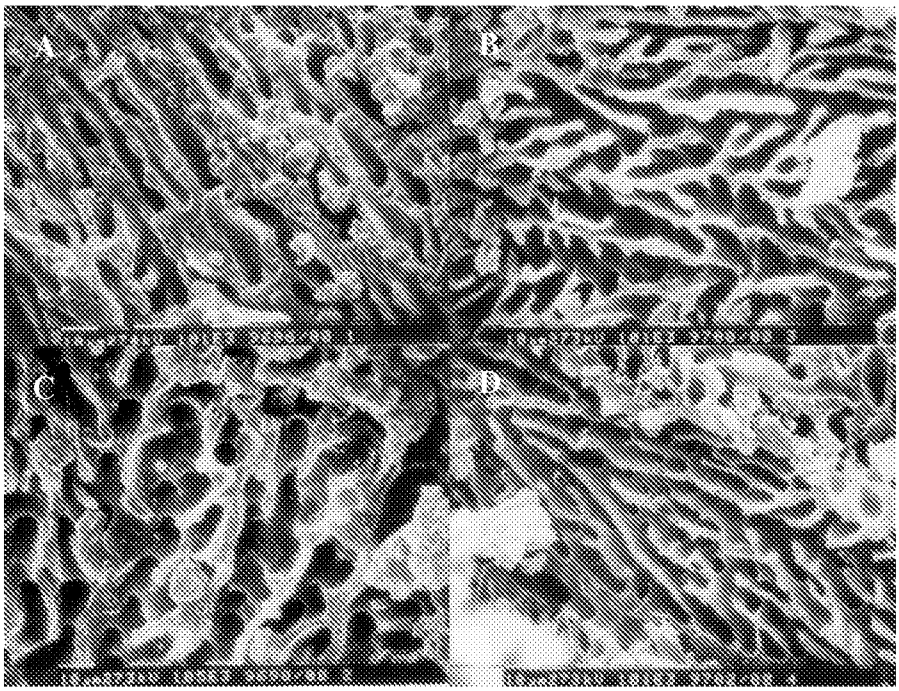

FIGURE 4

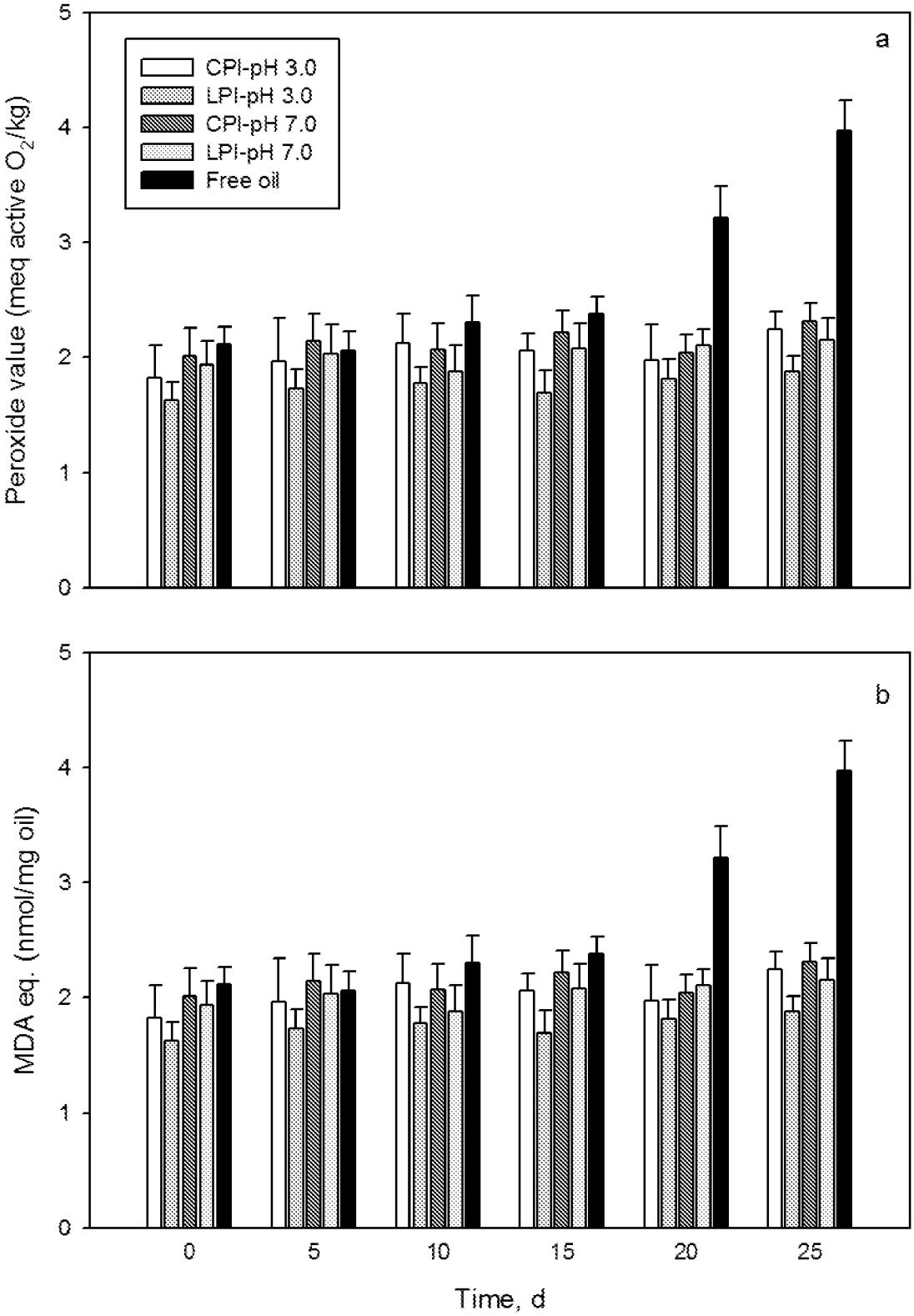
a
b
CPI-pH 3.0
LPI-pH 3.0
CPI-pH 7.0
LPI-pH 7.0
Free oil
Peroxide value (meq active O_2/kg)
MDA eq. (nmol/mg oil)
0
1
2
3
4
5
0
5
10
15
20
25
Time, d

FIGURE 5

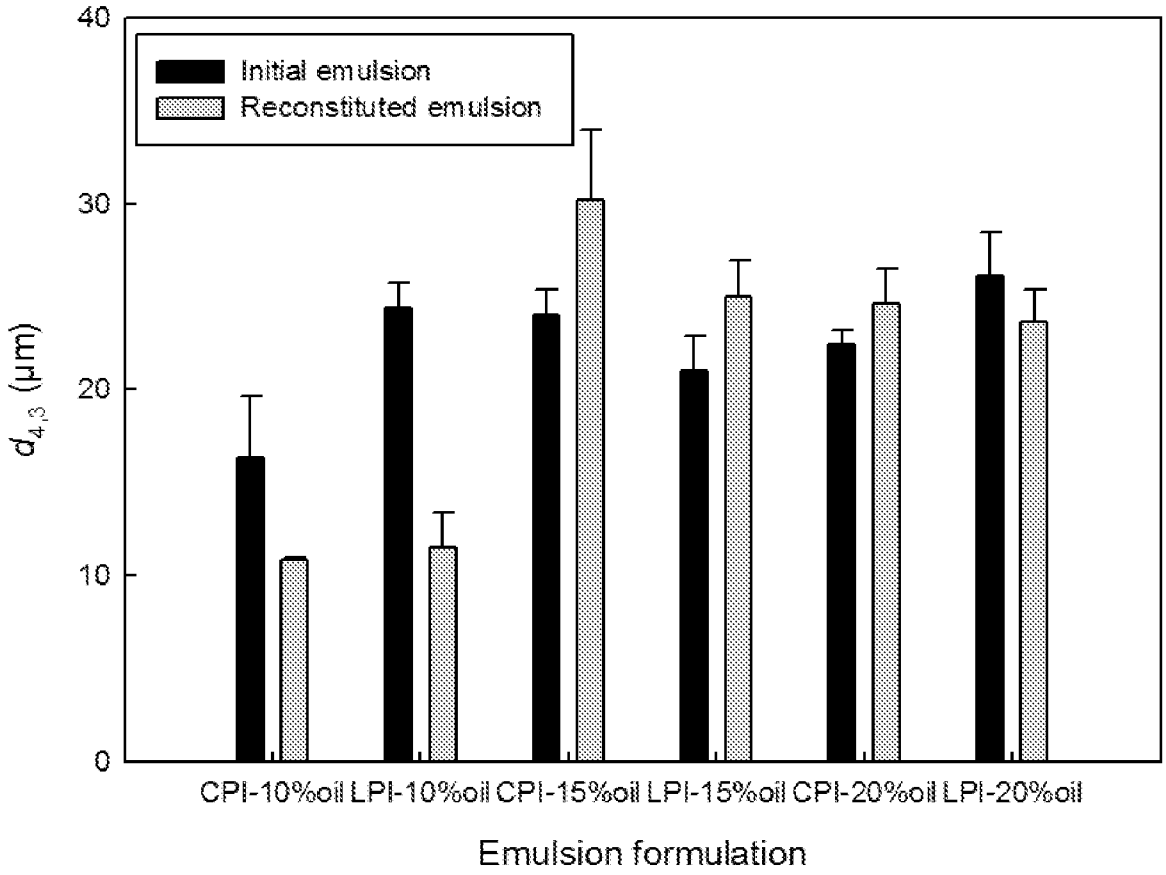
40
30
20
10
0
Initial emulsion
Reconstituted emulsion
$d_{4,3}$ (µm)
CPI-10%oil
LPI-10%oil
CPI-15%oil
LPI-15%oil
CPI-20%oil
LPI-20%oil
Emulsion formulation

FIGURE 6

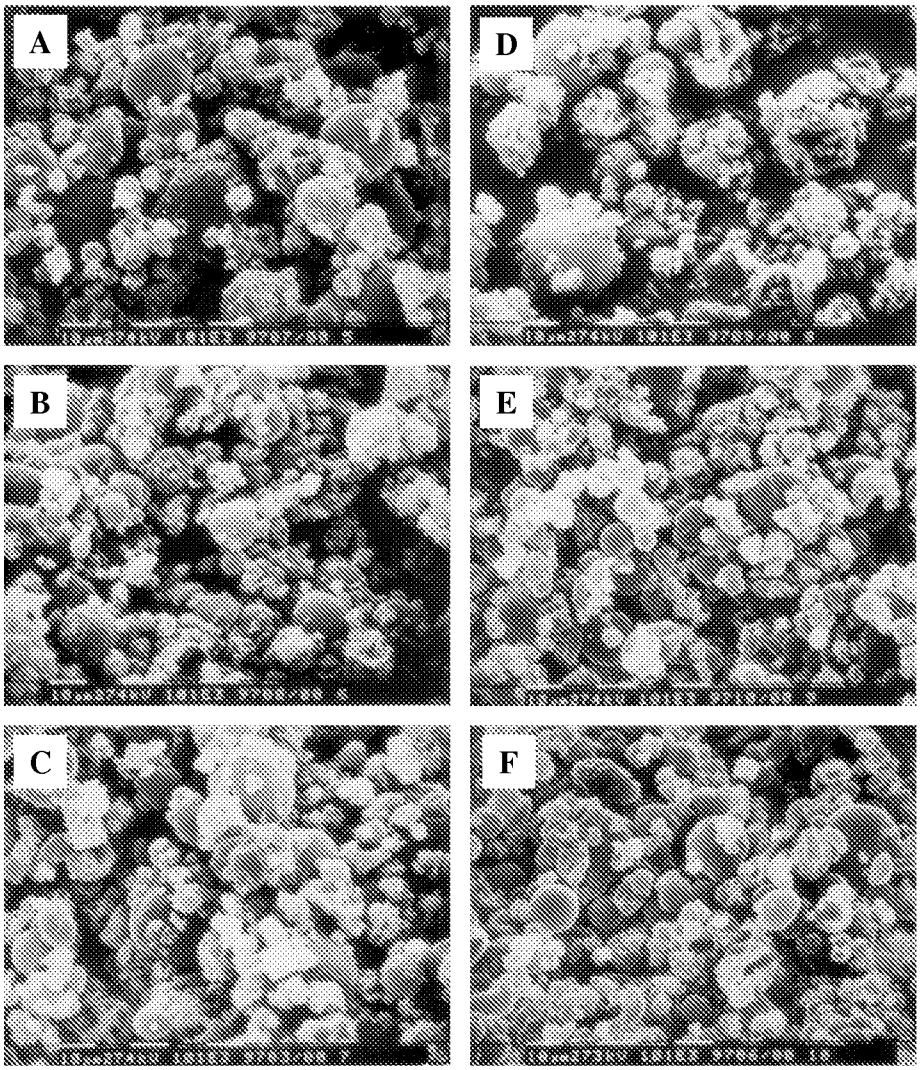

FIGURE 7

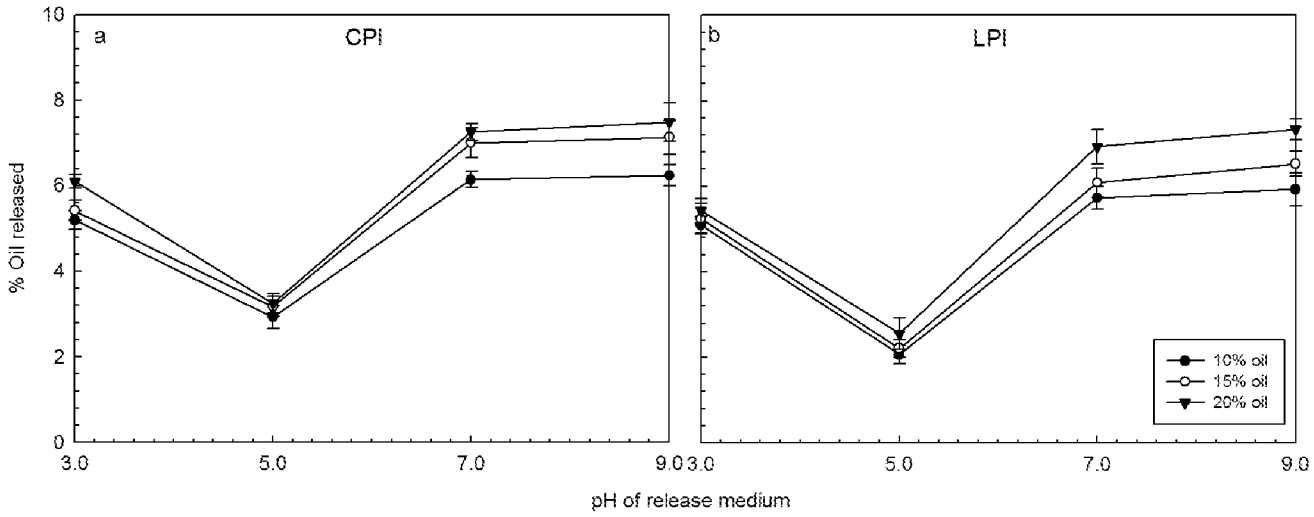

FIGURE 8

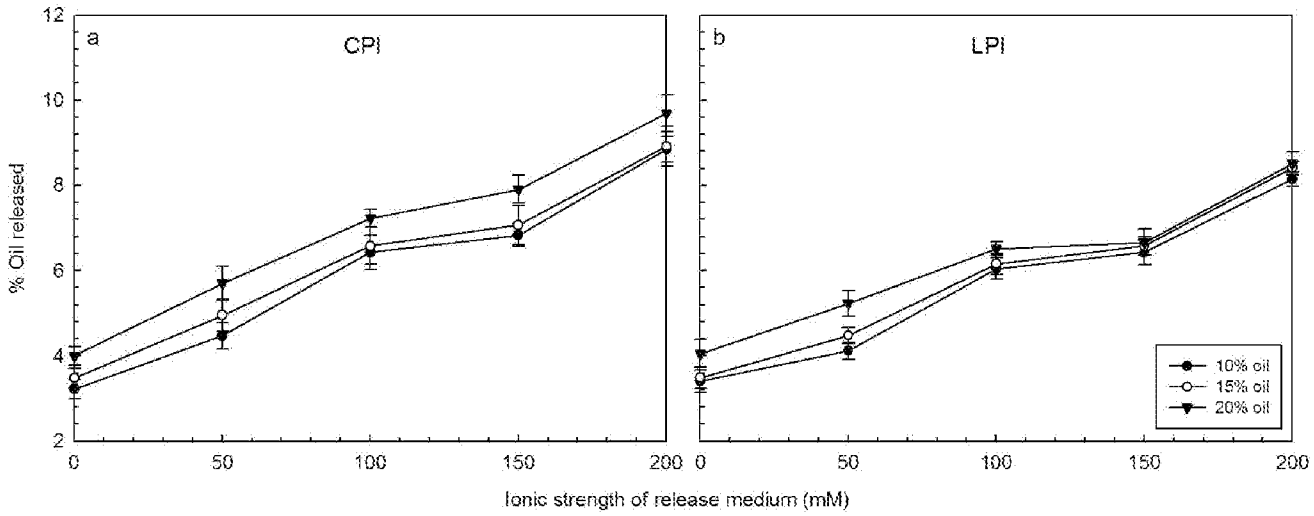

FIGURE 9

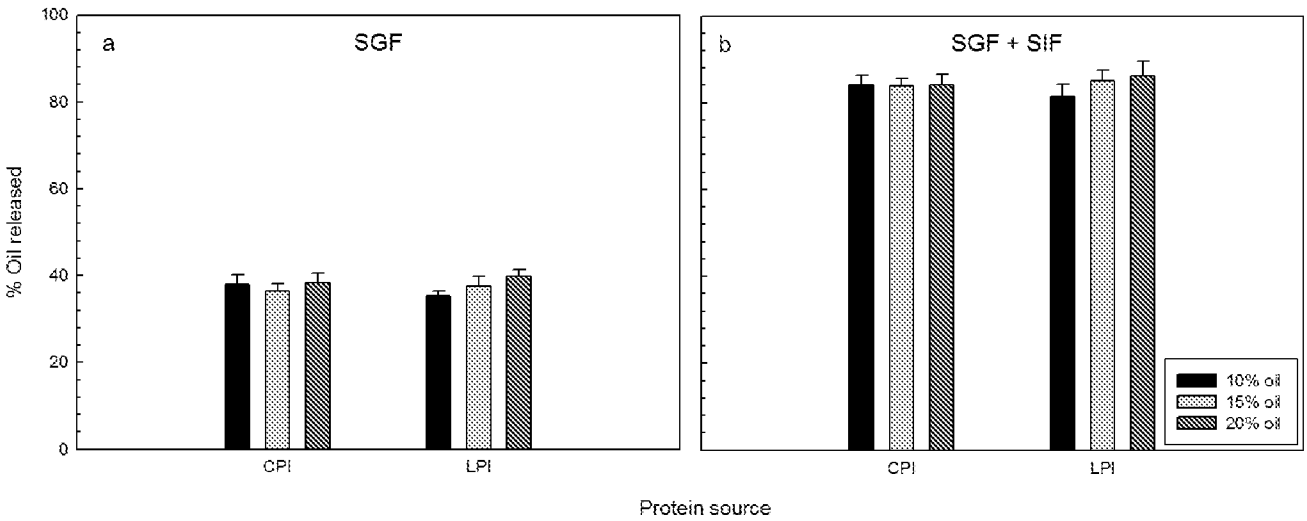
a
SGF
b
SGF + SIF
% Oil released
100
80
60
40
20
0
CPI
LPI
CPI
LPI
Protein source
10% oil
15% oil
20% oil

FIGURE 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2013/059458

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A23P 1/04*** (2006.01) , ***A23C 9/00*** (2006.01) , ***A23D 9/007*** (2006.01) , ***A23J 3/14*** (2006.01) , ***A23L 1/10*** (2006.01) , ***A23L 1/20*** (2006.01) (more IPCs on the last page)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23P 1/04, A23C 9/00, A23D 9/007, A23J 3/14, A23L 1/10, A23L 1/20, B01J 13/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
SCOPUS, TOTALPATENT, Canadian Patent Database. Search Terms used: chick, lentil, bean, carbohydrate, maltodextrin, core, coat, vegetable, legume, omega, flaxseed, fish, fish oil,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2009/070010 A (ALTING, A., FLORIS, T., WEINBRECK, F., GRANDIA, J., VAN DE VELDE, F.) 4 June 2009 (04-06-2009). p 7 l 31-33. p 8 l 26-28. examples 1, 4. claims.	1-24, 26-33, and 43-45 --- 1-52
X	EP 2 457 451 A1 (KOHANE, D., YEO, Y., GIVEN, P., LANGER, R.) 30 May 2012 (30-05-2012). Abstract. Claims 1, 4.	1-5, 12-16, 18, 19, 21-23, 27, and 30-33
X	US 6 596 337 B1 (VASLIN, S., GUERIN, G., MORVAN, M.) 22 July 2003 (22-07-2003). Abstract. Cols. 4, 6, 8. Example 1. Claims 1, 14, 15.	1, 2, 5, 6, 9, 12-16, 18, 22-26, 29, 42, 43 --- 1-52

[ ] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2014 (27-01-2014)	04 February 2014 (04-02-2014)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	ADAM MACKENZIE (819) 994-6514

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2013/059458

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		EP1051079B1	11 September 2002 (11-09-2002)
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		FR2774261B1	31 March 2000 (31-03-2000)
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		WO9938387A1	05 August 1999 (05-08-1999)

B01J 13/04 (2006.01)