ENCAPSULATION OF OMEGA FATTY ACID-RICH OILS USING PLANT PROTEIN-BASED MATRICES

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By
Chang Chang
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ABSTRACT

Oils rich in omega fatty acids (e.g., omega-3, -6, and -9) are both economically and nutritionally important to human beings, as they are playing significant roles in the prevention of various diseases (e.g., coronary artery disease, hypertension, and diabetes) and maintenance of mental health. However, due to their unsaturated nature, susceptibility to oxidation, and immiscibility in aqueous products, microencapsulation was introduced to entrap the oils to circumvent these challenges. The overall goal of this thesis was to encapsulate omega fatty acid-rich oils (e.g., canola, fish, and flaxseed oils) using plant protein-based (e.g., pea, soy, lentil, and canola protein isolates) matrices, in order to enhance storage stability.

In study one, the effect of pH (e.g., 3.0, 5.0, and 7.0) on the physicochemical (e.g., surface charge, hydrophobicity, and solubility), interfacial (e.g., interfacial tension and rheology), and emulsifying (e.g., droplet size and emulsion stability) properties of pea, soy, lentil, and canola protein isolates were determined to select one protein/pH to produce the most stable emulsion for encapsulation. Overall, proteins (at pH 7.0) with high surface charge, low hydrophobicity and high solubility showed a better ability to lower interfacial tension, whereas proteins (at pH 3.0) with high surface charge, hydrophobicity, and better solubility can form stronger viscoelastic films at the interface. All proteins could form stable emulsions away from their isoelectric point. Therefore, the selection of an effective plant protein emulsifier for encapsulation entails finding a balance between the properties needed to associate at the oil-water interface with those needed to develop a strong interfacial film. As such, lentil protein isolate (LPI) at pH 3.0 was selected as the most promising emulsifier to produce a stable emulsion, due to its high surface charge, solubility, and hydrophobicity.

In study two, the LPI-based wall materials (e.g., maltodextrin, sodium alginate, and lecithin) were used to encapsulate canola oil (as a model oil) using spray drying, in order to design a microcapsule formulation, which offered good physical properties (e.g., moisture content, water activity, color, wettability, particle size, surface oil, and entrapment efficiency)

and oxidative stability. Initially, mixtures of LPI (2-8%, w/w in initial emulsions) and maltodextrin (9.5-18%, w/w in initial emulsions) were used to entrap canola oil (20-30%, w/w in final microcapsules). Emulsion (e.g., emulsion stability, droplet size, viscosity) and microcapsule (e.g., surface oil and entrapment efficiency) properties were then characterized to determine a better capsule design. The microcapsules prepared with 20% oil, 2% LPI, and 18% maltodextrin were selected as a baseline to re-design better microcapsules using different preparation conditions and wall materials (e.g., sodium alginate and lecithin). Overall, the combination of LPI (2%), maltodextrin (17%), and sodium alginate (1%) presented the best capsule design to offer the highest entrapment efficiency (~88%) and oxidative stability, because of the formation of an electrostatic complex between negatively charged sodium alginate and positively charged LPI.

In study three, different omega fatty acid rich-oils (e.g., canola, fish, and flaxseed oils) were encapsulated by spray drying using the combination of LPI, sodium alginate, and maltodextrin. Physical properties, storage stability (e.g., free fatty acid content, peroxide value, 2-thiobarbituric acid reactive substances, and oxidative stability index) and *in vitro* release characteristics of encapsulated oils were investigated. Overall, all microcapsules displayed similar physical properties (except color). The combination of LPI, sodium alginate, and maltodextrin exhibited improved protection to susceptible oils from hydrolysis and oxidation in comparison with other microcapsules to entrap omega fatty acid-rich oils, and offered great antioxidative capacity, especially on fish oil, but oil-type had a significant effect on the rates of hydrolysis and oxidation. Minor amounts of encapsulated oils (~3.2-8.9%) were released under simulated gastric fluid, whereas the addition of simulated intestinal fluid resulted in significant oil release (~62.6-73.4%).

In summary, LPI with good physicochemical and functional properties represented as a promising emulsifier to alternate soy and animal-derived proteins and to produce a stable oil-in-water emulsion for the development of microcapsules. The combination of LPI, sodium alginate, and maltodextrin can be potentially used as a universal platform to encapsulate more omega fatty acid-rich oils to fortify omega fatty acids in commercial food and supplements.

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ALA	α-Linolenic acid
ANOVA	Analysis of variance
ANS	8-Anilino-1-naphthalenesulfonate
a.u.	Arbitrary unit
a_w	Water activity
BHT	Butylated hydroxytoluene
BSE	Bovine spongiform encephalopathy
ChPI	Chickpea protein isolate
CLSM	Confocal laser scanning microscope
cm	Centimeter
CPI	Canola protein isolate
d	Day
$d_{3,2}$	Surface-mass mean diameter
$d_{4,3}$	Volume-mass mean diameter
DE	Dextrose equivalent
DHA	Docosahexaenoic acid
d_i	Droplet diameter
DMSO	Dimethyl sulfoxide
EE	Entrapment efficiency
EPA	Eicosapentaenoic acid
ES	Emulsion stability
f(κα)	Smoluchowski approximation
FAMEs	Fatty acid methyl esters
Farad/m	Farad per minute
FFA	Free fatty acid
FI	Fluorescence intensity

F_{max}	Maximum force
g	Gram
$G_i´$	Storage modulus
$G_i´´$	Loss modulus
G_i^*	Complex modulus
h	Hour
HCl	Hydrogen chloride
H_E	Height of the emulsion
H_S	Height of the serum layer
kDa	Kilodalton
KH_2PO_4	Potassium phosphate
KI	Potassium iodide
L	Lecithin
L/h	Liter per hour
LPI	Lentil protein isolate
M	Molar
MD	Maltodextrin
MDA	Malondialdehyde
mg	Milligram
m^3/h	Cubic meter per hour
min	Minute
mM	Micromolar
mm	Millimeter
mN/m	Millinewton per meter
mPa	Millipascal
mPa·s	Millipascal per second
MUFA	Monounsaturated fatty acid
mV	Millivolt

N	Normality
NaCl	Sodium chloride
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
$Na_2S_2O_3$	Sodium thiosulfate
n_i	Number of droplet
NS	No separation
OC	Oil concentration in final microcapsules
OSI	Oxidative stability index
Pa·s	Pascal per second
pI	Isoelectric point
PPI	Pea protein isolate
PTFE	Polytetrafluoroethylene
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
R	Radius of the Du Noüy ring
rad/s	Radians per second
rpm	Revolutions per minute
s	Second
S	Svedberg unit
SA	Sodium alginate
SAFA	Saturated fatty acid
SC	Sodium caseinate
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SPI	Soy protein isolate

SRPH	Skipjack roe protein hydrolysate
TBA	2-thiobarbituric acid
TBARS	2-thiobarbituric acid reactive substances
TBHQ	*tert*-butylhydroquinone
TEP	1,1,3,3-tetraethoxypropane
TS	Total solids
U_E	Electrophoretic mobility
V	Volume
v/v	Volume by volume
W	Weight
w.b.	Wet basis
WPC	Whey protein concentrate
WPI	Whey protein isolate
w/v	Weight by volume
w/w	Weight by weight
α	Particle radius
γ	Interfacial tension
ε	Permittivity
ζ	Zeta potential
η	Dispersion viscosity
κ	Debye length
μL	Microliter
μmol/g	Micromole per gram

1. INTRODUCTION

1.1. Overview

Research activities surrounding microencapsulation of omega fatty acid-rich oils have attracted much attention over the past decade as they provide a number of potential advantages to the food industry. Microencapsulation offers protection to sensitive core materials, decreases nutrient loss of the encapsulant, and transforms liquid materials into an easily handled and dispersed solid powder (Desai and Park, 2005). The process typically involves coating individual active particles or droplets within wall materials comprised of proteins, polysaccharides and/or lipids to produce capsules in the micron to millimeter size range (Tyagi et al., 2011). Among the various microencapsulation techniques (e.g., spray drying, freeze drying, coacervation, extrusion, and fluidized-bed coating), spray drying is the most commonly applied, due to its low cost and wide availability of equipment (Desai and Park, 2005).

The selection of wall materials is meaningful for the production of microcapsules, because they greatly influence the stability of microcapsules, entrapment efficiency, and the degree of protection to the core materials. According to Nesterenko et al. (2013), no single wall material is able to provide high entrapment efficiency and effective protection, so, a combination of polysaccharides and proteins is most commonly studied to develop microcapsules, in which proteins serve as emulsifying and film-forming materials, whereas polysaccharides (e.g., maltodextrins, starches, pectin, chitosan, and sodium alginate) are applied as matrix forming materials (Young et al., 1993; Gharsallaoui et al., 2010). Proteins from animal sources (e.g., whey, gelatin, and casein) have been widely used for microencapsulation. However, plant proteins (e.g., soy, pea, and barley proteins) used as wall materials in microencapsulation have started to attract more attention and open up new

markets that restrict the use of animal proteins for religious, dietary, or ethical preferences and to reduce costs (Choi et al., 2010; Li et al., 2012; Nesterenko et al., 2013).

It is well known that omega fatty acids (e.g., omega-3, -6, and -9) play an essential role in human physiology, including the prevention and treatment of cardiovascular diseases and immune response disorders, development of the central nervous systems for infant growth, and maintenance of mental health (Shibasaki et al., 1999). However, because of their unsaturated nature, omega fatty acid-rich oils are chemically unstable, are susceptible to oxidative deterioration and readily produce free radicals, which are deemed to negatively affect the shelf-life, sensory properties, and overall acceptability of food products (Velasco et al., 2003). Therefore, microencapsulation of omega fatty acids-rich oils is considered as an effective way to reduce the susceptibility to environmental factors (e.g., oxygen, light, temperature, and moisture), increase shelf-life, mask the unpleasant taste, and supply the oils in the powder format in the marketplace (Calvo et al., 2012).

The overall goal of this research was to encapsulate omega fatty acids-rich oils (e.g., canola oil, fish oil, and flaxseed oil) within plant protein-based wall matrices, in order to produce stable microcapsules against oxidative reactions. Can Karaca et al. (2013a) designed a lentil protein-based wall material in combination with maltodextrin to entrap flaxseed oil that offered enhanced protection against a 25 d oxidative test and controlled release under simulated gastrointestinal fluids, but the oil payload (10%) was too low to be commercially viable. During the present research: a) different proteins (e.g., canola, soy, lentil and pea proteins) were considered through a pre-encapsulation screening process involving their effectiveness at stabilizing the oil-in-water interface during emulsion formation; b) the oil payload was increased to ≥20%; and c) a stable microencapsulation formulation was developed to entrap omega fatty acids-rich oils (e.g., canola, fish, and flaxseed oils).

1.2. Objectives

The overarching goal of this research project was to create microcapsules with plant proteins (e.g., canola protein isolate, soy protein isolate, lentil protein isolate, or pea protein isolate) as the wall materials to encapsulate omega fatty acids-rich oils (e.g., canola, fish, and flaxseed oils), in order to enhance storage stability. Specific objectives of this research were: a) to determine the effect of pH on the physicochemical, interfacial, and emulsifying properties of canola, soy, lentil, and pea protein isolates, in order to select one protein/pH to produce a stable emulsion; b) to develop a plant protein-based microcapsule formulation which effectively encapsulates an oil (e.g., canola oil), offers good physical properties (e.g.,

moisture, water activity, color, wettability, and particle size), and provides the protective nature against oxidation; and c) to entrap different omega fatty acids-rich oils (e.g., canola, fish, and flaxseed oils) using the developed microcapsules, and to assess the stability and *in vitro* release behavior of the encapsulated oils.

1.3. Hypotheses

The following hypotheses were tested as part of this research: a) plant proteins away from the isoelectric points will have better physicochemical, interfacial, and emulsifying properties; b) legume proteins will have better emulsifying properties than oilseed proteins; c) emulsion (e.g., droplet size, viscosity, and emulsion stability) and microcapsule (e.g., entrapment efficiency and surface oil) properties will be significantly affected by oil and protein concentrations; d) the encapsulation process will greatly improve the storage stability of oils; and e) the microcapsules will remain intact within simulated gastric fluid, but then release the oils in the presence of simulated intestinal fluid.

2. LITERATURE REVIEW

2.1. Microencapsulation

Microencapsulation has been used in numerous sectors, such as pharmaceuticals, biotechnology, agriculture and food to encapsulate essential oils, colorants, flavors, vitamins and microorganisms, in order to improve their applicable properties (e.g., shelf-life, handling properties and stability) (Ray et al., 2016). Microencapsulation is defined as a technology to isolate or embed bioactive particles (e.g., flavors, antioxidants, polyunsaturated oils, vitamins, and probiotics) by building a physical barrier or a homogeneous/heterogeneous matrix, to produce small capsules with various morphologies and of diameters between 0.2 and 5,000 μm. The encapsulated substances are known as the core, internal or payload phase, whereas the outer protective materials are considered as the wall, external, and coating phase. In general, microencapsulation is applied for different purposes: protecting the core material from surrounding environmental changes (e.g., pH, temperature, oxygen, light, and humidity), controlling the volatility and release properties of the core material, masking the unpleasant flavor and taste of the core material, transforming liquid compounds into solids for easy handling, and diluting the core material when only very small amounts are required (Desai and Park, 2005; Nesterenko et al., 2013; Bakry et al., 2016).

Depending on the physicochemical properties of the core materials, the composition of the wall materials, and the microencapsulation techniques, different morphologies can be obtained. For instance, Figure 2.1 shows: (a) a simple capsule design (where a core is surrounded by a single layer of wall material); (b) a multi-core capsule design (where multiple cores are dispersed within a continuous wall material matrix); (c) a multi-wall design (where a single core is surrounded by several layers of wall material); and (d) an assembled capsule design (where several distinct particles that consist of a single wall material layer coating a single core are embedded in a continuous matrix of wall material) (Raybaudi-Massilia and Mosqueda-Melgar, 2012).

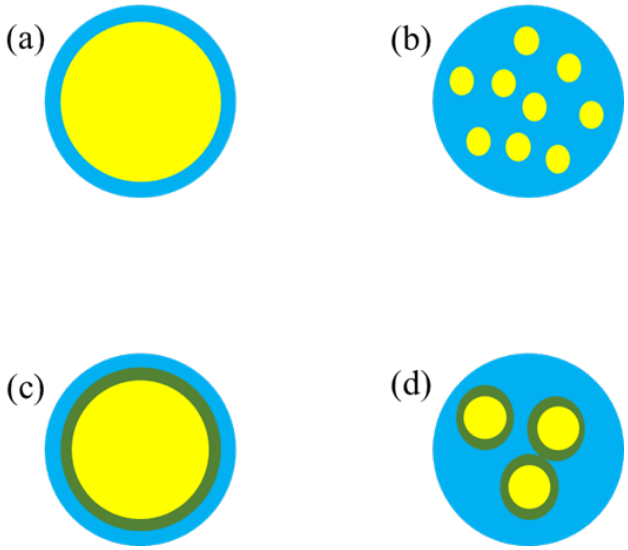

Figure 2.1. Different microcapsule model: (a) simple microcapsule, (b) multicore microcapsule, (c) multiwall microcapsule, and (d) assembled microcapsule.

2.1.1. Core materials

The health benefits of omega fatty acids-rich oils (e.g., derived from fish, flaxseed and canola) are substantiated through extensive studies that implicate their ability to prevent coronary artery disease, hypertension, diabetes, arthritis, inflammatory and autoimmune disorders (Connor, 2000; Tur et al., 2012). Many studies also encourage the daily intake of omega fatty acids (e.g., omega-3 and omega-6 fatty acids) by pregnant and lactating women to support the healthy development of both the retina and brain of the infant (Connor, 2000; Koletzko et al., 2011). Omega fatty acids belong to the family of unsaturated fatty acids, which have at least one double bond within the carbon chain. They include omega-3 (the first double bond placed at the third carbon starting from the methyl end of carbon chain, e.g., α-linolenic acid, eicosapentaenoic acid, and docosahaxaneoic acid), omega-6 (the first double bond placed at the sixth carbon starting from the methyl end of carbon chain, e.g., linoleic acid, arachidonic acid, docosapentaenoic acid), and omega-9 fatty acids (the first double bond placed at the ninth carbon starting from the methyl end of carbon chain, e.g., oleic acid). Marine oils (e.g., derived from fish and microalgae) and vegetable oils (e.g., derived from flaxseed, canola and soybean) represent the most widely available sources of omega fatty acids (Bakry et al., 2016). However, due to their unsaturated nature, omega fatty acids-rich oils are chemically unstable and susceptible to oxidative deterioration and readily produce free radicals and unpleasant tastes, which are deemed to negatively affect the shelf-life, sensory properties and overall acceptability of food products (Velasco et al., 2003). Therefore, microencapsulation technology is used as a viable method to maintain and improve the biological and functional characteristics of the oils, in order to further develop healthy food products fortified with omega fatty acids (e.g., bread, milk, and yogurt).

2.1.2. Wall materials

The selection of appropriate wall materials is an important aspect to consider in the design of microcapsules containing omega fatty acid-rich oils, because wall materials influence their stability, entrapment efficiency and the degree of protection. The ideal wall material should have the following characteristics: low viscosity under high concentrations; good emulsifying properties to stabilize the core materials; non-reactivity with the core materials; ability to hold core materials within the capsules; desired controlled release characteristics; ability to provide maximum protection to core materials against environmental conditions (e.g., oxygen, heat, light, and humidity); excellent solubility (in water or ethanol); have a plain taste; and be economically viable (Desai and Park, 2005).

Synthetic polymers (e.g., petroleum-derived polymers) and biopolymers (e.g., carbohydrates and proteins) are most commonly used as wall materials for microencapsulation, in which petroleum-derived polymers (e.g., polystyrenes, polyamides, and polyacrylates) are used in pharmacy and medicine, whereas biopolymers are used in the food industry (Dubey et al., 2009; Nesterenko et al., 2013). A number of polysaccharides have been studied as wall materials, such as starches, maltodextrin, gum Arabic, pectin, chitosan, and sodium alginate (Liu et al., 2010b; Sun-Waterhouse et al., 2011; Nesterenko et al., 2013). The major advantage of these polysaccharides is their excellent water solubility. However, they tend to have poor emulsifying properties that are important for microencapsulation (Nesterenko et al., 2013). Proteins from animal sources [e.g., milk (whey and casein) and gelatin] (Devi et al., 2012; Sanguansri et al., 2013; Silva et al., 2016) and plant sources (e.g., pulses, oilseeds and cereals) have also been used for oil microencapsulation (Wang et al., 2011; Can Karaca et al., 2013a; Tang and Li, 2013), because of their excellent emulsifying properties. Plant proteins are emerging in the industry as an alternative to animal proteins, due to perceived consumer safety concerns associated with the consumption of animal-derived proteins [e.g., bovine spongiform encephalopathy (BSE)], lower cost, abundant supply, and dietary preferences stemming from religious or moral concerns (Choi et al., 2010; Li et al., 2012; Nesterenko et al., 2013). Also maltodextrins are often used as a secondary material or ‘filler’ in the wall material, because of their excellent solubility and low viscosity at high concentrations, enabling the total solid contents to be raised to improve the drying efficiencies of the final powdered ingredient, especially during spray drying (Gharsallaoui et al., 2007). According to Nesterenko et al. (2013), no single wall material is able to present all the desired properties, where a combination of polysaccharides and proteins is most commonly studied to develop microcapsules. Utilization of proteins/polysaccharides mixtures allows the incorporation of specific properties of each polymer, to further improve their emulsion stability and produce microcapsules with better oxidative stability of the core material (Young et al., 1993; Gharsallaoui et al., 2010).

2.2. Protein-stabilized emulsions

Emulsion preparation is an important initial step during the microencapsulation of oils. During emulsion formation, oil droplets become dispersed through the input of mechanical energy (e.g., homogenization) within an aqueous continuous phase containing emulsifiers (e.g., protein). The formed oil-in-water emulsion has droplet diameters ranging

between 100 nm to 100 µm (McClements, 2005). Emulsions are considered to be thermodynamically unfavorable systems, which tend to break down over a period of time by a variety of destabilization mechanisms (e.g., gravitational separation, aggregation, and coalescence) (McClements, 2005). The role of emulsifiers is to prevent this instability by adsorbing to the interface to form a protective barrier around the oil droplets and can provide both electrostatic (at pHs away from the protein's isoelectric point) and steric stabilizing forces (McClements, 2005). During emulsion formation, proteins partially unfold to align at the oil-water interface and place their hydrophobic and hydrophilic moieties towards the oil and aqueous phases, respectively. Afterwards, the proteins at the interface aggregate to form a viscoelastic film which then may or may not be crosslinked using a fixative (e.g., transglutaminase) depending on the strength of the film (Morris and Gunning, 2008). It is recognized that flexible proteins (e.g., casein) that have more disordered structures are the most surface active proteins, whereas globular proteins (e.g., β-lactoglobulin or pea legumin) are more compact and rigid, and require some levels of unfolding before adsorbing to the interface (Dickinson, 1986). Several intrinsic characteristics of the proteins (e.g., flexibility, surface hydrophobicity, solubility, surface charge, and molecular size) affect the formation and stabilization of emulsions. For example, good solubility in the aqueous phase is a prerequisite for the proteins to be used as emulsifiers, because it determines the amount of proteins available to migrate to the oil-water interface to stabilize the oil droplets (Sikorski, 2001). Surface hydrophobicity also plays an important role, because the greater amount of hydrophobic patches on proteins allow the greater adsorption and retention at the oil-water interface (Sikorski, 2001). Due to non-toxicity, wide availability, and friendly labeling, proteins (e.g., caseins, whey proteins, and soy proteins) are widely used to stabilize emulsions to further produce microcapsules. Emulsion viscosity is another important parameter to control during encapsulation, since it can impact the stability and flow behavior of emulsions during the drying process. For instance, high viscosities of the feed emulsion can interfere the atomization during spray drying, prolong the drying process, lead to the formation of elongated particles, and cause air inclusion in the particles (Rosenberg et al., 1990). Moreover, emulsions should be stable over a certain period before drying process, so, smaller droplet sizes are necessary to prevent destabilization and air inclusion in the particles (Drusch, 2006).

2.3. Microencapsulation techniques

Omega fatty acids-rich oils have been microencapsulated using different techniques (e.g., spray drying, freeze drying, coacervation, extrusion, and fluidized-bed coating) (Table 2.1), however spray drying and coacervation are the most commonly used techniques in the food industry. The selection of a suitable technique depends on the type of core and wall materials, the size of final microcapsules, the desired physicochemical properties of microcapsules, the release profile, and the production scale and the cost (Bakry et al., 2016). Each technique produces microcapsules with specific properties (Table 2.2) (Desai and Park, 2005; Ray et al., 2016).

2.3.1. Spray drying

Due to the low cost, continuous operation and industrial scale, spray drying of emulsions containing omega fatty acids-rich oils has been extensively used as a microencapsulation and drying technology in the food industry (Bakry et al., 2016). Spray drying involves preparation of a stable emulsion prepared with the core materials and polymer solutions, pumping the emulsion into a spray dryer, atomization of the emulsion into droplets, and dehydration of the atomized droplets to produce microcapsules under extremely high inlet temperature (Desai and Park, 2005). Inlet and outlet temperatures should be critically controlled during spray drying, because inlet temperature greatly affects the efficiency of water evaporation to further determine the quality of microcapsules, whereas outlet temperature impacts the denaturation of wall materials of microcapsules (Broadhead et al., 1994; Kha et al., 2014). Spray drying offers several advantages: first, spray dryers are widely available and production cost is lower than other techniques (e.g., relative to freeze drying, the cost of spray drying is 30-50 × lower) (Gharsallaoui et al., 2007); second, spray drying is rapid and easily scaled-up (Pu et al., 2011); and third, spray drying is a flexible process, so as to offer substantial variation for the encapsulation matrix, while still producing dry powders with good quality (Desai and Park, 2005). However, a few limitations of spray drying include: a limited number of wall materials available, as they must require good solubility in water; and the high temperature during atomization could increase the level of lipid oxidation in the final product if proper care is not taken (Bakry et al., 2016). Sanguansri and co-workers (2013) demonstrated the use of sodium caseinate in combination with glucose and starch as wall materials to encapsulate tuna oil. They found the entrapment efficiency was affected by the bioactive mixtures (e.g., tributyrin and resveratrol) presented in the tuna

Table 2.1. Overview of microencapsulation of omega fatty acids-rich oils using different techniques.

Technique	Core material	Wall materials	Entrapment efficiency (%)	References
Spray drying	Tuna oil	Sodium caseinate, glucose, and starch	79-92	Sanguansri et al., 2013
	Flaxseed oil	Chickpea protein isolate or lentil protein isolate with maltodextrin	84-90	Can Karaca et al., 2013a
	Olive oil	Gelatin, sodium caseinate, gum Arabic, lactose, maltodextrin, and modified starch	33-53	Calvo et al., 2010
Freeze drying	Fish oil	Sodium caseinate and lactose or maltodextrin	29-82	Heinzelmann et al., 2000
	Flaxseed oil	Lentil protein isolate and maltodextrin	46-63	Avramenko et al., 2016
	Walnut oil	Sodium caseinate, carboxymethylcellulose, lecithin, and maltodextrin	37-69	Calvo et al., 2011
Complex coacervation	Fish oil	Gelatin and Acacia gum	17-92	Tamjidi et al., 2013
	Flaxseed oil	Gelatin and gum Arabic	84	Liu et al., 2010b
	Olive oil	Gelatin and sodium alginate	63-89	Devi et al., 2012
Extrusion	Olive oil	Sodium alginate	61	Sun-Waterhouse et al., 2011
Fluidized-bed coating	Fish oil	Soybean soluble polysaccharide, maltodextrin and octenyl succinic anhydride starch	96-99	Anwar and Kunz, 2011

Table 2.2. Comparison of various microencapsulation techniques used to encapsulate omega fatty acids-rich oils (Desai and Park, 2005; Ray et al., 2016).

Technique	Mechanism	Particle size (μm)	Oil payload (%)	Cost
Spray drying	Dehydration	5-150	5-50	Low
Freeze drying	Sublimation drying	-	Various	High
Coacervation	Electrostatic attraction	1-500	40-90	High
Extrusion	Immobilization by polysaccharide gel	150-2000	10-30	Moderate
Fluidized-bed coating	Coating of the solution	5-5000	5-50	Moderate

oil, which further influenced the amount of released oil (up to 86%) under simulated gastrointestinal fluid. Can Karaca et al. (2013a) encapsulated 10 to 20% of flaxseed oil within chickpea protein isolate or lentil protein isolate-based matrices, and found that oil concentration and protein source significantly impacted physicochemical properties, oxidative stability of core material and release characteristics from the microcapsules. Calvo and co-workers (2010) investigated the microencapsulation of olive oil using different wall materials (e.g., gelatin, sodium caseinate, gum Arabic, maltodextrin, lactose, and modified starch), and claimed that the combination of sodium caseinate and lactose produced microcapsules with better entrapment efficiency (~53%) and microcapsule yield (~50%) in comparison with other wall materials.

2.3.2. Freeze drying

Freeze drying is also known as lyophilisation, and is commonly used for the dehydration of heat-sensitive materials. For microencapsulation, the oil is homogenized with the coating materials to form an emulsion and is then frozen to be freeze dried (Heinzelmann et al., 2000). Frozen water is directly sublimated from the solid phase to a gas under a vacuum (Oetjen and Haseley, 2004). Because of simple operating procedures and reduced temperature, freeze drying is an attractive drying method to be used in microencapsulation. However, high energy use, long processing time, high production cost and poorer ability to protect encapsulated oils from oxidation (due to porous structure of the microcapsules) are major concerns for industrial application (Desobry et al., 1997). Heinzelmann and co-workers (2000) prepared a matrix of sodium caseinate with lactose or maltodextrin to encapsulate fish oil (26-33%) using different preparation conditions (e.g., homogenization passes and freezing rate), and observed that the microcapsules with highest core entrapment efficiency did not necessarily translate into better storage stability for the encapsulated oil, since other characteristics (e.g., moisture and particle size) were also influential. Avramenko and co-workers (2016) designed flaxseed oil (10-30%) microcapsules using native and pre-treated (heat and enzymatic hydrolysis) lentil protein isolate in combination with maltodextrin. They found it was not necessary to apply a pre-treatment of the lentil protein isolate, since the pre-treated lentil protein isolate (~47%) produced microcapsules with decreased entrapment efficiency when compared with native lentil protein isolate (~63%). Calvo et al. (2011) found that the fatty acid composition in walnut oil was not significantly affected by freeze drying and wall materials, in which the combination of lecithin, carboxymethylcellulose, and maltodextrin produced walnut oil microcapsules with higher entrapment efficiency (~69%).

2.3.3. Complex coacervation

Complex coacervation is a microencapsulation technique involving the electrostatic attraction between two oppositely charged biopolymers over a narrow pH range to produce a stable coacervates, which then separates to form a coacervate-rich and solvent-rich phase (Bakry et al., 2016). Coacervation is classified as simple or complex coacervation. In the former, one biopolymer comes out of solution to coat an oil droplet after homogenization via temperature changes, the addition of a water-miscible nonsolvent (e.g., ethanol) or the action of electrolytes (e.g., sodium sulfate) (Martins et al., 2009). However, in the case of complex coacervation, the oil is first emulsified in the aqueous solution at a pH where two biopolymers carry similar net charges, followed by the separation of the liquid phase during the formation of the coacervates as the adjustment of pH induces both biopolymers carry opposing net charges to further coat individual oil droplets. Finally, the solidification of wall materials is achieved by the addition of cross-linking agents (Piacentini et al., 2013). Capsules are then typically dried by spray drying to yield a flowable dried powder. Although simple coacervation is more economical than complex coacervation for microencapsulation, because of the lower cost to induce the reaction and phase separation, but formed capsules are less superior (Sutaphanit and Chitprasert, 2014). Those formed by complex coacervation achieve higher oil payloads (up to 99%), and have lower surface oil, better storage stability, and better controlled release properties than those formed by simple coacervation (Xiao et al., 2011). Complex coacervation has been successfully used in the microencapsulation of fish oil using gelatin and acacia gum as wall materials, in which the entrapment efficiency was significantly affected by the oil concentration and gelatin/acacia gum ratio (Tamjidi et al., 2013). Liu and co-workers (2010b) optimized the encapsulation of flaxseed oil within a gelatin-gum Arabic matrix via complex coacervation, and found that homogenization rate (3,000-15,000 rpm) and total biopolymer concentrations (1-2% w/v) had great effects on the structure, particle size, and surface oil of microcapsules. Olive oil was encapsulated within a gelatin and sodium alginate matrix using complex coacervation at pH 3.5-3.8, in which the entrapment efficiency and release characteristics of olive oil from microcapsules were greatly dependent on the polymer concentration and oil concentration (Devi et al., 2012).

2.3.4. Extrusion

Extrusion is a potential technology to encapsulate omega fatty acids-rich oils, in which a single or twin screw extruder is most commonly used to produce high density microcapsules with a less porous structure. Generally, the oil is dispersed within a molten

carbohydrate matrix (e.g., sucrose, maltodextrin, glucose syrup, and glucose) that stays in the glassy state (induced by high pressure, high temperature, and high shear rate), followed by extrusion through a series of dies into the dehydrating liquid (e.g., isopropanol and liquid nitrogen), which results in the solidification of wall materials to entrap the oil. The granules are then separated from the dehydrating liquid, followed by air-drying or vacuum-drying of the residual solvent (Saleeb and Arora, 1999; Nickerson et al., 2014). The advantage of extrusion is that the oil is completely surrounded by the wall materials to provide better oxidative stability and prolonged shelf-life. However, it is a relatively expensive process (twice that of spray drying) and produces microcapsules with larger particle sizes (150-2,000 μm), which limits its industrial applications (Desai and Park, 2005; Bakry et al., 2016). Extrusion techniques were previously used to encapsulate olive oil within a sodium alginate (with/without caffeic acid) matrix to limit the rate of oxidative degradation of the oil (Sun-Waterhouse et al., 2011).

2.3.5. Fluidized-bed coating

Fluidized-bed coating was originally used as a pharmaceutical technique, however it has recently been applied by the food industry to encapsulate a wide variety of essential oils and omega fatty acids-rich oils. In general, the powdered particles with core materials are suspended within a fluidized-bed chamber with high velocity air under controlled temperature and humidity. The wall materials are atomized within the chamber and gradually build outer layers on the surface of suspended particles (Desai and Park, 2005). Therefore, the wall materials (e.g., cellulose derivatives, dextrins, and starch derivatives) must have a lower viscosity to be atomized and pumped into the chamber and better thermal stability (Teunou and Poncelet, 2005). However, fluidized-bed coating has only been used as a secondary coating method for the microcapsules, which provides an additional coating on the surface of formed microcapsules (Kaushik et al., 2015). So, it is considered as a novel technique to produce microcapsules with increased shelf-life, controlled release characteristics, and improved aesthetics, taste, and color (Desai and Park, 2005). Recently, the fluidized-bed coating was used to encapsulate fish oil within a soybean soluble polysaccharide – maltodextrin. The prepared microcapsules had longer shelf-life at room temperature relative to capsules produced by freeze drying or spray drying (Anwar and Kunz, 2011).

2.4. Microcapsules properties

2.4.1. Physical properties

The physical properties of microcapsules are important to investigate in terms of both core bioavailability and applicability by the food industry. These properties include: moisture content, water activity, particle size, wettability, surface oil and entrapment efficiency (Kaushik et al., 2015). The moisture content is an important parameter to determine the storage stability of the microcapsules, where high moisture tends to lead to caking/agglomeration of the dried powder and lipid oxidation to induce off flavors (Lim et al., 2012). Moisture levels are controlled by the wall material (which abides water) and the drying temperature (e.g., inlet and outlet temperature in spray drying) (Reineccius, 2004a). The desired moisture content is 3-4% for shelf stable dried powders in the food industry (Klinkersorn et al., 2005). The water activity of the microcapsules can significantly affect microbial spoilage and lipid oxidation, which further determines shelf-life of the product (Sun-Waterhouse et al., 2013). Oxidative degradation is typically limited when the water activity falls between 0.2 and 0.3, because of the restriction of metal transition and retardation of hydroperoxide decomposition resulting from the quenching of free radicals and singlet oxygen (Velasco et al., 2003).

Particle size is an important parameter contributing to the flowability, compressibility, bulk density, and oxidative stability of the microcapsules (Koc et al., 2015). McNamee and co-workers (1998) reported that particle size of microcapsules is mainly dependent on the microencapsulation technique used. Therefore, it is affected by the size of nozzle, feeding rate, air pressure, and total solid content, when the microcapsules are produced using spray drying. In general, a decrease in surface area of microcapsules would delay the oxidation reaction as particle size increases (Desobry et al., 1997). The desirable particle size of microcapsules is below 40 μm in the food industry to avoid impacting the mouth feel of food products (Koc et al., 2015). The wettability of microcapsules is also primarily influenced by particle size and the wall materials, and can have a big impact on the dissolution and subsequent release of core materials (Vasisht, 2014). Generally, microcapsules with high surface oil and larger particle size are difficult to dissolve in the aqueous solution, because of the agglomeration of microcapsules. Moreover, the microcapsules produced by some wall materials (e.g., gum acacia) with lower dissolving ability in cold water exhibit poor wettability (Reineccius, 2004a).

Surface oil is defined as non-encapsulated oil on the surface of the dried particles. The measurement of surface oil is important as it can easily react with oxygen to produce

off-flavors and result in poor stability for the application. Surface oil can be determined by the gentle extraction of oil from microcapsules using an organic solvent (e.g., hexane, methanol, and ethyl acetate) (Kaushik et al., 2015). The ideal surface oil for microcapsules should be below 2% (w/w) for food industry applications (Nickerson et al., 2014). Entrapment efficiency is determined as the percentage of encapsulated oil within the wall matrix to the percentage of oil used in the formulation. Therefore, a higher entrapment efficiency indicates a lower surface oil on the microcapsules (Kaushik et al., 2015). Entrapment efficiency can be improved by increasing the wall materials content in the formulation, which is related to the formation of a stronger protective shell around the core material (Nesterenko et al., 2013). However, too much wall material can result in an abrupt increase in the viscosity of feeding emulsions used for spray drying; as such, drying process efficiency is greatly decreased resulting in microcapsules with lower entrapment efficiencies (Yu et al., 2007). Moreover, optimization of spray drying conditions (e.g., inlet and outlet temperatures) is important to improve the entrapment efficiency of microcapsules. For instance, the higher drying temperature promotes the formation of a rigid wall structure, in order to limit the migration and release of core materials (Rascon et al., 2010). Food industry aims to produce microcapsules with entrapment efficiency higher than 98% (Nickerson et al., 2014).

2.4.2. Oxidative stability

The primary purpose of encapsulating omega fatty acids-rich oils is to protect those oils against oxidative degradation by building a barrier around the oil droplets; so, lipid oxidation in microcapsules is meaningful to study, because it results in the loss of nutritional value and develops the undesirable flavors to further negatively affect their commercial application (Velasco et al., 2003). Lipid oxidation includes three steps: initiation, propagation, and termination. During initiation, the abstraction of hydrogen from polyunsaturated fatty acids (PUFA) produces the alkyl radical, on which the free radical is delocalized on the carbon chain, and upon molecular rearrangement, to form conjugated double bonds. Subsequently, the alkyl radical reacts with triplet state oxygen to produce a peroxyl radical, which has high energy to promote the abstraction of hydrogen from another PUFA. Therefore, the addition of hydrogen on the peroxyl radical produces hydroperoxide, and this step is known as propagation. In termination, the combination of two radicals (e.g., the combination of peroxyl radical and alkoxyl radical, and the combination between alkyl radicals) occurs to form non-radical species or the radical reacts with a chain-breaking

antioxidant (e.g., vitamin E) to form a relatively stable radical (McClements and Decker, 2007). In practice, primary lipid oxidation products (produced by the initiation and propagation steps of lipid oxidation, e.g., hydroperoxides, conjugated dienes and trienes) and secondary lipid oxidation products (produced by the decomposition of primary lipid oxidation products via β-scission reaction, e.g., aldehydes, carbonyls, and ketones) are monitored to determine the oxidative reaction (McClements and Decker, 2007) and the methods are summarized in Table 2.3. In general, different types of wall materials and microencapsulation techniques offer the different protective effects on the core materials, based on various ability of wall materials/structure to inhibit the oxygen transfer (Kaushik et al., 2015).

2.4.3. Release characteristics

According to Gouin (2004), another significant purpose for encapsulation is to control the release properties of the omega fatty acids-rich oils at the appropriate time and place, in order to improve their effectiveness and reduce the required dose (i.e., targeted delivery). Release maybe triggered by chemical, physical or mechanical means (e.g., shearing, solubilisation, heating, pH, enzymatic reaction) (Pothakamury and Barbosa-Canovas, 1995; Nesterenko et al., 2013). The main factors affecting the release characteristics of the core materials are related to interactions between the wall and core materials, physical properties of microcapsules (e.g., particle size and wettability), structure of microcapsules, viscosity and solubility of the wall materials (da Silva et al., 2014). Zuidam and Shimoni (2009) reported the cross-linking reagent used in wall materials provided a more gradual release upon the diffusion in water. The morphology of microcapsules also greatly affects the release characteristics of core materials. For example, in Figure 2.1, the core material is easily and quickly released from the simple microcapsules, followed by the multi-core microcapsules, whereas the multiple layers of wall materials in the multi-wall microcapsules can prolong the release process, but the core material can be still released quickly. However, the complex structure of assembled microcapsules greatly slow down the release action, and more serious chemical, physical or mechanical factors need to be involved to release the core material (Drusch and Mannino, 2009). Recently, the release of core materials has been investigated under various mechanisms, such as degradation, pH, salt, temperature, pressure, and simulated digestion (Desai and Park, 2005). For example, the degradation release is triggered by the addition of enzymes (e.g., proteases) to degrade the wall materials (e.g., proteins) (Hickey et al., 2007). The release of core materials has also

Table 2.3. Different methods to determine oxidative stability of encapsulated oils.

Method	Oxidation products	Desired industry target	Advantages	Disadvantages
Peroxide value	Primary	<5 meq/kg	Most commonly used	Relative insensitive with a detection limit of 0.5 meq/kg
Conjugated dienes and trienes	Primary	<2 mmol/kg	Simple, fast, and less samples required	Limited to samples with certain number of double bonds, ineffective in complex foods
Thiobarbituric acid reactive substances	Secondary	-	Simple and inexpensive	Non-specificity, not effective at lower oxidation level
Anisidine value	Secondary	<20	Able to measure non-volatile compounds with high molecular weight	Interference by other compounds having absorbance at 350 nm
Sensory panel	Secondary	-	Highly sensitive	Time consuming, expensive, and qualitative only

been studied under different pH and ionic strength conditions of the solvent, because those changes result in the alteration of wall material solubility (Can Karaca et al., 2013a). In practice, release of core materials from microcapsules can also occur under simulated gastrointestinal fluids (using pepsin and pancreatin), which involves degradation, pH, and temperature (Can Karaca et al., 2013a).

2.5. Choice of materials

2.5.1. Wall materials

Canola protein isolate

Canola (*Brassicaceae spp*.) is primarily grown for its oil content to be used for cooking and biodiesel purposes (Wu and Muir, 2008). Once the oil is pressed, the remaining meal (high in protein and fiber) is typically sold as low price feed products (Uruakpa and Arntfield, 2005). Canola meal is relatively high in protein (up to 50% protein on a dry basis) (Uppstrom, 1995), has a well-balanced amino acid profile, and good technologically functional properties (Aluko and McIntosh, 2001). Canola proteins are dominated by a salt-soluble globulin protein (cruciferin) and a water-soluble albumin protein (napin), constituting approximately 60% and 20% of the total protein, respectively (Hoglund et al., 1992). Cruciferin (12S; S is a Svedberg unit; molecular weight of 300 kDa; pI of 7.25) is a hexameric protein comprised of six subunits, each being composed of a heavy α-chain with 254 to 296 amino acids and a light β-chain with 189 to 191 amino acid residues linked by one disulfide bond. In contrast, napin (2S; molecular weight of 12.5-15 kDa; pI of 11) is a much small protein comprised of a 4.5 kDa polypeptide linked together with a 10 kDa polypeptide by two disulfide bonds (Wanasundara, 2011). It is characterized by strong alkalinity that is due to its high level of basic amino acid (e.g., histidine, lysine, and arginine), which leads to its very basic pI (Schmidt et al., 2004). Theoretically, cruciferin exhibits lower emulsifying ability than napin, because the globular conformation of cruciferin contributes to the low surface activity at the oil-water interface. Therefore, due to higher surface activity, napin is able to generate a greater initial surface coverage and have more intramolecular short-range interactions at the interface (Krause and Schwenke, 2001). Canola proteins have been used in many commercial products (e.g., beverages, dressing, baked goods, and protein snack bars) (Day, 2013), but very little information is available to use them as wall materials to develop microcapsules.

Soy protein isolate

Soybeans are used as a major source of edible oil, whereas the meal is primarily used as an animal feed. Because of the well-balanced amino acid profile, soy protein has been extracted from the remaining meal and widely used as nutritional and functional food ingredient (Singh et al., 2008). Soy protein provides all 9 essential amino acids (e.g., lysine, phenylalanine, tryptophan, valine, threonine, methionine, leucine, isoleucine, and histidine) and many techno-functional benefits, such as promoting moisture and flavor retention, emulsion stabilization, and enhancing the texture of food products (e.g., peanut butter and frozen desserts) (Anderson and Wolf, 1995). Soy protein (pI of 4.8) is dominated by glycinin and β-conglycinin proteins, constituting approximately 50 and 40% of the total protein, respectively (Koshiyama and Fukushima, 1976; Staswick et al., 1981). Glycinin (7S; molecular weight of 320-360 kDa) is comprised of five subunits, each being composed of acidic polypeptides (molecular weight of 35-43 kDa; pI of 4.8-5.5) and basic polypeptides (molecular weight of 20 kDa; pI of 6.5-8.5) linked by disulfide bonds (Badley et al., 1975; Staswick et al., 1981). β-conglycinin (11S; molecular weight of 180 kDa) contains 5% of carbohydrates moieties that relates to its immunoreactivity (Amigo-Benavent et al., 2009). It is comprised of α′ subunit (molecular weight of 76 kDa, pI of 4.9), α subunit (molecular weight of 72 kDa; pI of 5.2), and β subunit (molecular weight of 53 kDa, pI of 5.7-6.0) (Koshiyama and Fukushima, 1976). Soy proteins show interesting physicochemical and functional properties (e.g., water solubility, water and fat absorption, emulsion stabilization, and film forming properties) that attribute to the development of microcapsules (Gu et al., 2009). They have been studied as wall materials to produce microcapsules using spray drying (Augustin et al., 2006; Charve and Reineccius, 2009; Favaro-Trindade et al., 2010) and coacervation techniques (Chen and Subirade, 2009; Mendanha et al., 2009; Nori et al., 2010).

Pulse protein isolates

Pulse proteins are often eaten as a nutritional replacement for animal proteins, especially in countries in which the consumption of animal proteins is limited by non-availability or religious habits (Liener, 1962). Recently, it is reported that the consumption of pulses have a number of potential health benefits, such as reducing the risk of cardiovascular disease, cancer, diabetes, hypertension, and gastrointestinal disorders (Hu, 2003; Tharanathan and Mahadevamma, 2003). Pulses include peas, chickpeas, beans and lentils. Pulse proteins are dominated by salt-soluble globulin proteins (legumin and vicilin) and a water-soluble albumin protein (molecular weight of 16-483 kDa), constituting

approximately 70% and 10-20% of the total protein, respectively (Papalamprou et al., 2010; Roy et al., 2010). The globulin proteins in pulses are comprised of two major proteins: legumin (11S) and vicilin (7S). Legumin (pI of 4.8; molecular weight of 300-400 kDa) is a hexamer with subunits comprised of both acidic (molecular weight of approximately 40 kDa) and basic (molecular weight of approximately 20 kDa) chains linked by disulfide bridges. Vicilin (pI of 5.5; molecular weight of 150-180 kDa) is a trimer comprised of subunits with molecular weight of 50 kDa (Derbyshire et al., 1976; Sathe et al., 1984; Boye et al., 2010b). Generally, the ratios of albumin to globulin and legumin to vicilin are variable based on different pulse proteins. For example, the ratio of albumin to globulin is 1:3 for lentil protein; and the ratios of legumin to vicilin are 10.5:1 and 1:9 for lentil protein and pea protein, respectively (Gupta and Dhillon, 1993). These ratios affect the emulsifying properties of proteins. For example, Dagorn-Scaviner et al. (1987) found that purified pea vicilin proteins had higher emulsifying activity index (111 m^2/g) than purified pea legumin proteins (60 m^2/g). Pulse proteins have been successfully incorporated into microencapsulation processes as wall materials (Nesterenko et al., 2013; Can Karaca et al., 2015). Pulse proteins are often associated with polysaccharides to help improve their solubility and emulsifying properties to produce more stable emulsions with better droplet size distributions, in order to increase their entrapment efficiency during the microencapsulation process (Pereira et al., 2009; Gharsallaoui et al., 2010; Liu et al., 2010b).

Maltodextrin

Maltodextrins are defined as depolymerized starches [with dextrose equivalent (DE) value less than 20] produced by chemically and/or enzymatically hydrolysis of starches (Qi and Xu, 1999). Theoretically, the DE value significantly affects the browning tendency, oxidative protection property and hygroscopicity. Maltodextrins with high DE values provide better oxidation resistance, but they are more sweet and susceptible to browning reaction (Wang et al., 2014). Raja and co-workers (1989) found that maltodextrins with DE values between 10 to 20 were most suitable to produce microcapsules with higher entrapment efficiency using spray drying, whereas the maltodextrins with higher DE values were less acceptable for spray drying due to their caramelization characteristics and adhesive properties to clog the nozzle of spray dryer (Bayram et al., 2005). Because of their excellent solubility, little affinity to hydrophobic materials, and low viscosity at high concentrations, maltodextrins are most commonly used as a secondary wall material (also known as a filler) to improve the drying properties of microcapsules (Gharsallaoui et al., 2007). Desobry et al.

(1999) reported that maltodextrins with low molecular weight (high DE value) can effectively reduce the oxygen permeability by building the dense wall matrix to further increase the stability of encapsulated oils. However, since they lack of interfacial and emulsifying properties that are required for oil retention, they are generally associated with other wall materials (e.g., gum Arabic, whey proteins, sodium caseinate, and soy proteins) to provide high entrapment efficiency (Gharsallaoui et al., 2007). In the study of Jafari et al. (2007), they utilized maltodextrin together with modified starch or whey protein as wall materials to encapsulate fish oil, in order to achieve high oil retention and excellent oxidative protection.

Sodium alginate

Sodium alginate is an anionic polysaccharide consisting of α-L-guluronic acid and β-D-mannuronic acid residues that are linked by 1→4 linkages, which is derived from brown sea algae (Gaserod et al., 1998; Goh et al., 2012). Due to its hygroscopicity and innocuousness, sodium alginate is used as a gelling agent, stabilizer, and thickener for a wide range of products (e.g., jelly, ice cream, and chocolate milk) (Goh et al., 2012). It has been used as an encapsulating agent, because of its wide availability, low cost, and it is both tasteless and odorless (Etchepare et al., 2015). In the microencapsulation process, sodium alginate is added in the initial emulsions to improve stability by increasing the viscosity of the continuous phase, so as to reduce the movement of droplets (McClements, 2005). In most cases, positively charged proteins are utilized in combination with negatively charged sodium alginate to produce multilayers of polyelectrolytes (Kreft et al., 2007; Xiao et al., 2011). According to Zhang et al. (2015a), orange oil was encapsulated using β-lactoglobulin in combination with pectin or sodium alginate, in which the sodium alginate produced more stable double-layered emulsions with little changes on droplet size and turbidity after 4 weeks of storage.

2.5.2. Core materials

Canola oil

Canola oilseed is one of the most important oil source in the world. Canola oil is the third largest volume of vegetable oil produced worldwide after palm and soybean oils (Lin et al., 2013). In comparison with rapeseed, canola contains significant lower levels of erucic acid (< 2% compared to 54% in rapeseed) and glucosinolates (< 30 μmol/g compared to 55-115 μmol/g in rapeseed) which are beneficial for human and animal consumption (Mag, 1983; Velasco et al., 2008). Canola oil is characterized by its low level of saturated fatty acids

(7%) and larger amounts of unsaturated fatty acids [including oleic (61%), linoleic (21%), and α-linolenic acids (ALA, 11%)] (Dupont et al., 1989; Johnson et al., 2007). Therefore, canola oil is considered as a cardio-protective substance (Lin et al., 2013). Lin and co-workers (2013) stated that in comparison with other dietary fat sources, consumption of canola oil is beneficial to reduce total cholesterol and low-density lipoprotein cholesterol, in order to further decrease the risk of coronary heart disease and improve insulin sensitivity.

Fish oil

Fish oils are generally extracted from fresh oily fish or livers of lean fish, and contain polyunsaturated fatty acids [e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] (Calder, 2013). The amount of EPA and DHA are varying among fish, but most standard fish oils are comprised of 30% EPA and DHA in total at 1.5:1 ratio (Calder, 2013). Those long-chain polyunsaturated fatty acids provide a range of health benefits: 1) improving brain and cognition development at early childhood (Bakry et al., 2016); 2) providing anti-inflammatory effects to prevent cancer in human cell lines (Wendel and Heller, 2009); 3) maintaining cardiovascular and mental health (Ruxton et al., 2007); and 4) preventing several diseases (e.g., immune response disorders, ulcerative colitis, and Crohn's disease) (Eckert et al., 2010; Jordan, 2010). Specially, DHA presents as an essential structural lipid in sensory and vascular retina tissue (Lauterbach and Pawlik, 2014). However, due to insolubility in water, susceptibility to oxidative deterioration, and undesirable flavor, the incorporation of fish oil in food products is limited (Augustin et al., 2006). Therefore, encapsulation technology is investigated to utilize these high value oils in aqueous food systems (Patrick et al., 2013). For example, barley proteins-based microcapsules provided great protection on the fish oil to against oxidative reaction during accelerated storage test (at 40 °C) for 8 weeks (Wang et al., 2011).

Flaxseed oil

Flaxseed oil is known as a good source of ALA (45%-55%), oleic acid (21%), and linoleic acid (14%) (Rubilar et al., 2012; Calder, 2013). ALA is an essential fatty acid for human health, not only because it provides a number of health benefits (e.g., prevent cardiovascular diseases and immune response disorders), but it also acts as a precursor for the synthesis of long-chain polyunsaturated fatty acids (Calder, 2013). A number of studies demonstrated the conversion of ALA to EPA and DPA in plasma lipids, platelets, leukocytes and erythrocytes, but the conversion to DHA is limited (Arterburn et al., 2006; Burdge and

Calder, 2006). Flaxseed oil also contains vitamin E (tocopherols, 79 mg/100 g), which is dominated by γ-tocopherol. Therefore, due to the antioxidant functions of tocopherols, flaxseed oil also contributes to an essential nutrient to protect cell membranes against oxidative damage (Bozan and Temelli, 2008). Encapsulation of flaxseed oil has been studied using a range of wall materials (e.g., maltodextrin, whey protein concentration, modified starch, gum Arabic, and pulse proteins) by different techniques (e.g., spray drying and complex coacervation) to improve the oxidative stability, handling properties, and acceptability to consumers (Rubilar et al., 2012; Can Karaca et al., 2013a; Carneiro et al., 2013).

3. EFFECT OF PH ON THE INTER-RELATIONSHIPS BETWEEN THE PHYSICOCHEMICAL, INTERFACIAL AND EMULSIFYING PROPERTIES OF PEA, SOY, LENTIL AND CANOLA PROTEIN ISOLATES[1]

3.1. Abstract

The inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates as a function of pHs (3.0, 5.0, and 7.0) were investigated. Surface charge, hydrophobicity, solubility, interfacial tension, rheology, droplet size and emulsion stability were all studied. Conditions that favored the protein to have a high charge, low hydrophobicity and high solubility (pH 7.0) were better able to associate with the oil–water interface to lower interfacial tension. However, conditions that fostered the protein to have a high charge, high hydrophobicity and high solubility (pH 3.0) led to stronger interfacial viscoelastic films. Findings suggest that a balance of the surface active properties is most ideal for using plant protein emulsifiers in a food application. Overall, findings from this study indicated that all proteins could form stable emulsions away from its isoelectric point (pH 3.0 or pH 7.0), although the ones formed at pH 3.0 displayed much better interfacial rheology. Of the protein-types studied, the most promising alternative to soy protein isolate as an emulsifier was lentil protein isolate because it had high charge, solubility and hydrophobicity at pH 3.0. The low solubility of pea protein at acidic pH could cause sedimentation issues in products, whereas allergen concerns are still associated with the napin protein from canola.

3.2. Introduction

Food emulsions are mixtures of two (or more) immiscible liquids (e.g., oil and water), where one liquid is dispersed as droplets within a continuous phase of the other, formed in the presence of emulsifiers (e.g., proteins) under mechanical shear (McClements, 2005). Although the emulsifying properties of plant proteins have been previously studied

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[1.] Chang, C., Tu, S., Ghosh, S., & Nickerson, M. T. (2015). Effect of pH on the inter-relationships between the physicochemical, interfacial, and emulsifying properties of pea, soy, lentil and canola protein isolates. *Food Research International, 77,* 360-367.

(Adebiyi and Aluko, 2011; Avramenko et al., 2013; Barac et al., 2010; Can Karaca et al., 2011a; Cheung et al., 2014; Liang and Tang, 2013), little information is available relating to how the surface properties of a protein, their interfacial characteristics (e.g., interfacial tension and interfacial rheology), and their ability to stabilize an emulsion interrelate. Knowledge of these inter-relationships involving plant proteins may be useful in emulsion preparation, prediction of long-term stability, and quality control in food products.

Plant protein-based emulsifiers typically involve the use of soy protein products (e.g., concentrates or isolates), however because of allergen concerns industry is searching for other alternatives. Some emerging protein ingredients include those derived from pea, lentil and canola. Soy, pea and lentil proteins are all dominated by 11S (S denotes a Svedberg unit; molecular mass ~350 kDa) and 7S (molecular mass ~150-180 kDa) globulin-type storage proteins (Boye et al., 2010a). In soy, these are known as glycinin and β-conglycinin, respectively, whereas in pea and lentil these are referred to as legumin and vicilin, respectively. In contrast, canola proteins are dominated by a 12S globulin protein (known as cruciferin, molecular mass ~300 kDa) and a 2S albumin protein (known as napin, molecular mass ~14-16 kDa) (Wanasundara, 2011). Similar to other protein based emulsifiers, all of these proteins act by diffusing to the interface, re-orienting to become better integrated with the interface and then form a viscoelastic film to stabilize the oil droplets by either charge repulsion at pHs away from the protein's isoelectric point (pI) or through steric stabilization (Tcholakova et al., 2006; Morris and Gunning, 2008).

The overall goal of this study was to investigate the effect of pH on the physicochemical, interfacial, and emulsifying properties of pea, soy, lentil and canola protein isolates separately, in order to elucidate potential inter-relationships that exist to better tailor their use in the future.

3.3. Materials and methods

3.3.1. Materials

Pea (Propulse™) (PPI), lentil (LPI) and soy (PRO-FAM 974) (SPI) protein isolates were kindly donated by Nutri-Pea Limited (Portage la Prairie, MB, Canada), POS Bio-Sciences (Saskatoon, SK, Canada) and Archer Daniels Midland Company (Decatur, IL, USA), respectively. Canola seeds (Brassica napus/variety VI-500) were kindly donated by Viterra (Saskatoon, SK, Canada) for use in preparation of the canola protein isolate (CPI). According to the Association of Official Analytical Chemists Method 920.87 (AOAC, 2003), the crude protein contents of PPI, SPI and LPI were determined to be 78.30% (wet basis,

w.b.), 94.87% w.b., and 79.36% w.b. (%N × 6.25), respectively. Concentrations used in this study reflect the protein contents rather than powder weight. Canola oil used in this study was purchased from a local supermarket. Milli-Q water was obtained from a Millipore Milli-Q™ water purification system (Millipore Corporation, Milford, MA, USA).

3.3.2. Preparation of a canola protein isolate

Prior to use, canola seeds (stored at 4 °C in a sealed container) were initially screened using a #8 (2.63 mm) followed by a #12 (1.70 mm) Tyler mesh filters (Tyler, Mentor, OH, USA) to remove smaller seeds. The screened seed was then frozen at -40 °C for overnight before the cracking by using a stone mill (Morehouse-Cowles stone mill, Chino, CA, USA). The cotyledons were separated from the seed coat using an air classifier (Agriculex Inc., Guelph, ON, Canada). The cotyledons oil (~13%) was extracted using a continuous screw expeller (Komet, Type CA59 C; IBG Monforts Oekotec GmbH & Co., Mönchengladbach, Germany) at 59 rpm with a 3.50 mm choke. The hexane extraction (× 3) at 1:3 meal to hexane ratio for 8 h was used to remove the residual oil from canola meal, and the meal was then air-dried for another 8 h to evaporate the residual hexane to prepare defatted canola meal.

CPI was extracted from the defatted canola meal according to Klassen et al. (2011). In brief, defatted canola meal and 0.05 M Tris-HCl buffer (pH = 7.0) containing 0.1 M NaCl were mixed at 1:10 ratio and mechanically stirred at 500 rpm (IKAMAG RET-G, Janke & Kunkel GmbH & Co. KG, IKA-Labortechnik, Germany) for 2 h under room temperature (21-23 °C). The supernatant was then separated from the mixture using a centrifuge (Sorvall RC Plus Superspeed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) at 3000 × g for 1 h, followed by the filtration using #1 Whatman filter paper (Whatman International Ltd., Maidstone, England), and dialysis with Spectra/Por molecular porous membrane tubing (6-8 kDa cut off, Spectrum Medical Industries, Inc., USA) at 4 °C for 72 h in a fresh Milli-Q water (Millipore Corporation, MA, USA) environment to remove the salt. The dialyzed solution was then centrifuged (× 2) at 3000 × g for 1 h to collect the sediments. Finally, the sediments were freeze-dried (Labconco Corporation, Kansas City, Missouri 64132) for 24 h under a temperature difference of 35 °C to produce the CPI for the later experiments. The crude protein content of CPI was measured to be 99.11% w.b. (%N × 6.25).

3.3.3. Sample preparation

Protein solutions (0.05%, 0.01%, and 2.0%, w/w) were prepared by dissolving protein powders (PPI, SPI, LPI, and CPI) into Milli-Q water. The solution pH was adjusted to pH 3.0, pH 5.0, and pH 7.0 using either 1.0 M HCl or 1.0 M NaOH. The protein solutions were then mechanically stirred at 500 rpm for 2 h at room temperature (21-23 °C).

3.3.4. Zeta potential

A Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA, USA) was used to measure the electrophoretic mobility (U_E) of the protein isolate solutions (0.05%, w/w), and the zeta potential (ζ, mV) was determined as a function of pH and protein type through Henry's equation (3.1):

$$U_E = \frac{2\varepsilon \cdot \zeta \cdot f(\kappa\alpha)}{3\eta} \qquad [3.1.]$$

where, ε (Farad/m) is the permittivity, $f(\kappa\alpha)$ is a function related to the ratio of particle radius (α, nm) and the Debye length (κ, nm^{-1}), and η (mPa·s) is the dispersion viscosity (constant at 1.002 mPa·s). For this study, the Smoluchowski approximation $f(\kappa\alpha)$ equaled to 1.5 as is typically done when using folded capillary cells with point scatters larger than 200 nm in a dispersant with electrolyte concentrations of > 1 mmol/L. This approximation assumes that the point scatters (i.e., the protein) is at high enough levels so that the Debye length (or thickness of the electric double layer) is small relative to the particle size ($\kappa\alpha \gg 1$). The approximation also assumes that the zeta potential is linearly related to the electrophoretic mobility. Measurements were made in triplicate, and reported as the mean ± one standard deviation (n = 3).

3.3.5. Solubility

Solubility was investigated as a function of pH for all isolates using the modified technique of Morr et al. (1985). In brief, a 2.0% (w/w) protein solution was transferred to a 15 mL centrifuge tube and centrifuged (Clinical 200, VWR International, Germany) at 9100 × g for 10 min at room temperature (21-23 °C) to remove insoluble residues. Protein solubility was calculated based on the protein content in the supernatant divided by the protein content in the original protein sample. All measurements are reported as the mean ± one standard deviation (n = 3).

3.3.6. Surface hydrophobicity

Surface hydrophobicity for all protein isolates was measured as a function of pH using the modified method described by Kato and Nakai (1980), based on the interaction between 8-anilino-1-naphthalenesulfonate (ANS) probe and hydrophobic moieties on the protein's surface to give a fluorescent signal. In brief, the stock protein solution (0.01%, w/w) was diluted to 0.002% (w/w), 0.004% (w/w), 0.006% (w/w), and 0.008% (w/w) with Milli-Q water. 20 μL of 8 mM ANS solution was mixed with 4 mL of protein solutions by vortexing (S/P Vortex Mixer, Baxter Diagnostics Inc., USA) for 10 s, and kept in the dark for 15 min. 4 mL of protein solutions with 20 μL of Milli-Q water mixture were used as controls, and 4 mL of Milli-Q water with 20 μL of 8 mM ANS mixture was used as a blank and kept in the dark for 15 min before the test. Fluorescence intensity (FI) of samples was measured using a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) with the excitation wavelength at 390 nm and the emission wavelength at 470 nm at a slit width of 1 nm. Net FI was calculated by subtracting FI values for the control and blank samples from the FI value for the mixture of protein solutions with ANS. An index of relative surface hydrophobicity for the protein isolate was presented as the initial slope of the net FI versus protein concentration of the protein solutions. All measurements were reported as the mean ± one standard deviation (n = 3).

3.3.7. Interfacial tension

Interfacial tension between protein solutions (2.0%, w/w) and canola oil was determined as a function of pH using a semi-automatic tensiometer (Lauda TD2, GmbH & Co., Lauda-Königshofen, Germany) with a Du Noüy ring (20 mm diameter). In brief, a 20 mL protein solution was added into the glass sample cup (57 mm diameter), and then the Du Noüy ring was lowered into the protein solution, followed by the addition of canola oil (20 mL). The maximum force measured while the ring was pulling upwards to stretch the oil–protein interface without breaking the interface was recorded. Three consecutive maximum force readings were made on each time of interface stretching at 3 min intervals, and the measurement was stopped until the standard deviation lower than 0.10 mN/m. The interfacial tension was then calculated from the maximum force (F_{max}) using the following formula:

$$\gamma = \frac{F_{max}}{4\pi R\beta} \quad [3.2.]$$

where γ is the interfacial tension (mN/m); R is the radius of the ring (10 mm); β is a correction factor that depends on the dimensions of the ring and the density of the liquid involved. All measurements were reported as the mean ± one standard deviation (n = 3).

3.3.8. Interfacial rheology

Oscillatory interfacial dilatational rheological properties of all isolates as a function of pH were evaluated using a rheometer (AR-G2 Rheometer, TA Instruments Ltd., New Castle, DE, USA). The interface between a protein solution (2.0%, w/w) and canola oil was subjected to be an infinitesimal sinusoidal compression and expansion surface area, and the rheological behavior of the interface was characterized by measuring the interfacial storage modulus [G_i' (Pa)] and the loss modulus [G_i'' (Pa)] through time and frequency sweep tests. The overall response of the sample against the interfacial deformation was expressed as complex modulus (G_i^*) that was calculated by the following formula:

$$G_i^* = \sqrt{(G_i')^2 + (G_i'')^2} \quad [3.3.]$$

A bicone geometry (diameter = 68 mm, angle = 10°) and a polytetrafluoroethylene (PTFE) cup (inner diameter = 80 mm, depth = 45 mm) were used for the experiment. The protein solution was poured into the PTFE cup at the height of 19,500 μm, followed by immersing the bicone geometry into the solution without touching the bottom. Then, the similar amount of canola oil was added to cover the exposed protein solution surface. The interface location was determined by the dramatic normal force drop while the geometry moving upwards to 15,000 μm relative to the original position. Time sweep test was carried out with the controlled strain (0.1%) and frequency (0.1 rad/s) at room temperature (21-23 °C) for 30 min to determine the formation of viscoelastic film at interface in 30 min. This was then followed immediately by a frequency sweep test over a frequency range (0.1-10 rad/s) with controlled strain (0.1%) at room temperature (21-23 °C) on the same sample to measure the strength of the viscoelastic film at interface.

3.3.9. Emulsion preparation

Oil-in-water emulsions were prepared by homogenizing 2.0% (w/w) protein solutions with canola oil. In brief, 9 g of 2.0% (w/w) protein solutions and 1 g of canola oil were homogenized using an Omni Macro Homogenizer (Omni International Inc., Marietta, GA, USA.) equipped with a 20 mm saw tooth at speed 4 (~7200 rpm) for 5 min to prepare 10.0% (w/w) oil-in-water emulsions in a 50 mL plastic centrifuge tube.

3.3.10. Droplet size distribution

Droplet size distribution of freshly prepared emulsions was determined using a Mastersizer 2000 laser light scattering instrument (Malvern Instruments Ltd., Worcestershire, UK) with a Hydro 2000S sample handling unit as described by Can Karaca et al. (2011a,b). The droplet size distribution was measured immediately after the emulsion samples were prepared. Distilled water was used as the dispersant in the sample handling unit, and the obscuration was brought up to ~14% by sample addition. The relative refractive index of emulsion, which is the ratio of the refractive index of canola oil (1.470) to the refractive index of the dispersant (1.330) was 1.105. The droplet size was reported as surface-average diameter ($d_{3,2}$) that is expressed as:

$$d_{3,2} = \frac{\sum_{i=1} n_i \cdot d_i^3}{\sum_{i=1} n_i \cdot d_i^2} \qquad [3.4.]$$

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

3.3.11. Emulsion stability

Emulsion stability (ES) was determined according to Liu et al. (2010a) with minor modifications. In brief, homogenized samples (10 mL) were immediately filled into a 10 mL sealed graduated glass cylinders (inner diameter = 10.5 mm, height = 160 mm), and then stored for 24 h at room temperature. During storage, the emulsions separated into a cream upper layer and a serum bottom layer which included protein sediments. Emulsion stability was measured as ES (%) and expressed as:

$$ES\ (\%) = H_S / H_E \times 100 \qquad [3.5.]$$

where H_S is the height of the serum layer, and H_E is the height of the emulsion, as measured using a digital micrometer (Model 62379-531, Control Company, USA) having a precision of 0.01 mm. Figure 3.1 gives an image of a CPI stabilized emulsion at pH 3.0 immediately after homogenization (A) and after 24 h storage (B). Figure 3.1 (C) shows an image of a destabilized CPI emulsion found to occur at pH 7.0 (see Results and discussion section).

3.3.12. Statistics

All experiments were performed in triplicate and reported as the mean ± one standard deviation. A two-way analysis of variance (ANOVA) was used to measure statistical differences in physicochemical properties, interfacial properties, droplet size distribution, and emulsion stability as a function of pHs (3.0, 5.0, and 7.0) and protein types (PPI, SPI, LPI, and CPI). A simple Pearson correlation was calculated to describe the relationship between different properties [i.e., solubility, charge (absolute value), hydrophobicity, interfacial tension, interfacial complex modulus, droplet size and emulsion stability] of all protein isolates as a function of pH. All statistics were analyzed using Systat 10.0 software (Systat Software, Inc., Chicago, IL).

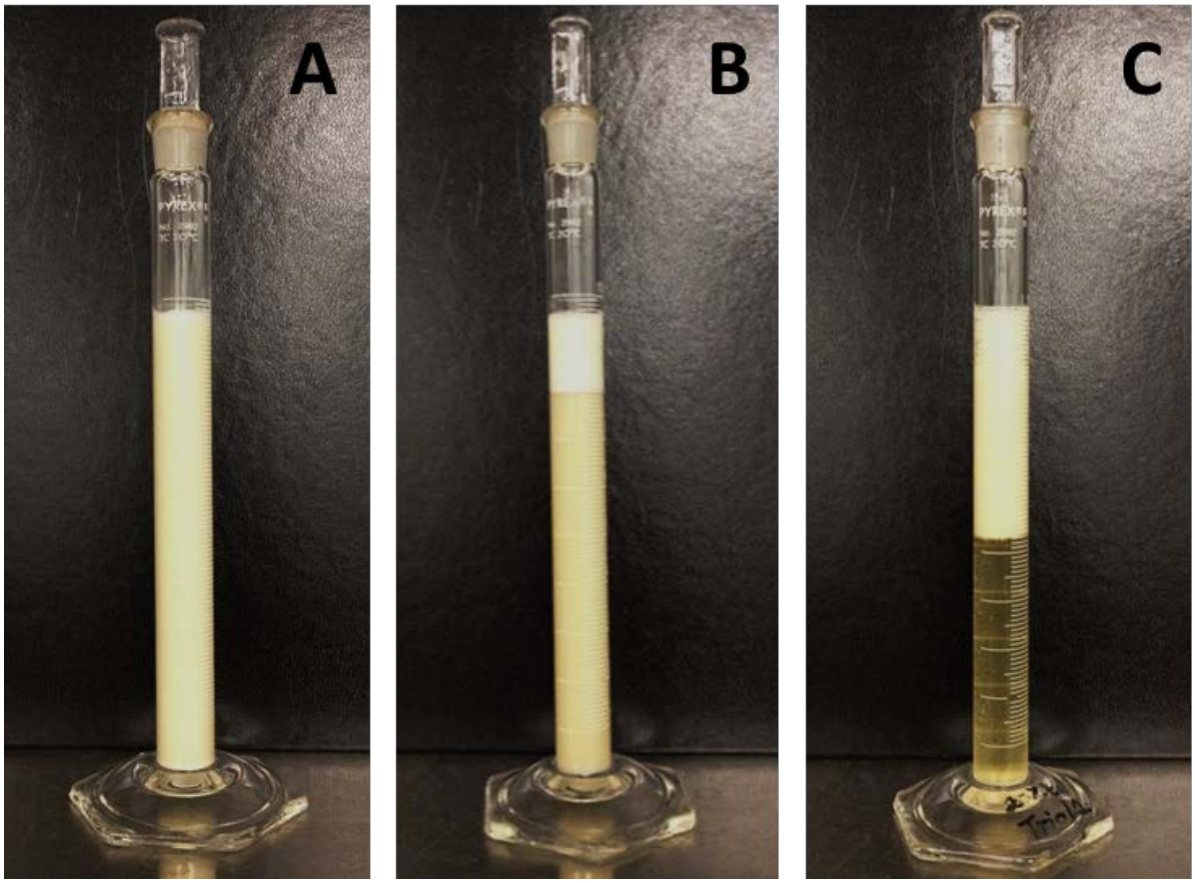


Figure 3.1. An image of a freshly prepared oil-in-water emulsion stabilized with a canola protein isolate (A – regardless of the pH), and that after a 24 h storage period for an emulsion prepared at pH 3.0 (B) and pH 7.0 (C).

3.4. Results and discussion

3.4.1. Physicochemical properties

Physicochemical properties, such as charge, hydrophobicity and solubility are important factors contributing to the diffusion and association of the plant proteins to an oil–water interface. Protein charge, hydrophobicity and solubility for all protein isolates (e.g., PPI, SPI, LPI, and CPI) were measured as a function of pH [Figure 3.2 (A-C)]. An analysis of variance indicated that all physicochemical properties were affected by pH and protein-type, along with their interaction ($p < 0.001$). A Pearson correlation found that solubility was strongly associated with charge ($r = 0.711$; $p < 0.001$) indicating that more highly charged proteins were more soluble.

At pH 3.0, all isolates had similar net positive charges (~ +32.5 mV) [Figure 3.2 (A)]. Hydrophobicity at this pH was the highest relative to other pHs for all isolates, with LPI showing the greatest hydrophobicity followed by SPI, CPI and then PPI [Figure 3.2 (B)]. It is presumed that the dissociation of protein subunits at pH 3.0 might contribute to the higher hydrophobicity relative to the other pHs. Differences in hydrophobicity among the proteins studied are hypothesized to reflect inherent differences in protein composition (e.g., percentage of 11S vs 7S proteins, or ratio of globulin and albumin proteins). For instance, globulin proteins tend to be more hydrophobic than albumins (Papalamprou et al., 2009), and 11S proteins are more hydrophobic than 7S proteins (Liang and Tang, 2013). In the case of solubility, all isolates were found to be the highest and similar in magnitude at pH 3.0 and pH 7.0, with the exception of CPI at the latter pH [Figure 3.2 (C)]. At pH 3.0, CPI showed the highest protein solubility, followed by SPI, LPI, and PPI [Figure 3.2 (C)].

At pH 5.0, there were almost no net charges (~ ±5 mV) for PPI, SPI, and LPI indicating that proteins were near the pI values. In contrast, at pH 5.0 CPI carried a net charge of ~ +18.0 mV [Figure 3.2 (A)]. Net neutrality for CPI occurred near pH 6.2 [Figure 3.2 (A)]. Hydrophobicity was also found to be reduced at pH 5.0 relative to pH 3.0, and followed a similar trend in terms of protein-type at pH 3.0 [Figure 3.2 (B)]. Due to the reduced surface charge, protein–protein interactions dominated leading a reduction in surface hydrophobicity (i.e., hydrophobic moieties on the surface of smaller individual proteins become buried again as larger aggregates) and a minimal solubility of ~4% for SPI, LPI and PPI at pH 5.0. In the case of CPI, solubility remained near ~37% since it was still away from its pI value of 6.2.

At pH 7.0, all isolates carried a net negative charge which differed depending on the protein-type. SPI was found to display the greatest charge, followed by LPI, PPI and CPI [Figure 3.2 (A)]. With the exception of CPI, all other proteins were away from their pI value.

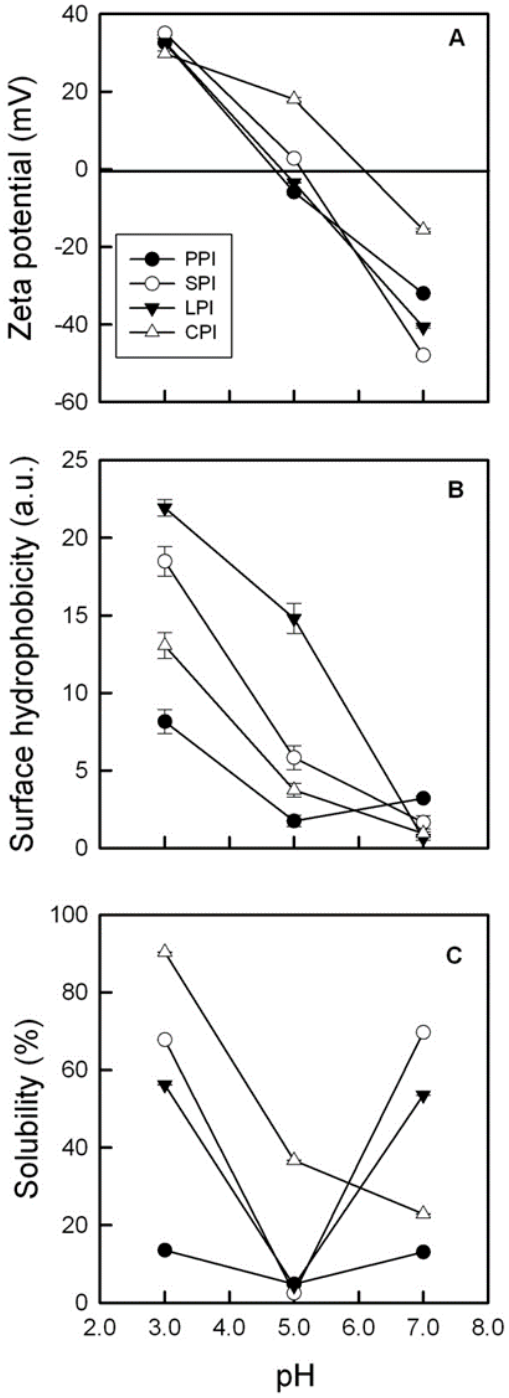


Figure 3.2. Zeta potential (mV) (A), surface hydrophobicity (arbitrary units, a.u.) (B), and solubility (%) (C) for protein solutions as a function of pH and protein-type. Data present the mean ± one standard deviation (n = 3). Abbreviations include: pea protein isolate (PPI), soy protein isolate (SPI), lentil protein isolate (LPI) and canola protein isolate (CPI).

Overall, hydrophobicity was found to be the lowest at pH 7.0 for all isolates relative to the other pHs, with the exception of CPI which was slightly higher than at pH 5.0. At pH 7.0, all isolates were of similar magnitude (± 2 arbitrary units) [Figure 3.2 (B)]. Hydrophobicity values may have been lowered at pH 7.0, because both the isolates and ANS probes carried a net negative charge. The net electrostatic repulsive forces in the solution may disrupt the interaction between aromatic moieties on protein isolates with the ANS probe to give poorer estimates of the true value (Alizadeh-Pasdar and Li-Chen, 2000). Solubility at pH 7.0 was similar to that of pH 3.0 for all isolates with the exception of CPI which was at its lowest (~23%) [Figure 3.2 (C)]. Although not measured, it is presumed that solubility would be minimal for CPI at pH 6.2, which corresponds to where its surface charge was neutral.

Overall, solubility is dependent upon the balance between protein–protein and protein–solvent interactions. A Pearson correlation found that solubility was strongly associated with charge (r = 0.711; p < 0.001) indicating that more highly charged proteins (whether negative or positive) were more soluble, and that hydrophobicity was not strongly linked to solubility (r = 0.320; p > 0.05), although hydrophobic interactions are expected to play an important role in stabilizing protein–protein aggregates as they form under more neutral conditions.

3.4.2. Interfacial properties

During emulsion formation, proteins migrate and accumulate at the oil–water interface to lower the interfacial tension (Damodaran, 1996). Changes to the interfacial tension as a function of pH for all isolates is given in Figure 3.3. An analysis of variance indicated that both pH (p < 0.001) and protein-type (p < 0.001), along with their interaction (p < 0.05) were significant. Overall, the addition of isolates into the aqueous phase at all pHs was found to lower the interfacial tension from ~ 22.5 mN/m (control, no proteins) to 8-16 mN/m. The ability for all proteins to lower the interfacial tension was similar at pH 3.0 and pH 5.0 regardless of their differences in physicochemical properties, however they significantly improved at pH 7.0 (Figure 3.3). Furthermore at each pH, PPI was the most effective at reducing interfacial tension, followed by LPI and SPI which were similar, and then by CPI which was the least effective (Figure 3.3). In the present study, interfacial tension was negatively correlated with surface charge (r = -0.372; p < 0.05) and positively correlated with surface hydrophobicity (r = 0.494; p < 0.01). This suggests that the effectiveness of the protein to reduce the interfacial tension (i.e., lower values) is better when

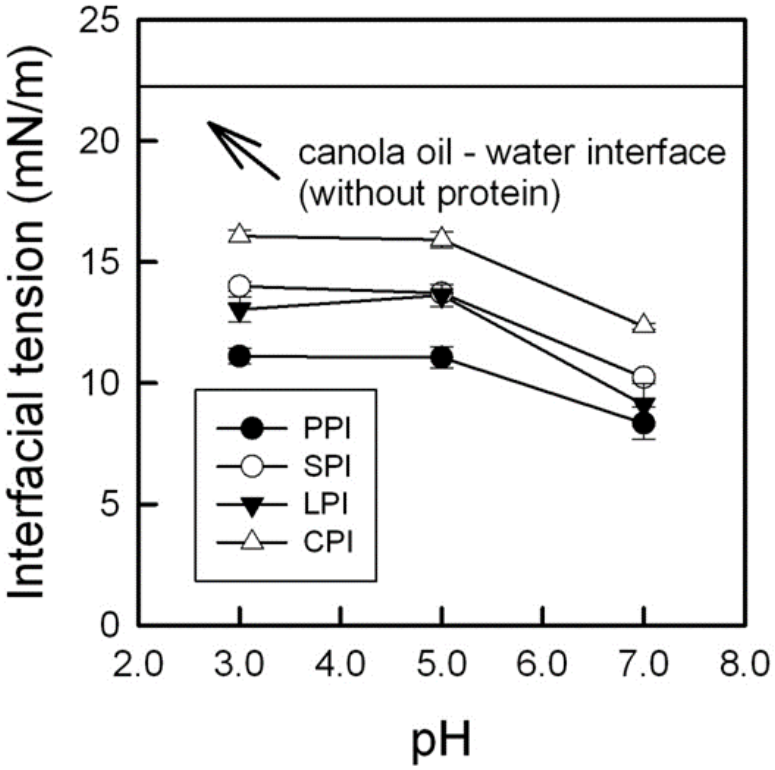


Figure 3.3. Interfacial tension (mN/m) for protein solutions as a function of pH and protein-type at a canola oil-water interface. Data represent the mean ± one standard deviation (n = 3). Abbreviations include: pea protein isolate (PPI), soy protein isolate (SPI), lentil protein isolate (LPI) and canola protein isolate (CPI).

the protein carries a higher charge and reduced hydrophobicity. However, it's important to note that reduced hydrophobicity does not mean any surface activity. No correlation was seen between solubility and interfacial tension (r = 0.260; p > 0.05) was found. It is hypothesized that interfacial tension is more related to an optimum balance between charge and hydrophobicity on the protein.

At pH 3.0, proteins have both high charge and hydrophobicity, and therefore were only moderately able to reduce interfacial tension driven most likely by the charge on the proteins. In contrast, at pH 5.0 charges were reduced to neutrality for the legume proteins (SPI, PPI and LPI) and to low levels for CPI, whereas hydrophobicity was also reduced to more moderate levels. In this case, interfacial tension was able to be reduced moderately driven most likely by the lower hydrophobicity on the proteins. At pH 7.0, proteins were more effective at reducing interfacial tension, since hydrophobicity was generally lower and charge was again high. In all cases, interfacial tension was lowered by both soluble and insoluble protein dispersed in the aqueous medium, in which the ratio of soluble-to-insoluble proteins would be pH dependent. For instance, at pH 5.0 the legume proteins were not very soluble [Figure 3.2 (C)], however they were still effective at reducing interfacial tension by the small amount of soluble protein and the insoluble protein that did not sediment yet in the time frame of the experiment. Lam and Nickerson (2014) also reported the interfacial tension of β-lactoglobulin at pH 3.0 (~17.8 mN/m) to be similar at pH 5.0 (~18.6 mN/m), even though they had large differences on the physicochemical properties.

Interfacial rheology is an important physical parameter related with the long-term stability of emulsions stabilized by proteins (Bos and Van Vliet, 2001). The dynamic interfacial storage modulus (G_i') and loss modulus (G_i'') of interfacial layers of SPI with time and frequency sweep as a function of pH are presented in Figure 3.4. All other proteins (PPI, LPI, and CPI) followed a similar trend except for differences in magnitude (not shown). For time sweep data, G_i' and G_i'' showed a slight upward and downward trend, respectively over time, suggesting that SPI at pH 3.0 and pH 5.0 [Figure 3.4 (A and C)] reached the interface relatively quickly, and formed a viscoelastic film as evidenced by $G_i' > G_i''$. At the interface, it is presumed that protein–protein interactions and the rearrangement of the protein's tertiary structure lead to the formation of an intermolecular network to keep G_i' and G_i'' at the equilibrium state (Ruiz-Henestrosa et al., 2008). However, for SPI at pH 7.0, $G_i' < G_i''$ indicated that no protein network was formed at the interface [Figure 3.4 (E)].

Once the protein isolates were adsorbed and attained the equilibrium states at the interface, the strength of the viscoelastic protein film at the interface was investigated as a

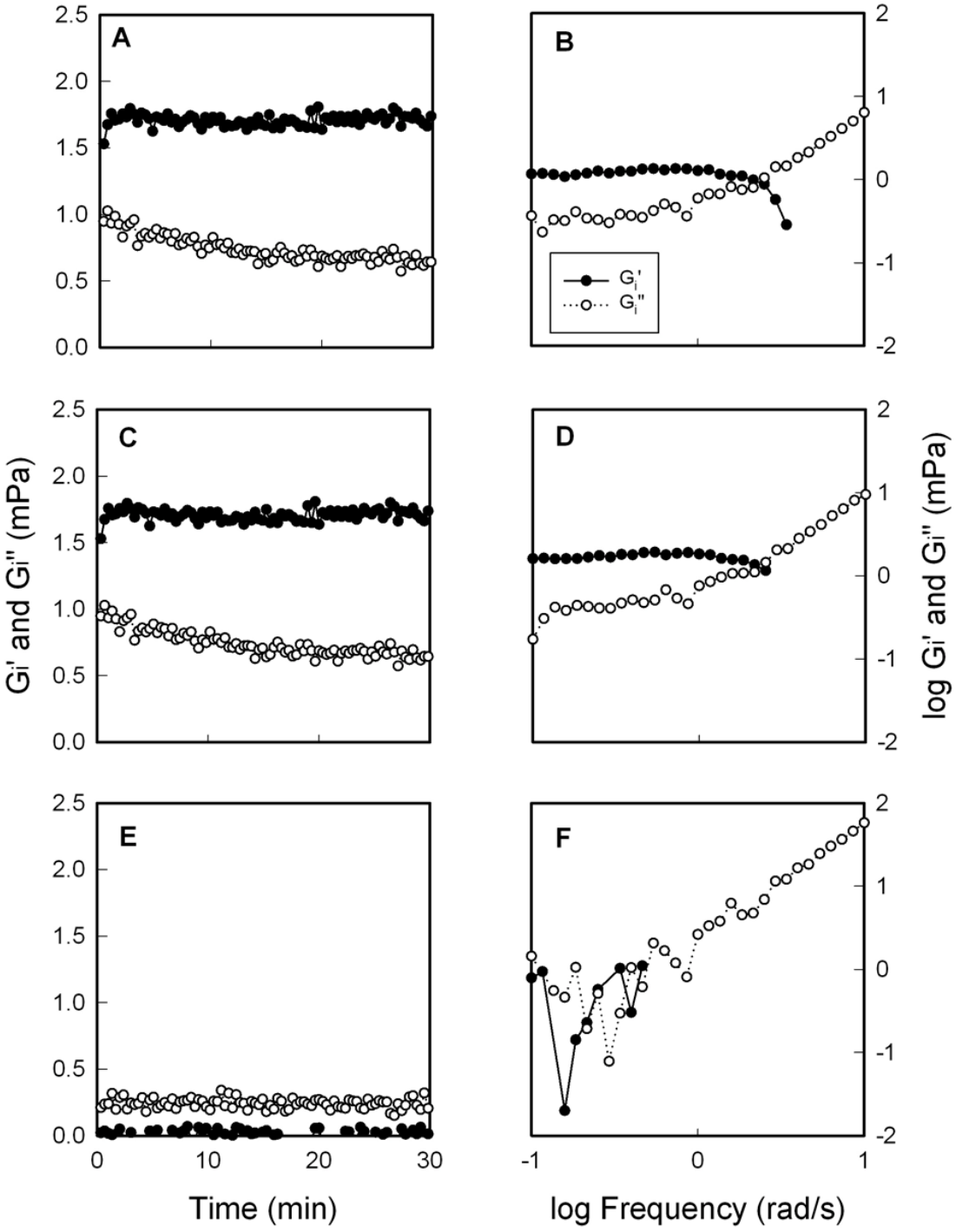


Figure 3.4. Dilatational storage modulus (G_i') and loss modulus (G_i'') for SPI solutions at pH 3.0 (A and B), pH 5.0 (C and D), and pH 7.0 (E and F) at the oil-water interface as a function of time (left) and frequency (right).

function of frequency [Figure 3.4 (B, D and F at pH 3.0, pH 5.0 and pH 7.0, respectively)]. G_i' and G_i'' for SPI at both pH 3.0 and pH 5.0 were relatively constant as a function of frequency, until a cross-over point was reached at ~2.24 rad/s, after which $G_i' < G_i''$ and the viscoelastic film began showing rubbery-like behavior where G_i'' increases and G_i' starts to decrease suddenly [Figure 3.4 (B and D)]. At pH 7.0, no film network was formed at the interface leading to fluid-like rheological behavior of the protein network at the interface ($G_i' < G_i''$) [Figure 3.4 (F)]. It is surmised that SPI at pH 3.0 and pH 5.0, PPI at pH 3.0, LPI at pH 3.0 and pH 5.0, and CPI at pH 3.0 and pH 5.0 (data were not shown) could form the viscoelastic films at the interface which is significant for the long-term stability of the emulsions, and the strength of the viscoelastic films as a function of pH and protein-type at 1 rad/s was evaluated in Table 3.1. An analysis of variance of complex modulus (G_i^*) data at a frequency of 1 rad/s indicated that pH ($p < 0.001$) and protein-type ($p < 0.001$), along with their interaction ($p < 0.05$) were all significant (Table 3.1). G_i^* expresses the energy involved at the interface through relaxation processes (Lucassen and van den Tempel, 1972; Seta et al., 2012). G_i^* of CPI at pH 3.0 and pH 5.0 was much larger than LPI, followed by PPI and SPI at both pHs, suggesting that CPI at pH 3.0 and pH 5.0 formed stronger viscoelastic films that may result in an emulsion with better long-term stability than the others (Table 3.1).

A simple Pearson correlation indicated that the interfacial tension was positively correlated with G_i^* ($r = 0.705$; $p < 0.001$) suggesting that the higher the interfacial tension value (or the least effective at reducing interfacial tension the protein was) the stronger and thicker the viscoelastic film will be. Findings suggest that despite the protein's ability to lower interfacial tension further at pH 7.0, an interconnected network was unable to form, possibly due to the lower surface hydrophobicity [Figure 3.2 (B)] which would stabilize protein–protein aggregation at the interface. In contrast, it is hypothesized that stronger interfacial films form at pH 3.0, since proteins experience a greater amount of protein–protein interactions as the hydrophobic forces are more abundant. The higher molecular interaction between adsorbed proteins at the interface could contribute to this result (Lucassen-Reynders et al., 1975), which was also demonstrated by the study of interfacial properties of β-casein and β-lactoglobulin (Seta et al., 2014).

Table 3.1. Effect of pH and protein-type on storage modulus (G_i'), loss modulus (G_i''), and complex modulus (G_i^*) (Units: milliPascal) of the O/W model system emulsion at 1 rad/s. Data represent the mean values of triplicate samples. Standard deviations were not reported since no differences were observed until after the second decimal position. Different letters (a ~ d) in the column indicate significant ($p < 0.05$) differences among protein solutions. Abbreviations include: pea protein isolate (PPI), soy protein isolate (SPI), lentil protein isolate (LPI), canola protein isolate (CPI), storage modulus (G_i'), loss modulus (G_i'') and complex modulus (G_i^*).

	pH 3.0			**pH 5.0**			**pH 7.0**		
	G_i' (mPa)	G_i'' (mPa)	G_i^* (mPa)	G_i' (mPa)	G_i'' (mPa)	G_i^* (mPa)	G_i' (mPa)	G_i'' (mPa)	G_i^* (mPa)
PPI	2.3	0.7	2.4^{ab}	-	0.3	0.4^{a}	-	0.3	0.2^{a}
SPI	1.3	0.6	1.4^{a}	1.8	0.8	2.0^{b}	-	0.3	0.3^{b}
LPI	2.5	0.9	2.7^{ab}	0.8	0.6	1.0^{c}	0.3	0.4	0.8^{c}
CPI	4.5	1.6	4.8^{b}	4.9	2.2	5.4^{d}	0	0.4	0.4^{d}

3.4.3. Emulsifying properties

Because of droplet flocculation and excess protein aggregation in the aqueous phase, the droplet size distributions for all emulsions showed multimodal size distributions, with each of them having one prominent peak (Figure 3.5). However, the magnitude and location of the peak varied based on the pH and protein-type. It is hypothesized that for the legume proteins (SPI, PPI and LPI) a shift in the distribution towards larger particles at pH 5.0 from pH 3.0 and pH 7.0 reflects protein–protein aggregation occurring due to reduced solubility. In the case of CPI, solubility continually declines as pH is raised from 3.0 to 7.0 resulting in a continuous pH-dependent shift within the size distribution. A similar multimodal size distribution was also previously reported in PPI stabilized emulsions at pH 3.0 – pH 9.0 (Liang and Tang, 2013), SPI and LPI stabilized emulsions at pH 7.0 (Can Karaca et al., 2011a), and CPI stabilized emulsions at pH 7.0 (Can Karaca et al., 2011b).

The average droplet diameter ($d_{3,2}$) for all protein-stabilized emulsions as a function of pH is shown in Figure 3.6. An analysis of variance indicated that pH and protein-type, along with their interaction ($p < 0.001$) were significant. Overall, oil droplets stabilized by PPI and LPI at pH 5.0 were significantly larger than those at pH 3.0 and pH 7.0 which were similar in magnitude. SPI behaved similarly, except that droplets were slightly larger at pH 7.0 than at pH 3.0 possibly. At pH 3.0, CPI and SPI produced similar size droplets (~5 μm) which were smaller than PPI and LPI stabilized oil droplets which were also similar in magnitude (~9 μm) (Figure 3.6). A Pearson correlation revealed the droplet size to be negatively correlated with surface charge (r = -0.740; $p < 0.001$), the strength of the interfacial film (G_i^*) (r = -0.323; $p < 0.05$), solubility (r = -0.817; $p < 0.001$) and hydrophobicity (r = -0.372; $p < 0.05$). Findings suggest that smaller sized droplets can be obtained using proteins that: a) are highly charged to facilitate movement to the oil–water interface to lower interfacial tension and to increase charge repulsion between droplets once integrated to the interface; b) are highly soluble to allow quicker diffusion to the interface and to afford greater conformational flexibility needed to rearrange at the interface; and c) have high hydrophobicity to produce stronger interfacial films.

Gravitational separation driven by density differences between oil and aqueous phases is one of the most common mechanisms for instability (McClements, 2007). Because of the significantly ($p < 0.001$) different protein solubility, the emulsions either separated into a 2- phase emulsion or destabilized. Figure 3.1 shows an example involving CPI only. It was observed that the emulsion with CPI at pH 3.0 separated into a cream layer (at the top) and a turbid serum layer (at the bottom) [Figure 3.1 (B)] after 24 h from time zero [Figure 3.1 (A)].

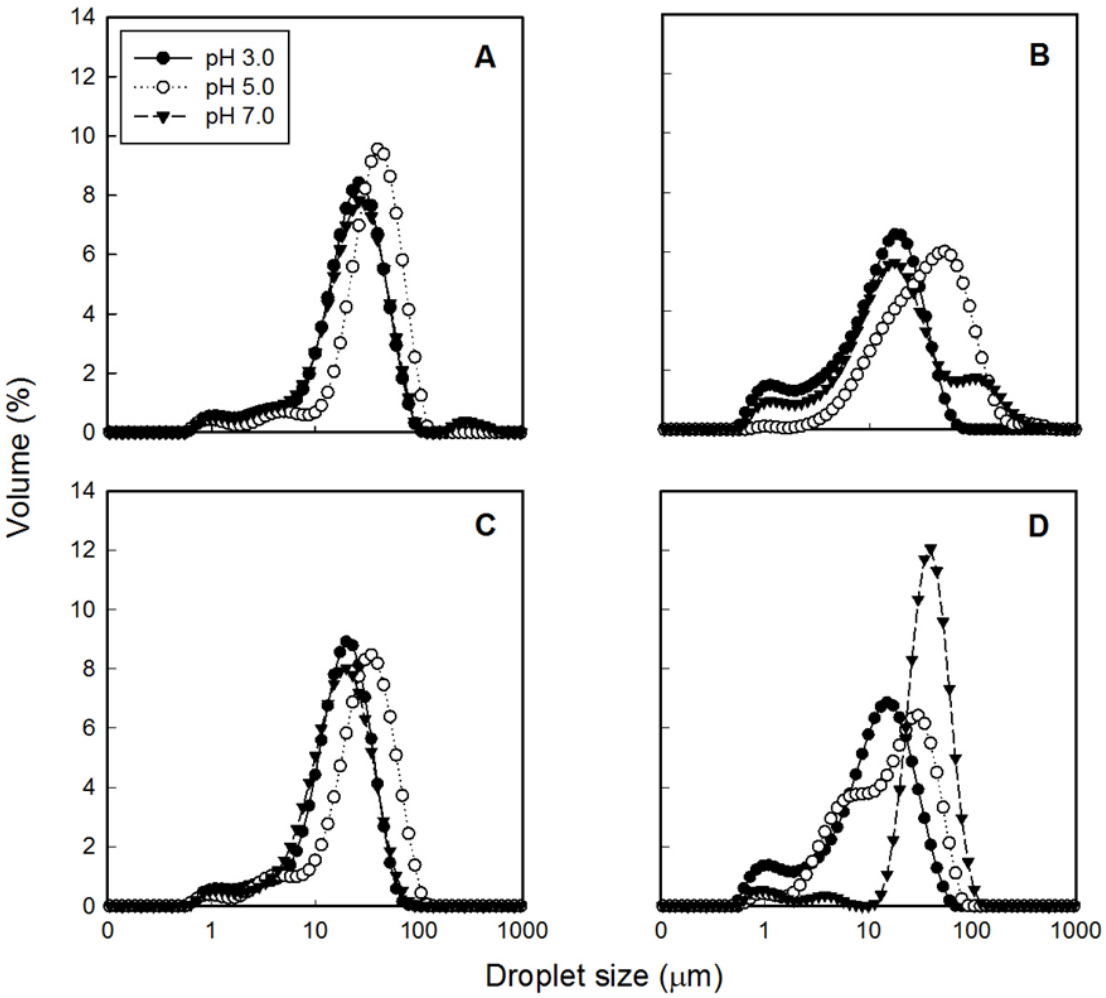


Figure 3.5. Droplet size distribution of PPI (A), SPI (B), LPI (C), and CPI (D) (2.0%, w/w) stabilized emulsions prepared at a 1:9 (w/w) oil-to-water ratio with canola oil.

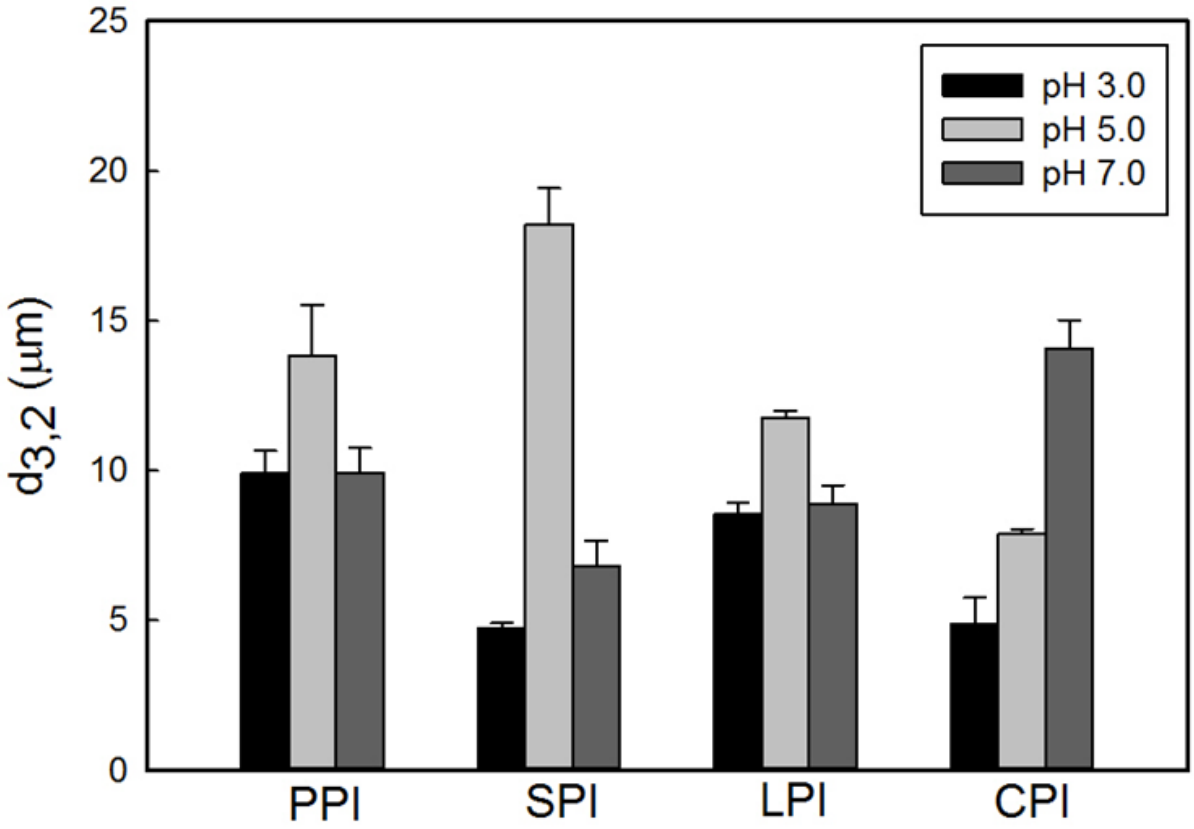


Figure 3.6. Mean droplet diameter ($d_{3,2}$, μm) of different proteins (2.0%, w/w) stabilized emulsion prepared at a 1:9 (w/w) canola oil-to-water ratio. Data represent the mean ± one standard deviation (n = 3). Abbreviations include: pea protein isolate (PPI), soy protein isolate (SPI), lentil protein isolate (LPI) and canola protein isolate (CPI).

The emulsions with PPI, SPI, and LPI at pH 3.0 and pH 7.0 separated in a similar manner. However, the emulsion prepared with CPI at pH 5.0 (not shown) and pH 7.0 [Figure 3.1 (C)] destabilized into a cloudy emulsion layer with oil–protein flocculates (at the top) and a clear serum layer (at the bottom). This destabilization occurred with emulsions prepared with PPI, SPI, and LPI at pH 5.0, which is close to the protein's isoelectric point.

Overall, ES was found to be similar in magnitude at pH 3.0 and pH 7.0 for PPI (~85% and ~87%, respectively), SPI (~90% and ~86%, respectively), and LPI (~87% and ~83%, respectively). However, all of them were found to be unstable at pH 5.0 which was close to the pI for the legume proteins. In contrast, ES for CPI at pH 3.0 was found to be ~85%, whereas the emulsions were unstable at both pH 5.0 and pH 7.0 which were closer to the isoelectric point (~pH 6.2) [Figure 3.2 (A)]. Despite differences seen in the physicochemical and interfacial properties at pH 3.0 and pH 7.0 for the legume proteins, emulsion stability over the 24 h time frame remained similar. It was surmised that this may be due to the high charge (negative or positive) on the protein's surface that coated the oil droplets. Most likely the neutral charge on the protein's surface at pH 5.0 resulted in flocculation of the oil droplets during the gravitational creaming experiment. Droplet size within range of ~5 to 9 μm did not seem to play a key role in altering stability, however the small reduction in stability for SPI from 90% to 86% may be the result of a slightly smaller droplet size at pH 3.0. In the case of CPI, the charge on the protein declined as pH was raised from pH 3.0 to pH 7.0, leading to droplet flocculation and instability at pH 5.0 and pH 7.0.

3.5. Conclusions

The generic consensus of how a protein stabilizes an emulsion involves its migration to the interface, where it then unravels and rearranges to position its hydrophobic moieties towards the apolar phase and its hydrophilic moieties towards the polar phase. An interfacial viscoelastic film then forms by protein–protein aggregation to coat the oil droplet and stabilize the emulsion via charge repulsion (at pHs away from the protein's pI) and/or steric forces. Findings from this study indicate that the ability for a protein (specifically isolates from pea, soy, lentil and canola) to initially associate with the oil–water interface during the initial stage of emulsion formation to lower the interfacial tension requires them to have a high surface charge and low hydrophobicity (e.g., pH 7.0 in the present study). It's important to note that low hydrophobicity does not imply that the protein is not surface active. However the properties of a protein to form a strong viscoelastic interfacial film are different, where proteins require a high surface charge and high hydrophobicity (e.g., pH 3.0 in the present

study). Therefore selection of an effective plant protein emulsifier really entails finding a balance between properties needed to associate at the oil–water interface with those needed to develop a strong interfacial film.

The most prudent way to find this balance is to consider the oil droplet size, where smaller sized droplets typically lead to improved stability. Findings from this study suggest that proteins should be: (i) highly charged so they can associate with the interface and to provide charge repulsion once the oil droplet is coated; (ii) have good solubility to allow for easier diffusion to the interface and conformational flexibility during the rearrangement step; and (iii) have moderately high hydrophobicity to produce stronger interfacial films. Although a long-term stability trial was not performed as part of this study, it is presumed that the stronger interfacial films formed at pH 3.0 than pH 7.0 would lead to more stable emulsion, which may find applications in protein-rich acidic beverages or similar type products. Of the protein-types studied, the most promising alternative to SPI as an emulsifier is LPI because it showed high charge, solubility and hydrophobicity at pH 3.0. The low solubility of pea protein at this pH could result in precipitation issues of the emulsifier for product developers within the continuous phase (although not impacting emulsion stability), whereas CPI would have issues lowering interfacial tension during emulsion formation and allergen concerns associated with its 2S protein.

3.6. Linkage

From the experiments reported in this manuscript, LPI at pH 3.0 was selected for further study as a promising emulsifier to stabilize an oil-in-water emulsion, because it has high surface charge, hydrophobicity, and good solubility to effectively lower the interfacial tension and produce a strong interfacial film to coat on the oil droplet. The focus of the second study of this research project was to develop LPI-based microcapsules to entrap canola oil using spray drying. The effect of LPI and oil concentrations on the emulsion (e.g., droplet size, viscosity, and emulsion stability) and microcapsule (e.g., surface oil and entrapment efficiency) properties were first investigated to determine an appropriate capsule design, which was further re-designed by using different wall materials (e.g., LPI, maltodextrin, lecithin, and sodium alginate) and preparation methods, in order to produce a best capsule formulation that offered good physical properties (e.g., moisture content, water activity, color, wettability, particle size) and protective nature against oxidation.

4. MICROENCAPSULATION OF CANOLA OIL BY LENTIL PROTEIN ISOLATE-BASED WALL MATERIALS[2]

4.1. Abstract

The overall goal was to encapsulate canola oil using a mixture of lentil protein isolate and maltodextrin with/without lecithin and/or sodium alginate by spray drying. Initially, emulsion and microcapsule properties as a function of oil (20%-30%), protein (2%-8%) and maltodextrin concentration (9.5%-18%) were characterized by emulsion stability, droplet size, viscosity, surface oil and entrapment efficiency. Microcapsules with 20% oil, 2% protein and 18% maltodextrin were shown to have the highest entrapment efficiency, and selected for further re-design using different preparation conditions and wall ingredients (lentil protein isolate, maltodextrin, lecithin and/or sodium alginate). The combination of lentil protein isolate, maltodextrin, and sodium alginate represented as the best wall material to produce microcapsules with the highest entrapment efficiency (~88%). The lentil protein isolate-maltodextrin-sodium alginate microcapsules showed better oxidative stability and had a stronger wall structure than the lentil protein isolate-maltodextrin microcapsules.

4.2. Introduction

Canola oil is rich in unsaturated fatty acids (e.g., oleic acid, linoleic acid and α-linolenic acid), which provide a variety of health benefits, including the reduction of cardiovascular disease, type 2 diabetes, and osteoporosis risk (Rajaram, 2014). However, the susceptibility of unsaturated fatty acids to oxidation represents a major challenge in its application, since lipid oxidation leads to the formation of free radicals and volatile compounds resulting in undesirable flavor in food products (Pegg, 2005). Microencapsulation is a process that helps circumvent this issue by offering protection to oils during food processing and storage, increasing their shelf-life, and transforming a liquid into a more easily handled and dispersed solid powder (Desai and Park, 2005).

[2.] Chang, C., Varankovich, N., & Nickerson, M. T. (2016). Microencapsulation of canola oil by lentil protein isolate-based wall materials. *Food Chemistry, 212,* 264-273.

Microencapsulation is defined as a process involving the coating of individual active particles or droplets within an edible wall material comprised of proteins, polysaccharides and/or lipids; to produce capsules in the micron to millimeter size range (Tyagi et al., 2011). Among the various microencapsulation techniques (e.g., spray drying, extrusion coating, complex coacervation, and liposome entrapment), the most commonly one applied is spray drying, due to its low cost and wide availability of equipment (Desai and Park, 2005). Wall material formulations and emulsification conditions (e.g., emulsion stability, droplet size, and emulsion viscosity) are the most important factors impacting the quality of spray dried microcapsules in terms of their entrapment efficiency, physicochemical properties and storage stability (Koc et al., 2015). Hogan et al. (2001) found the emulsions prepared by soya oil, sodium caseinate, and corn syrup solids had lower viscosity, which further produced the microcapsules with significantly higher entrapment efficiency, in comparison with the microcapsules prepared by maize starch. Can Karaca et al. (2013a) also demonstrated the emulsions prepared by flaxseed oil and legume proteins with larger droplet size resulted in the microcapsules with better oxidative stability and lower surface oil.

Wall materials act as barriers to protect the core material and to control diffusion, playing an essential role in producing stable microcapsules with high entrapment efficiency. They require to have good emulsifying properties, solubility, drying properties and proper rheological properties to be easily used in the spray dryer (Gharsallaoui et al., 2007). The most commonly studied wall materials for microencapsulation in the food industry are whey proteins, sodium caseinate, soy protein, gelatin, maltodextrin, starches and gum Arabic (Gharsallaoui et al., 2007; Koc et al., 2015). Hogan et al. (2001) stated that it was impossible to produce soya oil microcapsules only using sodium caseinate, and the addition of maize starch ideally increased entrapment efficiency. There is no single material providing all properties required for an ideal encapsulating agent, therefore the combination of proteins and polysaccharides as wall materials is commonly studied to offer enhanced entrapment efficiency.

Because of its low cost, good solubility, neutral aroma and taste, low viscosity at high concentrations and poor emulsifying capacity, maltodextrin (a hydrolysed starch) is desirable to be used in combination with other wall materials in the microencapsulation process as a processing aid (Madene et al., 2006). The degree of hydrolysis [dextrose equivalent (DE) of 5.0-20.0] of corn starch to produce maltodextrin exhibits significant effects on the microcapsules' characteristics (Dokic et al., 2004), in which microcapsules prepared by maltodextrin with lower DE value (e.g., DE of 9.0) had lower surface oil in

comparison with microcapsules containing maltodextrin with higher DE value (e.g., DE of 18.0), due to the formation of more hydrophilic microcapsule surface structure resulting from the higher molecular weight glucose oligomers (Can Karaca et al., 2013b). Lecithin, an ionic phospholipid, is widely used in the preparation of single-layered and bi-layered microcapsules (Carvalho et al., 2014), because of non-toxicity, well compatibility and nutritional effects (e.g., lowering the cholesterol level in the blood) (Wilson et al., 1998). The addition of lecithin in the production of microcapsules has been previously reported to improve microcapsules' properties, such as higher entrapment efficiency, better oxidative stability, and smaller particle size (Carvalho et al., 2014). Sodium alginate, which contains two monomeric units of β-D-mannuronic acid and α-L-guluronic acid, is a natural anionic polysaccharide extracted from brown algae (Liu et al., 2013). It is commonly used in the production of microcapsules to form the rigid wall matrix with multivalent cations to increase oxidative stability of encapsulated oils (e.g., olive oil) (Liu et al., 2013; Sun-Waterhouse et al., 2013). Very little information is available about the microencapsulation of canola oil using pulse proteins-based wall materials in the literature. Lentil protein isolate (LPI) is considered as a promising emerging protein used by the food industry, due to its nutritional value, low cost and functional properties (e.g., water holding capacity and oil binding capacity) (Boye et al., 2010a). Can Karaca and co-workers (2013a) designed a lentil protein-based wall material in combination with maltodextrin to entrap 10% flaxseed oil which is far too low to be commercially viable.

The objective of this study was to improve the oil concentration by developing a LPI-based wall material which provides the protective nature to against oxidation for the delivery of healthy oils (e.g., canola oil), beyond that of what Can Karaca et al. (2013a) could achieve (10% oil concentration).

4.3. Materials and methods

4.3.1. Materials

LPI and maltodextrin (MALTRIN M100, dextrose equivalent of 9.0-12.0) were kindly donated by POS Bio-Sciences (Saskatoon, SK, Canada) and Grain Processing Corporation (Muscatine, IA, USA), respectively. The crude protein content of LPI was determined to be 78.97% w.b. (%N × 6.25) as described by the Association of Official Analytical Chemists Method 920.87 (AOAC, 2003). Soy lecithin (L-alpha-Lecithin from soybean oil), canola oil, SA and all chemicals used in this study were purchased from Fisher Scientific (Ottawa, ON, Canada), a local supermarket, and Sigma-Aldrich (Oakville, ON,

Canada), respectively. A Millipore Milli-Q^{TM} water purification system (Millipore Corporation, Milford, MA, USA) was used to produce Milli-Q water.

4.3.2. Emulsion preparation

Phase one

The formulations of initial emulsions were prepared with different oil, LPI and maltodextrin concentrations [Table 4.1 (a)]. LPI was first dispersed in Milli-Q water at the specified concentration (corrected for protein level within the powder) and adjusted to pH 3.0 with 2.0 M HCl or 2.0 M NaOH, followed by stirring at 500 rpm for overnight at 4 °C to ensure complete dispersion. pH of the LPI solutions was re-adjusted to 3.0 prior to sample homogenization. In a preliminary experiment, the LPI concentration in the emulsion was restricted <10% (w/w), since at levels ≥10% (w/w), LPI solutions were too viscous to be used for pH adjustment and emulsion preparation (data not shown). A pH 3.0 protein solution was used based on work by Chang et al. (2015). Maltodextrin was then dissolved into LPI solution at levels outlined in Table 4.1 (a) and stirred at 500 rpm for 3 h at room temperature (22-23 °C). Oil-in-water emulsions were prepared by homogenizing varying amounts of oil (20% vs 30% oil concentration), maltodextrin, and LPI solutions using a Polytron PT 2100 Homogenizer (Kinematica AG, Lucerne, Switzerland) equipped with a 12 mm PT-DA 2112/2EC generating probe at 15,000 rpm for 5 min at room temperature [Table 4.1 (a)].

Phase two

Stemming from the results in phase one, a wall formulation of 2% LPI and 18% maltodextrin with 20% oil concentration was selected as the base formulation (*See Results and Discussion*) for further reformulation using different homogenization conditions and additional ingredients (lecithin, and/or sodium alginate) in wall material. LPI solutions were prepared in the same manner as described above. A soy lecithin solution was prepared by dissolving it in Milli-Q water and adjusting to pH 3.0 (at which the lecithin has better dissociation behavior, because the phosphate groups on the lecithin have a pK_a value of ~1.5) (Chuah et al., 2009) with 1.0 M HCl or 1.0 M NaOH, followed by stirring at 500 rpm for overnight at 4 °C. In a preliminary experiment, the soy lecithin concentration in the emulsion was restricted ≤3.0% (w/w), since at levels >3.0% (w/w), the soy lecithin cannot be completely solubilized after stirring overnight, and the solution was too thick to be used for emulsion preparation. pHs of the LPI and the lecithin solutions were re-adjusted to 3.0 prior to sample homogenization. Sodium alginate and maltodextrin were separately dissolved in

Table 4.1. Formulations used for developing the initial emulsions to deliver 20% and 30% oil within microcapsules (phase one) (a), and with different wall materials (b) and emulsion preparation conditions [all solutions were mixed with the emulsions for 10 min at 500 rpm, and then homogenized at 15,000 rpm under room temperature (21-23 °C)] (c) to deliver 20% oil within microcapsules (phase two). Abbreviations include: lentil protein isolate (LPI), oil concentration in final microcapsules (OC), maltodextrin (MD), and total solids (TS), lecithin (L), and sodium alginate (SA).

a. Formulations of initial emulsions to deliver 20% and 30% oil in the spray-dried microcapsules (phase one).

Formulation		**Initial emulsions (%, w/w)**					**Spray-dried microcapsules (%, w/w)**			
		Oil	**LPI**	**MD**	**TS**	**Core : Wall**	**Oil**	**LPI**	**MD**	**TS**
20% OC	2% LPI	5	2	18	25	1:4	20	8	72	100
	4% LPI	5	4	16	25	1:4	20	16	64	100
	6% LPI	5	6	14	25	1:4	20	24	56	100
	8% LPI	5	8	12	25	1:4	20	32	48	100
30% OC	2% LPI	7.5	2	15.5	25	1:2.3	30	8	62	100
	4% LPI	7.5	4	13.5	25	1:2.3	30	16	54	100
	6% LPI	7.5	6	11.5	25	1:2.3	30	24	46	100
	8% LPI	7.5	8	9.5	25	1:2.3	30	32	38	100

Table 4.1. (continued).

b. Formulations of initial emulsions with different wall materials to deliver 20% oil in the spray-dried microcapsules (phase two).

Strategy	Formulation	Before Spray Drying (%, w/w)							After Spray Drying (%, w/w)					
		Oil	LPI	MD	L	SA	TS	Core:Wall	Oil	LPI	MD	L	SA	TS
A	LPI-MD	5	2	18	-	-	25	1:4	20	8	72	-	-	100
B	LPI-MD	5	2	18	-	-	25	1:4	20	8	72	-	-	100
C	LPI-MD	5	2	18	-	-	25	1:4	20	8	72	-	-	100
D	LPI-MD-L	5	2	15	3	-	25	1:4	20	8	60	12	-	100
E	LPI-MD-L	5	2	17	1	-	25	1:4	20	8	68	4	-	100
F	LPI-MD-SA	5	2	17	-	1	25	1:4	20	8	68	-	4	100
G	LPI-MD-L-SA	5	2	14	3	1	25	1:4	20	8	56	12	4	100
H	LPI-MD-L-SA	5	2	16	1	1	25	1:4	20	8	64	4	4	100

Table 4.1. (continued).

c. Emulsion preparation conditions to deliver 20% oil in microcapsules (phase two).

Strategy	Formulation	Approach			
		Primary emulsion	**Secondary emulsion**	**Tertiary emulsion**	**Quaternary emulsion**
A	LPI-MD[a]	Dissolve MD in LPI solution and stir for 3 h; then homogenize with the oil for 5 min	-	-	-
B	LPI-MD[a]	Homogenize LPI solution with the oil for 5 min	Homogenize MD solution with the primary emulsion for 3 min	-	-
C	LPI-MD[a]	Homogenize LPI solution with the oil for 5 min and stir for 3 h at 4 °C	Homogenize MD solution with the primary emulsion for 3 min	-	-
D	LPI-MD-L[b]	Homogenize L solution with the oil for 3 min	Homogenize LPI solution with the primary emulsion for 5 min	Homogenize MD solution with the secondary emulsion for 3 min	-
E	LPI-MD-L[c]	Prepared as described in strategy D (*but at a different L concentration*)			
F	LPI-MD-SA[d]	Homogenize LPI solution with the oil for 5 min	Homogenize SA solution with the primary emulsion for 3 min	Homogenize MD solution with secondary emulsion for 3 min	-
G	LPI-MD-L-SA[e]	Homogenize L solution with the oil for 3 min	Homogenize LPI solution with the primary emulsion for 5 min	Homogenize SA solution with the secondary emulsion for 3 min	Homogenize MD solution with the tertiary emulsion for 3 min
H	LPI-MD-L-SA[f]	Prepared as described in strategy G (*but at a different L concentration*)			

[a]. The initial emulsions contain 5% canola oil, 2% LPI, and 18% maltodextrin.
[b]. The initial emulsion contains 5% canola oil, 2% LPI, 15% maltodextrin, and 3% lecithin.
[c]. The initial emulsion contains 5% canola oil, 2% LPI, 17% maltodextrin, and 1% lecithin.
[d]. The initial emulsion contains 5% canola oil, 2% LPI, 17% maltodextrin, and 1% sodium alginate.
[e]. The initial emulsion contains 5% canola oil, 2% LPI, 14% maltodextrin, 3% lecithin, and 1% sodium alginate.
[f]. The initial emulsion contains 5% canola oil, 2% LPI, 16% maltodextrin, 1% lecithin, and 1% sodium alginate.

Milli-Q water and stirring at 500 rpm for 3 h at room temperature. The initial oil-in-water emulsions with 20% (w/w) oil concentration and different wall material components [Table 4.1 (b)] were prepared as described in Table 4.1 (c) using the Polytron PT 2100 Homogenizer.

4.3.3. Emulsion characteristics

Emulsion stability

Emulsion stability (ES) was measured as described by Liu et al. (2010a) with minor modification. In brief, freshly prepared emulsions (10 mL) were filled into a 10 mL sealed graduated glass cylinders (inner diameter = 10.5 mm, height = 160 mm), and then stored for 24 h at room temperature. During storage, the emulsions were separated into a cream upper layer and a serum bottom layer. The visual observation was done after 24 h of storage. Emulsion stability was measured as ES (%) and expressed as:

$$ES\ (\%) = H_S / H_E \times 100 \qquad [4.1.]$$

where H_S is the height of the serum layer, and H_E is the height of the emulsion, as measured using a digital micrometer (Model 62379-531, Control Company, USA) having a precision of 0.01 mm. All data was reported as the mean ± one standard deviation from triplicate emulsion preparations (n = 3).

Emulsion droplet size

Droplet size of freshly prepared emulsions was measured using a Mastersizer 2000 laser light scattering instrument (Malvern Instruments Ltd., Worcestershire, UK) equipped with a Hydro 2000S sample handling unit as described by Can Karaca et al. (2013a) with minor modification. Droplet size measurements were taken immediately after the production of emulsions and obscuration in all measurements was performed at ~14% by sample addition. According to Mie Theory, droplet size was calculated by using the refractive index difference between droplets and dispersing medium to predict scattering light intensity. The ratio of refractive index of canola oil (1.470) to Milli-Q water (1.330) was 1.105. Droplet size was presented as volume-mass mean diameter ($d_{4,3}$) that is expressed as:

$$d_{4,3} = \frac{\sum_{i=1} n_i \cdot d_i^4}{\sum_{i=1} n_i \cdot d_i^3} \quad [4.2.]$$

where n_i is the number of droplets of diameter (d_i) (McClements, 2005). All data was reported as the mean ± one standard deviation from triplicate emulsion preparations (n = 3).

Emulsion viscosity

The viscosity of emulsions as a function of shear rate (1-200 s^{-1}) was evaluated using a rheometer (AR-G2 Rheometer, TA Instruments Ltd., New Castle, DE, USA) equipped with a geometry (40 mm cone diameter, 1° cone angle) at room temperature, in order to determine if the emulsions were able to be fed into the benchtop spray dryer (B-290, Buchi Labortechnik AG, Flawil, Switzerland), which requires the viscosity of sample to be lower than 0.3 Pa s. The viscosity of emulsions was determined using shear stress divided by shear rate (at 50 s^{-1}). All data was reported as the mean ± one standard deviation from triplicate emulsion preparations (n = 3).

4.3.4. Spray drying

The emulsions were spray dried using a benchtop Buchi Advanced Mini Spray Drier B-290 (Buchi Labortechnik AG, Flawil, Switzerland) equipped with an atomizing nozzle (0.7 mm diameter). The emulsions were fed into the primary chamber (65 × 110 × 70 cm) through a peristaltic pump under constant mechanically stirring at 300 rpm to maintain homogeneity and prevent droplet coalescence. The drying air flow rate was 35,000 L/h, and the compressed air pressure was adjusted to 5 bars. The inlet temperature was set up to 180 °C, and the outlet temperature was kept at 85 ± 1 °C by adjusting pump rate (5-20%). Finally, the formulation of spray-dried microcapsules was shown in Table 4.1. The production of microcapsules by spray drying was performed in triplicate.

4.3.5. Microcapsule properties

Physical characteristics

The moisture content of microcapsules was determined gravimetrically after drying the microcapsules in a gravity convention oven (APTLine ED, Binder GmbH, Tuttlingen, Germany) at 105 °C for overnight. The water activity (a_w) of microcapsules was measured using an AquaLab 4TE water activity meter (Decagon Devices, Inc., Pullman, WA, USA) with a 0.001 sensitivity at 22 °C. The colour of microcapsules was measured using a Hunter

Colorimeter (ColorFlex EZ 45/0, Hunter Associates Laboratory, Inc., Reston, VA, USA) and reported in term of the *L* (lightness), *a* (redness), and *b* (yellowness) color system. Moisture, water activity and colour were reported as the mean ± one standard deviation from triplicate capsule preparations (n = 3).

Wettability of microcapsules was measured using the method described by Balmaceda et al. (1976). In brief, ~1 g of microcapsules was transferred into 80 mL Milli-Q water through a small sieve. The ability for microcapsules to dissolve into water was observed for 30 min at room temperature without mechanical stirring, followed by the stirring at 500 rpm for 1 min. Finally, the wettability of microcapsules was graded as excellent (e.g., the sample immediately wets as it contacts the water, followed by complete dispersion after 30 min), good (e.g., the sample slightly wets as it contacts the water, followed by partial dispersion and sedimentation at the bottom after 30 min), fair (e.g., the sample is very slightly wet and tends to clump at the surface of water, however, after 30 min, a small amount of sample is still on the surface), and poor (e.g., the sample hardly wets and clumps when it contacts with water; most of the sample remains on the surface of water after 30 min), based on the performance of microcapsules dissolving into water.

Particle size of microcapsules was measured using Microtrac II Particle Size Analyzer (Models 7997-10, Leeds & Northrup). According to the instrument manual, ~1 g of microcapsules was diluted into Milli-Q water until the laser attenuation was within the range from 0.8 to 0.85. Particle size was presented as volume-mass mean diameter ($d_{4,3}$). Data was reported as the mean ± one standard deviation from triplicate capsule preparations (n = 3).

Surface oil and entrapment efficiency

One gram of microcapsules was weighted in a 50 mL centrifuge tube and 15 mL of hexane was added and shaken for 15 s to extract surface oil. The solvent was then filtered twice through #3 Whatman filter paper (Whatman International Ltd., Maidstone, UK), collected the clear organic solvent in a 30 mL beaker, and evaporated in a fume hood for overnight. Finally, the residual hexane was completely removed by heating in a gravity convection oven (APTLine ED, Binder GmbH, Tuttlingen, Germany) at 105 °C for 30 min. The surface oil of microcapsules was determined gravimetrically. The entrapment efficiency (EE) was calculated by the following formula (Anwar and Kunz, 2011):

$$EE = (\text{Total oil} - \text{Surface oil}) / \text{Total oil} \times 100\% \quad [4.3.]$$

where total oil is the oil payload (20% or 30%, w/w) in the microcapsules. Data was reported as the mean ± one standard deviation from triplicate capsule preparations (n = 3).

4.3.6. Oxidative stability

Oil extraction

Oxidative stability of encapsulated canola oil in microcapsules and free canola oil (control sample) was determined by measuring peroxide value (PV) and 2-thiobarbituric acid reactive substances (TBARS) as a function of storage time over 30 d for triplicate capsule preparations. After spray drying, the microcapsules (~ 4 g) along with free canola oil (~ 2 g) were stored in unflushed sealed amber glass bottles (10 mL) at room temperature. The extraction of encapsulated oils was performed prior to PV and TBARS tests as described by Can Karaca et al. (2013a, b) and Klinkesorn et al. (2005) with some modifications. In brief, microcapsules (~ 4 g) were dissolved in Milli-Q water (30 mL) and stirring at 500 rpm for 5 min, followed by the addition of hexane/isopropanol (3:1, v/v) mixture (40 mL) and stirring for 15 min to extract the oil. The resulting mixture (in a 250 mL centrifuge tube) was then centrifuged (Sorvall RC Plus Superspeed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) at 4,193 × *g* for 10 min at 20 °C. The clear organic solvent was syringed out and filtered through #1 Whatman filter paper (Whatman International Ltd., Maidstone, England) with anhydrous Na_2SO_4 into a 125 mL Erlenmeyer flask. Afterwards, the organic solvent was dried under a stream of nitrogen in the fume hood. PV and TBARS tests were carried out immediately after oil extraction on every 5 d of storage over a 30 d period.

Peroxide value (PV)

In brief, the extracted and free canola oil (~0.2 g) were mixed with 30 mL of acetic acid/chloroform solution (3:2, v/v) in a 125 mL Erlenmeyer flask, followed by the addition of saturated potassium iodide (KI, 0.5 mL). The solution was left to stand exactly for 1 min with occasional shaking to release iodine from chloroform layer, followed by the addition of Milli-Q water (30 mL) to stop the reaction. Afterwards, 1% (w/v) starch indicator (0.5 mL) prepared by corn starch was applied into the mixture. Finally, the resulting solution was titrated with 0.001 N sodium thiosulfate ($Na_2S_2O_3$) until the violet color derived from the iodine disappeared. A blank sample as a control was carried out through all the steps. PV of encapsulated and free canola oils was calculated using the following formula (Pegg, 2005):

$$PV = (S - B) \times N \times 1000 / W \quad [4.4.]$$

where S is the volume (mL) of $Na_2S_2O_3$ solution used to titrate the encapsulated and free oils, B is the volume (mL) of $Na_2S_2O_3$ solution used to titrate the blank (without oils), N is the normality of $Na_2S_2O_3$ solution, and W is the oil weight (g). Data was reported as the mean ± one standard deviation from triplicate capsule preparations (n = 3).

2-Thiobarbituric acid reactive substances (TBARS)

TBARS test was performed based on the reaction between malondialdehyde (MDA, a compound that results from the decomposition of peroxides) and 2-thiobarbituric acid (TBA). Specifically, the extracted and free canola oils (~ 40 mg) were first mixed with 2-butanol in the 10 mL volumetric flasks. 50 μL of 8.1% (w/v) sodium dodecyl sulfate (SDS), 375 μL of 20% acetic acid (pH 3.5), 375 μL of 0.8% (w/v) TBA, 8.25 μL of 0.02% (w/v) butylated hydroxytoluene (BHT) (in dimethyl sulfoxide (DMSO)), and 200 μL of the oil-butanol mixture were added into a 2.0 mL Eppendorf tube. MDA standards (200 μL) were prepared by diluting 100 μM of 1,1,3,3-tetraethoxypropane (TEP) (in 1% (v/v) sulfuric acid) in a 2-butanol at a concentration range of 1.25-50.00 μM under the same experimental conditions as the oils. The blank was prepared by applying 200 μL of 2-butanol under the same experimental conditions as the oils. Afterwards, oil samples, MDA standards, and the blank were heated at 95 °C for 1 h, and then cooled down in the cold water for 5 min, followed by the addition of 2-butanol/pyridine (15:1, v/v) mixture (0.9 mL) with vigorously mixing for 30 s using an analog vortex mixer at speed of 3 (VWR Vortexer Mini, USA). Then, oil samples, MDA standards, and the blank were centrifuged (Eppendorf Centrifuge 5424, Hamburg, Germany) at 4,000 × *g* for 10 min. The absorbance of the upper organic solvent at 532 nm was measured against a 2-butanol blank using a spectrophotometer (Genesys 10uv, Thermo Fisher Scientific). Finally, a standard curve was plotted using the net absorbance of MDA standards (subtracting the absorbance value of the blank from the MDA standards) versus the MDA concentration to get the equation of the trend line. TBA values of samples were expressed as MDA content (nmol)/sample oil weight (mg), in which MDA concentration of samples was calculated by using the equation of the trend line on the standard curve and net absorbance value of oil samples (subtracting the absorbance value of blank from the sample) (Akhlaghi and Bandy, 2010; Pegg, 2005). Data was reported as the mean ± one standard deviation from triplicate capsule preparations (n = 3).

4.3.*7. Emulsion and microcapsule morphology*

The morphology of fluorescently-labeled emulsion droplets stabilized by (a) LPI, (b) LPI-MD, (c) LPI-SA and (d) LPI-MD-SA solutions were examined using a Nikon C2 Confocal Laser Scanning Microscope (CLSM) (Nikon, Tokyo, Japan). In brief, 0.1% (w/w, basis on labeling materials) of Nile Red (staining the oil) and Fast Green FCF (staining the LPI) were dissolved in the canola oil and LPI solution, respectively, and stirred at 500 rpm for 10 min in the dark. Maltodextrin and sodium alginate could not be visualized since the oligo- and polysaccharides were below the size resolution of the microscope. Emulsions were then prepared as described in Section 4.3.2. [Strategies B and F, in Tables 4.1 (b and c), with and without sodium alginate]. Fluorescently-labeled emulsions were observed using CLSM with two lasers at the excitation and emission wavelengths for Nile Red (excitation λ = 530 nm; emission λ = 635 nm) and Fast Green FCF (excitation λ = 633 nm; emission λ = 740 nm).

Surface morphology of spray dried (a) LPI, (b) LPI-MD, (c) LPI-SA, and (d) LPI-MD-SA microcapsules was taken using a scanning electron microscope (SEM) (JSM-840A, JEOL, Japan) operated at 10 kV with a sample chamber pressure of 5.0×10^{-6} Torr. The microcapsules were coated with approximately 200 angstroms of gold (Edwards S-150B Plasma Sputter Coater) in order to make samples conductive, and observed at 8000 × magnification with a working distance of 15 mm.

4.3.8. Statistics

All experiments were performed on triplicate batches of capsules and emulsions (except particle size and wettability tests, which were performed in duplicate), and reported as the mean ± one standard deviation. A two-way analysis of variance (ANOVA) and Tukey Test were used to measure statistical difference in the emulsion properties (e.g., emulsion stability, emulsion droplet size, and emulsion viscosity), surface oil and entrapment efficiency of the microcapsules prepared in phase one as a function of LPI concentration (2-8%) and oil content (20% vs. 30%).

A one-way ANOVA was used to analyze statistical differences in the emulsion properties, surface oil and entrapment efficiency of the microcapsules prepared in phase two, and physical properties (e.g., moisture content, water activity, color, wettability, and particle size) and oxidative stability (e.g., PV and TBARS) of selected microcapsules. All statistics were performed using Systat v10 software (San Jose, CA, USA).

4.4. Results and discussion

4.4.1. Encapsulation of oil using a LPI-MD wall material (phase one)

The emulsion stability, droplet size, and viscosity of emulsions prepared with different LPI concentrations and oil contents are shown in Table 4.2 (a). In terms of emulsion stability, emulsions prepared with a 20% oil were slightly more stable than those with a 30% oil ($p < 0.05$). For both oil concentrations, a slight increasing trend was observed as LPI concentration was increased ($p < 0.05$), where at the 8% LPI level no separation occurred. Overall droplet size was found to be greater at the 30% oil content (~18.1 µm diameter) relative to the 20% oil content (~16.8 µm diameter) ($p < 0.05$), however the effect was different depending on the LPI concentration. At the 20% oil level, all droplets were of similar size ($p > 0.05$). However, at the 30% oil level, droplets were all similar in size for 2% and 4% LPI concentrations, whereas droplets were slightly smaller in magnitude for 6% and 8% LPI concentrations ($p < 0.05$) [Table 4.2 (a)]. In terms of viscosity, the effect of payload was not significant ($p > 0.05$), however a rise in viscosity was observed as the LPI concentration increased from 2% LPI (~6.5 mPa s) to 8% LPI (~11.4 mPa s) ($p < 0.05$). Overall, better stability of the formed emulsions is thought to be attributed to slightly higher viscosity of the continuous phase, and slightly smaller droplets at the higher LPI concentrations.

Effects of oil content and LPI concentration on the surface oil and entrapment efficiency are shown in Table 4.2 (b). Overall, the surface oil content was found greater with 30% oil (~12%) relative to the 20% oil (~7%) ($p < 0.05$), and was found to increase with increasing LPI concentration ($p < 0.05$). Surface oil was the lowest (~5.4%) at the 2% LPI concentration with a 20% oil content. Overall, entrapment efficiency was found greater with the 20% oil (~65%) relative to the higher oil concentration (~60%) ($p < 0.05$), and decreased with increasing LPI concentration at each oil content ($p < 0.05$). Only at the 8% LPI concentration, the entrapment efficiency was similar in magnitude for both payloads (~56%) ($p > 0.05$). The highest entrapment efficiency (~73%) was found in the microcapsules with 2% LPI concentration and 20% oil content.

Gharsallaoui and co-workers (2007) indicated wall materials with lower emulsion viscosity display better coating properties with higher entrapment efficiency. Rosenberg et al. (1990) reported that higher viscosity of emulsions can cause the atomization step during spray drying to be prolonged, which adversely affects the drying rate of the powder to give higher surface oil on the dry powder. It is proposed the decline in entrapment efficiency with increased payload may be due to: a) the lack of sufficient wall material to form a tightly

Table 4.2. Effect of oil and lentil protein isolate concentrations on the properties of the initial emulsion and the spray-dried microcapsules. Data represent the mean ± one standard deviation (n = 3). Different small letters in the same column indicate a significant difference, as well as different capital letters in the same line ($p \leq 0.05$). Abbreviations include: lentil protein isolate (LPI), oil concentration in the final microcapsule (OC), and no separation due to evident creaming (NS).

a. Emulsion characteristics of initial emulsions to deliver 20% and 30% oil in the spray-dried microcapsules (phase one)

LPI (%)	Emulsion stability (%)		Droplet size(μm)		Viscosity (mPa s)	
	20% OC	30% OC	20% OC	30% OC	20% OC	30% OC
2.0	92.8 ± 0.7^{aA}	87.6 ± 0.1^{aB}	17.1 ± 0.7^{aA}	18.6 ± 0.4^{aB}	6.6 ± 1.6^{aA}	6.4 ± 1.0^{aA}
4.0	92.9 ± 0.7^{aA}	89.4 ± 0.2^{bB}	16.8 ± 0.4^{aA}	18.7 ± 0.0^{aB}	7.4 ± 1.1^{abA}	6.9 ± 0.9^{aA}
6.0	94.3 ± 1.3^{bA}	90.4 ± 0.3^{bB}	16.5 ± 0.3^{aA}	17.8 ± 0.2^{bB}	8.8 ± 0.3^{bA}	9.5 ± 0.7^{bA}
8.0	NS	NS	16.8 ± 0.2^{aA}	17.3 ± 0.4^{bA}	10.9 ± 0.6^{cA}	11.9 ± 0.8^{cA}

b. Physical properties of spray-dried microcapsules to deliver 20% and 30% oil (phase one)

LPI (%)	Surface oil (%)		Entrapment efficiency (%)	
	20% OC	30% OC	20% OC	30% OC
2.0	5.4 ± 0.2^{aA}	10.4 ± 0.4^{aB}	73.0 ± 0.9^{aA}	65.2 ± 1.4^{aB}
4.0	6.9 ± 0.2^{bA}	11.6 ± 0.3^{bB}	65.5 ± 1.1^{bA}	61.5 ± 1.1^{bB}
6.0	7.2 ± 0.1^{bA}	12.3 ± 0.1^{cB}	63.9 ± 0.6^{bA}	58.9 ± 0.3^{cB}
8.0	8.8 ± 0.1^{cA}	13.7 ± 0.5^{dB}	55.8 ± 0.5^{cA}	56.1 ± 1.7^{dA}

packed wall structure around the oil droplets (Polavarapu et al., 2011); and b) the entrapment procedure itself. During the formulation, LPI and maltodextrin were mixed together prior to homogenization to form the wall material, however Sansone et al. (2011) demonstrated that maltodextrin is capable to break the structural integrity to cause agglomeration in wall matrix while studying pectin-maltodextrin wall materials for entrapping functional extracts from *Fadogia ancylantha*, *Melissa officinalis*, and *Tussilago farfara*. As such, a more complex wall material design may be necessary to avoid damage caused by maltodextrin on the wall integrity for plant protein based capsules. The food industry aims to produce microcapsules with < 2% surface oil and > 98% entrapment efficiency (Drusch and Berg, 2008), so, the microcapsules with 20% oil and 2% LPI were carried forward with the aim of significantly improving their properties.

4.4.2. Encapsulation of oil using re-formulated LPI-MD-based wall materials (phase two)

Changes in the emulsion stability, droplet size and viscosity as a function of new wall formulations and preparation conditions as described in Tables 4.1 (b and c) are given in Table 4.3. Emulsions prepared with LPI and maltodextrin [Strategies A-C, Table 4.1 (b and c)] had relatively similar emulsion stability (~92%), droplet size (~17 µm) and viscosity (~7 mPa s), resulting in similar surface oil (~5.3%) and entrapment efficiencies (~73%) for the final spray dried microcapsules ($p > 0.05$). The addition of lecithin (L) [Strategy D, Tables 4.1 (b and c)] at the 3% level to the LPI and maltodextrin mixture led to a decrease in emulsion stability from ~92% to ~40%, an increase in droplet size from ~17 µm to ~84 µm, and an increase in viscosity from ~7 mPa s to ~17 mPa s ($p < 0.05$). Although changes in emulsion characteristics were found, this did not translate into different surface oil or entrapment efficiencies compared with LPI-MD ($p > 0.05$). A reduction in lecithin content from 3% to 1% [Strategy E, Tables 4.1 (b and c)] was found to follow a similar trend in the emulsion properties relative to LPI-MD [Strategies A-C, Tables 4.1 (b and c)], however to a lesser magnitude. Surface oil of the LPI-MD-L (1%) was found to increase from ~5.5% to ~8.5% relative to LPI-MD-L (3%) and entrapment efficiency reduced from ~72% to ~58% ($p < 0.05$). The presence of lecithin (a phospholipid molecule) is hypothesized to disrupt the LPI-stabilized oil-water interface by outcompeting with the LPI molecules. As such, emulsions became less stable, had greater coalescence occurring (as evident by larger droplets) which resulted in greater emulsion viscosities. The end result was that surface oil and entrapment efficiency became more and less, respectively. When lecithin was present at 3% level, changes were not seen in surface oil and entrapment efficiency value in comparison

Table 4.3. Effect of wall materials on the properties of initial emulsion and the microencapsulated powder after spray drying. Data represent the mean ± one standard deviation (n = 3). Different small letters in the same column indicate a significant difference ($p \leq 0.05$). Abbreviations include: lentil protein isolate (LPI), maltodextrin (MD), lecithin (L), sodium alginate (SA), and no separation due to evident creaming (NS).

Strategy	Formulation	Emulsion stability (%)	Droplet size (μm)	Viscosity (mPa s)	Surface oil (%)	Entrapment efficiency (%)
A	LPI-MD	92.8 ± 0.7^{a}	17.1 ± 0.7^{ab}	6.6 ± 1.6^{a}	5.4 ± 0.2^{a}	73.0 ± 0.9^{a}
B	LPI-MD	93.0 ± 0.3^{a}	15.2 ± 0.5^{b}	6.6 ± 0.4^{a}	4.9 ± 0.5^{a}	75.3 ± 2.7^{a}
C	LPI-MD	91.1 ± 0.0^{b}	17.4 ± 0.5^{ab}	7.3 ± 0.9^{ab}	5.7 ± 0.1^{a}	71.3 ± 0.4^{a}
D	LPI-MD-L	39.7 ± 0.7^{c}	83.8 ± 3.0^{c}	16.6 ± 1.9^{c}	5.5 ± 0.7^{a}	72.3 ± 3.3^{a}
E	LPI-MD-L	54.1 ± 0.1^{d}	37.1 ± 1.2^{d}	13.0 ± 1.0^{bc}	8.5 ± 1.2^{b}	57.5 ± 6.0^{b}
F	LPI-MD-SA	NS	58.8 ± 3.8^{e}	48.9 ± 0.1^{d}	2.4 ± 0.0^{c}	87.9 ± 0.2^{c}
G	LPI-MD-L-SA	NS	18.4 ± 0.6^{ab}	172.2 ± 9.8^{e}	11.5 ± 1.7^{d}	42.3 ± 8.5^{d}
H	LPI-MD-L-SA	NS	19.1 ± 0.3^{a}	53.6 ± 1.2^{d}	5.5 ± 0.3^{a}	72.4 ± 1.4^{a}

with LPI-MD (Strategies A-C, Table 4.3), possibly since the higher concentration lead to the formation of phospholipid micelles within the emulsion.

The addition of sodium alginate (SA) to the LPI-MD [Strategy F, Tables 4.1 (b and c)] resulted in improved emulsion stability from ~92% to ~100% relative to the LPI-MD formulations, increased droplet size from ~17 μm to ~59 μm and increased viscosity from ~7 mPa s to ~49 mPa s ($p < 0.05$). Emulsion stability was improved, postulated due to the significant rise in viscosity relative to the LPI-MD emulsions. Upon spray drying, surface oil was at a minimum (~2.4%) and entrapment efficiency was the maximum (~88%) relative to the other capsule formulations. Sodium alginate in the proposed design is expected to act in two ways: (1) during emulsion formation, the primary emulsion is stabilized by LPI at the oil-water interface. At pH 3.0, LPI has positive charges. During the second homogenization step, the negatively charged alginate polysaccharide forms an electrostatic complex with the LPI (Guzey and McClements, 2006; Chang et al., 2015), leading to the condensation of the alginate on the droplet surface forming a complex matrix. The thicker interfacial membrane would prevent droplet coalescence via electrostatic repulsion and steric stabilization. Maltodextrin is considered as a hydrophilic non-ionic polysaccharide, therefore is not thought to contribute to the opposite charge adsorption (Carvalho et al., 2014). And (2) alginate is very hydroscopic and acts as a thickener to increase the viscosity of the continuous phase of the emulsion, limiting the diffusion of oil droplets and decelerating the velocity of gravitational separation (Zhang et al., 2015a). The addition of lecithin to the LPI-MD-SA at the 3% concentration [Strategy G, Tables 4.1 (b and c)] acted to lower droplet size from ~59 μm to ~18 μm and raise viscosity from ~49 mPa s to ~172 mPa s ($p < 0.05$), without impacting emulsion stability ($p > 0.05$). Surface oil was also increased ~5 fold to ~11.5% and entrapment efficiency was reduced from ~88% to ~42% ($p < 0.05$). The substantial rise in viscosity with this formulation is proposed to be associated with a combination of micelles, aggregated protein-protein and protein-alginate aggregates within the continuous phase as the lecithin outcompetes with the protein to reside on the interface. The 1% lecithin level [Strategy H, Tables 4.1 (b and c)] showed a similar trend, but was less effective.

4.4.3. Comparison between LPI-MD and LPI-MD-SA microcapsules with entrapped oils

Based on the previous experiments, the capsule comprised of LPI-MD-SA [Strategy F, Tables 4.1 (b and c)] was selected, along with LPI-MD [Strategy B, Tables 4.1 (b and c)] as a control were studied further in terms their physical properties, their ability to against degradative oxidative reactions during storage and morphology.

The physical properties (e.g., moisture content, water activity, color, wettability and particle size) of the LPI-MD and LPI-MD-SA microcapsules are shown in Table 4.4. Microcapsules having high moisture levels may lead to caking/agglomeration of dried powder, and lipid oxidation to induce off flavors (Lim et al., 2012). Those with high water activity could increase the risk of microbial spoilage and result in shorter shelf-life (Sun-Waterhouse et al., 2013). In the current study, LPI-MD microcapsules had significantly ($p < 0.05$) higher moisture content and water activity than LPI-MD-SA microcapsules, probably due to the greater amount of maltodextrin which abides water. The moisture content of the developed LPI-MD-SA microcapsules (~3.50%) falls within the desired range (3-4%) for shelf stable dried powders in the food industry (Klinkesorn et al., 2005). The *L* (lightness), *a* (redness), and *b* (yellowness) color values are also reported in Table 4. LPI-MD microcapsules were found to be slightly yellower in color than LPI-MD-SA microcapsules, as the *b* value (~7.15) was significantly ($p < 0.05$) higher than the LPI-MD-SA microcapsules (~5.57), most likely due to the higher surface oil content on LPI-MD microcapsules. The wettability of microcapsules is primarily influenced by particle size and wall materials, and can have a big impact on the dissolution and release of active ingredients (Vasisht, 2014). LPI-MD microcapsules were found to have better wettability than LPI-MD-SA microcapsules (Table 4.4) postulated because of the higher levels of maltodextrin present. Particle size of the LPI-MD and LPI-MD-SA microcapsules were similar (< 10 μm) ($p > 0.05$), and in the range of the desired particle size (< 40 μm) for microcapsules in the food industry (Koc et al., 2015). Particle size is an important parameter contributing to the flowability, compressibility, bulk density, and oxidative stability of the microcapsules (Koc et al., 2015). McNamee et al. (1998) reported that particle size of microcapsules could be affected by the size of nozzle, feeding rate, air pressure, and total solid content. In this study, all of emulsions were spray dried under same conditions, regardless of wall materials used.

The ‘peroxide value’ test is an indicator of primary lipid oxidation associated with the production of hydroperoxides, whereas ‘TBARS’ test is used to measure secondary lipid oxidation products, such as aldehydes, ketones, cyclic compounds, alcohols, and hydrocarbons (Pegg, 2005). Oxidative stability of the LPI-MD and LPI-MD-SA microcapsules in comparison with the free canola oil is presented in Figure 4.1. An analysis of variance indicated that both peroxide value and TBARS value of the microcapsules were affected by wall materials and storage time, along with their interaction ($p < 0.05$). Overall, the free canola oil and the encapsulated oil in LPI-MD microcapsules experienced similar ($p >$

Table 4.4. Physical properties of selected microcapsules. Data represent the mean ± one standard deviation. Different small letters in the same column indicate a significant difference ($p \leq 0.05$). Abbreviations include: lentil protein isolate (LPI), maltodextrin (MD), and sodium alginate (SA).

Strategy	Formulations	Moisture (%)	Water activity	Color			Wettability	Particle size (µm)
				L	*a*	*b*		
B	LPI-MD	4.30 ± 0.17[a]	0.42 ± 0.00[a]	91.71 ± 0.39[a]	0.38 ± 0.07[a]	7.15 ± 0.11[a]	Good	9.15 ± 0.29[a]
F	LPI-MD-SA	3.50 ± 0.09[b]	0.36 ± 0.01[b]	92.43 ± 0.31[a]	0.12 ± 0.03[b]	5.57 ± 0.25[b]	Fair	9.04 ± 0.11[a]

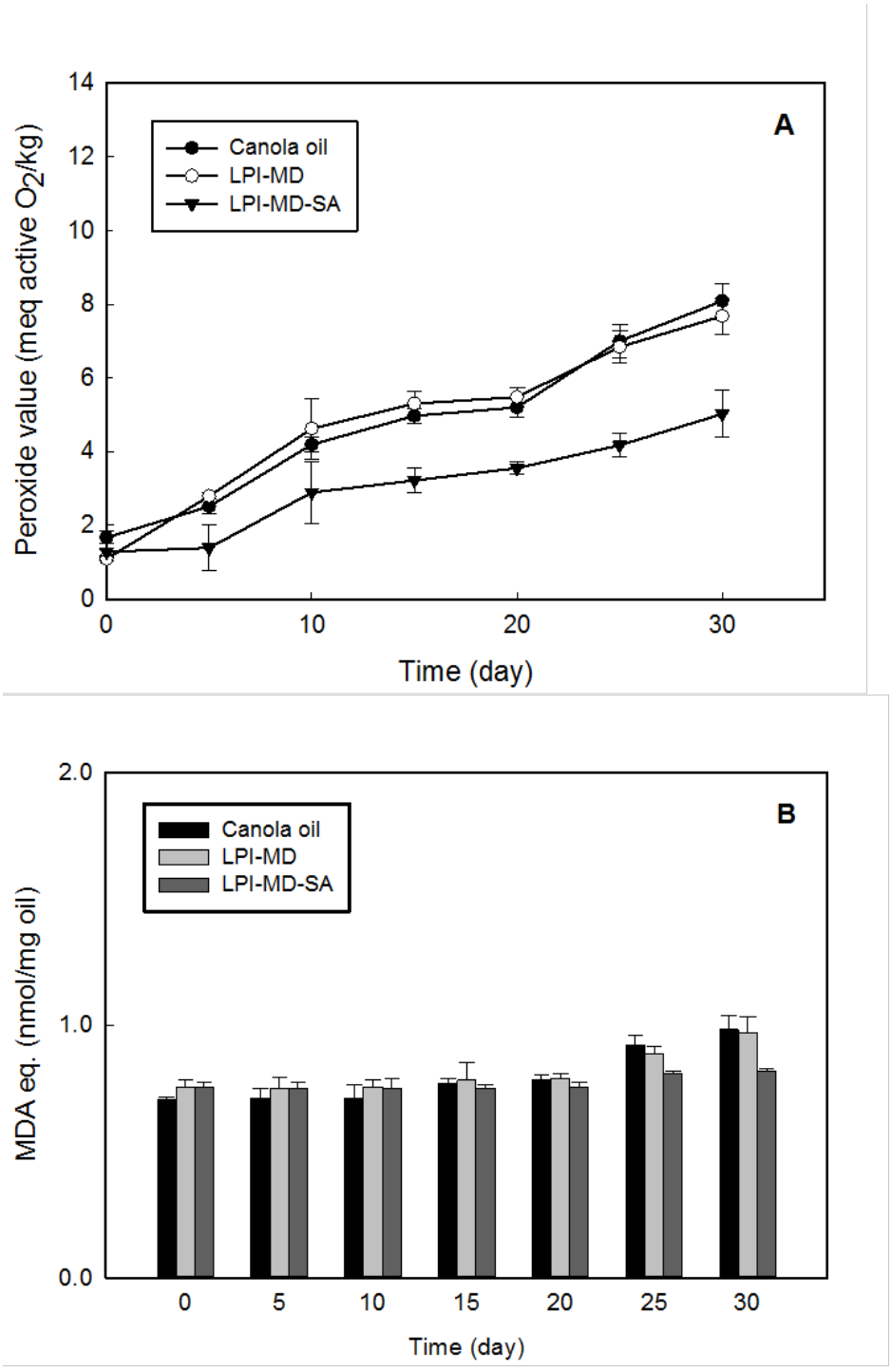
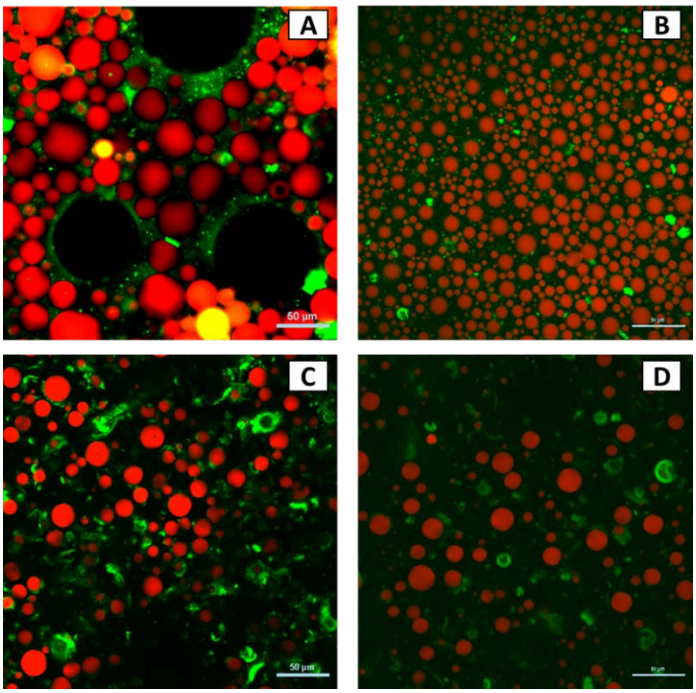


Figure 4.1. Changes in (A) peroxide value (PV) and (B) 2-thiobarbituric acid reactive substances (TBARS) for the free and encapsulated canola oil in LPI-MD and LPI-MD-SA microcapsules over 30 d of storage. Data represent the mean ± one standard deviation (n = 3). Abbreviations include: lentil protein isolate (LPI), maltodextrin (MD), and sodium alginate (SA).

0.05) oxidative stability, which were significantly ($p < 0.05$) less stable than oil entrapped within the LPI-MD-SA microcapsules. LPI-MD-SA microcapsules had a significantly ($p < 0.05$) lower peroxide value than LPI-MD microcapsules and free oil over the 30 d storage period. In all cases, the peroxide values were gradually increased during storage from ~1.5 to ~7.5 meq active O_2 kg^{-1} for the oil entrapped within LPI-MD capsules and the free oil; and from ~1.5 to ~5 meq active O_2 kg^{-1} for the oil entrapped within the LPI-MD-SA capsules over the 30 d storage period [Figure 4.1 (A)]. In contrast, TBARS values for the oil entrapped within both microcapsules and the free oil were similar ($p > 0.05$) over the first 20 d of storage. However, by day 25, TBARS values for both of the free canola oil and that entrapped within the LPI-MD microcapsule significantly increased relative to the oil entrapped within the LPI-MD-SA microcapsule ($p < 0.05$), which remained unchanged from early times [Figure 4.1 (B)]. A possible reason why the LPI-MD-SA microcapsules are better may be due to: (a) the larger droplet sizes than the LPI-MD emulsions (Table 4.3) that would have less surface area to limit the contact between oil and oxygen (Heinzelmann and Franke, 1999); (b) the lower surface oil and better entrapment efficiency than the LPI-MD microcapsules (Table 4.3); and (c) the thicker complex interface. Labuza et al. (1972) also suggested that when water activity is close to 0.3, lipid oxidation was limited, which contribute to the better oxidative stability of LPI-MD-SA microcapsules (a_w of ~0.36) versus the LPI-MD microcapsules (a_w of ~0.42). In the present study, peroxide value of the encapsulated oil in LPI-MD-SA microcapsules after 30 days storage met the desired industry target (5 meq active O_2 kg^{-1}) (Nickerson et al., 2014).

The morphology of emulsions prepared with a) LPI, b) LPI-MD, c) LPI-SA and d) LPI-MD-SA stabilizing solutions, imaged by CLSM is given in Figure 4.2 (a). Without maltodextrin, the LPI only solution resulted in oil droplets (red color staining) with a wide droplet distribution dispersed within the protein solution (green color staining) [Figure 4.2 (a-A)]. It is postulated the LPI binds to the oil-water interface as evident by well-defined darker edges around each droplet, which are then further surrounded by a LPI-rich continuous phase [Figure 4.2 (a-A)]. The large dark circles within the image represent air bubbles. The addition of maltodextrin created smaller, more uniformly distributed oil droplets (red color staining) with similar morphology as without maltodextrin [Figure 4.2 (a-B)]. The addition of sodium alginate to the LPI solution (without maltodextrin) resulted in a more structured continuous phase with larger green aggregates most likely comprised of LPI-SA electrostatic complexes formed under the acidic conditions [Figure 4.2 (a-C)]. The addition of maltodextrin to the solution, led to a more uniform continuous phase with fewer large LPI-SA

a. Confocal laser scanning microscopy images of emulsions.



b. Scanning electron microscopy images of microcapsules.

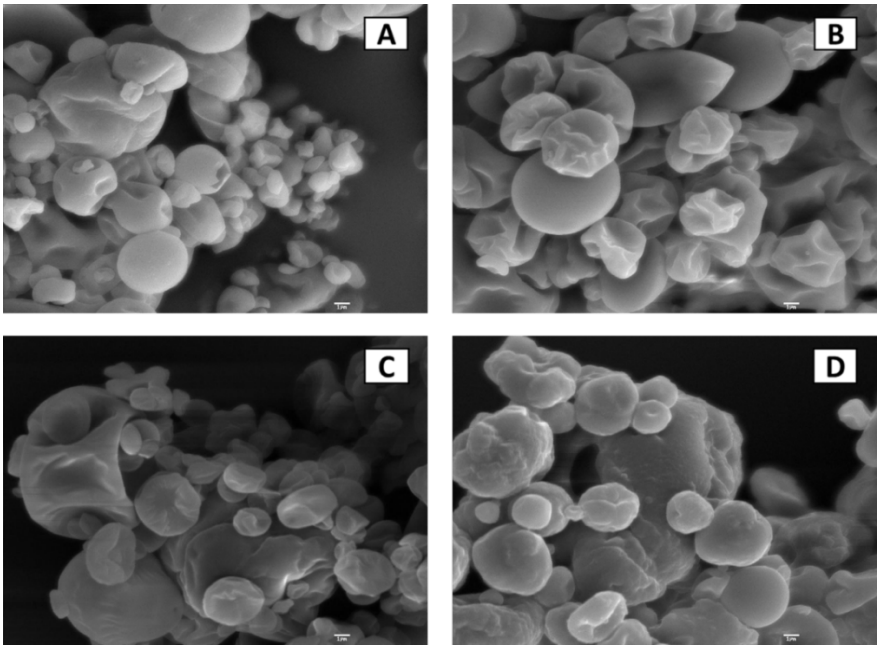


Figure 4.2. Confocal laser scanning microscopy images of the emulsions (a) and scanning electron microscopy images (at 8000 × magnification) of the microcapsules (b) prepared by LPI (A), LPI-MD (B), LPI-SA (C) and LPI-MD-SA (D). Abbreviations include: lentil protein isolate (LPI), maltodextrin (MD), and sodium alginate (SA).

structures, postulated due to a disruption of protein-polysaccharide interaction in the presence of maltodextrin [Figure 4.2 (a-D)]. Based on the CLSM images, it is difficult to discern the distribution of sodium alginate chains within LPI-MD-SA stabilized emulsions, whether a mixed interfacial film or a bilayer is formed.

To resolve this, the surface morphology of a) LPI, b) LPI-MD, c) LPI-SA, and d) LPI-MD-SA microcapsules were imaged by SEM after spray drying, and shown in Figure 4.2 (b). All microcapsules exhibited spherical geometry and irregular surface with various size that are typical characteristics resulting from the spray drying process (Carvalho et al., 2014). Without maltodextrin, the microcapsules prepared with LPI (only) were spherical shaped and had smooth surface. The capsules showed few indentations indicating the microcapsules had thinner walls with lower retention of the core material [Figure 4.2 (b-A)]. LPI-MD microcapsules were characterized by deeper invaginations and wrinkles with slight agglomerations [Figure 4.2 (b-B)], whereas LPI-SA microcapsules presented larger particles with relative smooth surface with few concavity [Figure 4.2 (b-C)]. Theoretically, invagination and concavity are considered as the result of uneven shrinkage of the wall during spray drying (Sheu and Rosenberg, 1998). It is postulated that the more tightly bound wall of the LPI-SA microcapsules caused less invaginations than the LPI-MD microcapsules. In general, capsules with greater amounts of invaginations result in poor reconstitution and greater surface area. The latter allows higher air permeability through the wall, to reduce the effectiveness of protection from degradative oxidative reactions (Rosenberg and Sheu, 1996; Walton and Mumford, 1999). The addition of maltodextrin on the combination of LPI and sodium alginate greatly strengthened the wall structure and resulted in the thicker wall material to encapsulate the oil with relative rough surface [Figure 4.2 (b-D)], which indicates the LPI-MD-SA microcapsules had lower air permeability and better protection to the core material. According to the SEM images, the combination of LPI, maltodextrin, and sodium alginate formed stronger wall structure to protect the oil from deteriorative oxidative reactions in comparison with LPI-MD microcapsules, which was also demonstrated in Figure 4.1. The increased surface roughness on the LPI-SA [Figure 4.2 (b-C)] and LPI-MD-SA [Figure 4.2 (b-D)] microcapsules relative to the LPI-MD microcapsules [Figure 4.2 (b-B)] suggests that a bi-layer type wall material is more probable.

4.5. Conclusions

Overall, the LPI-based wall material comprised of 2% LPI, 17 % maltodextrin and 1% sodium alginate with a 20% oil proved to be the most effective encapsulation design from the current study, which greatly increased the oil concentration comparing with the lentil protein-based microcapsules developed by Can Karaca and co-workers (2013a) to encapsulate 10% flaxseed oil. The wall material was found capable to offering protection to against degradative oxidative reactions much better than LPI-MD alone, suggesting that a plant protein wall material needs to be strengthened by means of electrostatic complexation with an opposite charged polysaccharide to reduce the level of wall shrinkage during spray drying. Although the production of microcapsules using a benchtop spray dryer should be explored for its scale up potential, the capsule design could be incorporated into a wide range of food products, ranging from dairy, to snack foods and baked good applications, and also be used as a feed supplement to enhance the delivery of healthy oils.

4.6. Linkage

LPI-based microcapsules were prepared with different concentrations of LPI and maltodextrin as wall materials, and preparation methods to entrap 20 and 30% of canola oil, in which the combination of LPI, maltodextrin, and sodium alginate was demonstrated as the best capsule design to encapsulate 20% oil, because it offered the highest entrapment efficiency (~88%), good physical properties, and protective nature against oxidation. The focus of the third study of this research project was to encapsulate different omega fatty acids-rich oils (e.g., canola oil, fish oil, and flaxseed oils) using the combination of LPI, maltodextrin, and sodium alginate in the previous study, and to investigate the physical properties of microcapsules, storage stability, and *in vitro* release behavior of the encapsulated oils, in order to further determine its potentiality to be used as a universal platform to deliver healthy oils.

5. STABILITY AND IN VITRO RELEASE BEHAVIOR OF ENCAPSULATED OMEGA FATTY ACIDS-RICH OILS IN LENTIL PROTEIN ISOLATE-BASED MICROCAPSULES

5.1. Abstract

The objective of this study was to investigate the use of a lentil protein isolate-based microcapsule design as a platform for entrapping different types of omega fatty acids-rich oils (e.g., canola, fish, and flaxseed oils), and to characterize differences in the physical properties (e.g., moisture content, water activity, color, wettability, particle size, surface oil, and entrapment efficiency), storage stability, and *in vitro* release behavior of the entrapped oils. All microcapsules displayed similar physical properties regardless of the core material. Free fatty acid content, peroxide value, 2-thiobarbituric acid reactive substances, and Rancimat test were investigated between the free and encapsulated oils to determine protective effects from microencapsulation, and found the wall material provided the greatest protective effect to fish oils relative to the others. Overall, only a minor amount of encapsulated oil (~3.2-8.9%) was released within simulated gastric fluid, with the majority (~62.6-73.4%) being released after sequential exposure to simulated intestinal fluid, which promoted the release of polyunsaturated fatty acids.

5.2. Introduction

Oils rich in omega fatty acids (e.g., omega-3, -6 and -9) are of high economic importance to the food industry, due to their roles in the prevention of cardiovascular diseases, hypertension and diabetes, and in brain and ocular development during fetal and infant growth (Larsen et al., 2011). Over the last few decades, an increasing number of studies have focused on metabolic relationships, nutritional benefits, handling and distribution, and recommend intakes of long chain polyunsaturated fatty acids (PUFAs) from plant and marine origins, particularly linoleic acid, α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Canola and flaxseed oils typically contain 5-10% and 45-55% of ALA, respectively. EPA and DHA are mainly present in fish oils at different amounts and ratios depending on various metabolic characteristics of the fish itself.

Most commercial fish oils are formulated to have 30% EPA and DHA at a ratio of 1.5:1 (Calder, 2013). However, their susceptibility to degradative oxidative reactions can lead to rancidity, off-flavors, off-odours, loss in the bioavailability and poor product quality (Nickerson et al., 2014). Furthermore, the immiscibility of the oils in various aqueous products causes significant challenges in food formulations. To help circumvent these challenges, microencapsulation can be applied to entrap the oils within a physical barrier to help minimize fatty acid degradation.

Microencapsulation is defined as a technology that utilizes proteins, carbohydrates, or lipids to enclose or package active core ingredients (e.g., essential fatty acids, antioxidants, vitamins, and flavors) within matrices in the diameter range of micro- to millimeter. The technology helps to improve the handling properties of the oils, control their release during transit through the gastrointestinal tract, mask the undesirable flavours, and offer protection from environmental factors (e.g., temperature, air, moisture, and light) present during food processing and storage (Desai and Park, 2005). Microencapsulation of fish, flaxseed and canola oils using different proteins-based wall materials (e.g., chickpea protein isolate, lentil protein isolate, and sodium caseinate) has been studied previously (Can Karaca et al., 2013a; Gallardo et al., 2013; Pourashouri et al., 2014; Goyal et al., 2015; Chang et al., 2016). However, more intense research is being focused towards the use of plant proteins in order to address changing consumer demands and niche markets (e.g., vegan) (Pelser et al., 2007; Can Karaca et al., 2013a; Chang et al., 2016). Lentil protein is one of the plant-based alternatives to animal derived proteins that has potential as an encapsulating agent, because of its excellent emulsifying properties, low cost, good nutritional value and low risk of allergen (Boye et al., 2010a). Can Karaca et al. (2013a) found lentil protein (20%)-maltodextin (70%) wall materials were able to entrap flaxseed oil to give low surface oil (~1.1%) and high entrapment efficiencies (~90.4%), however the payload (also known as oil concentration) remained low (10%). Chang and co-workers (2016) investigated different wall formulations and emulsion preparation conditions for entrapping canola oil using a benchtop spray dryer. The authors found that a wall material comprised of 8% lentil protein isolate, 4% sodium alginate and 68% maltodextrin provided the best capsule formulation to give low surface oil (~2.4%), high entrapment efficiencies (~87.9%) and good protection to against oxidation over a 30 d storage study. Payloads were also increased to 20%.

The overall goal of this research was to build on work by Chang et al. (2016), to investigate the effect of oil-type on the physical characteristics, stability properties (against oxidation), and *in vitro* release behavior of encapsulated oils from microcapsules containing

LPI, sodium alginate and maltodextrin as the wall material. Further, changes to the fatty acid profile throughout processing and release were examined to determine if some fatty acids had a greater affinity to the protein matrix than others. Evaluation of the different oil-types from the wall material will describe its utility as a universal platform for delivering healthy oils in food products.

5.3. Materials and methods

5.3.1. Materials

In the microcapsules, the core materials used included canola, fish, and flaxseed oils, in which both of fish and flaxseed oils were procured from Bioriginal Food and Science Corp. (Saskatoon, SK, Canada), whereas canola oil was obtained from a local supermarket. The combination of LPI, maltodextrin, and sodium alginate was selected as the wall material based on the work by Chang et al. (2016), in which LPI [containing 78.97% (%N × 6.25) of protein, 0.49% of lipid, 6.24% of moisture, 7.19% of ash, and 7.11% of carbohydrates on wet basis] and maltodextrin [MALTRIN M100, dextrose equivalent (DE) of 9.0-12.0] were produced by POS Bio-Sciences (Saskatoon, SK, Canada) and the Grain Processing Corporation (Muscatine, IA, USA), respectively, whereas sodium alginate and other chemicals (on analytical grade) were purchased from Sigma-Aldrich, Canada. A Millipore Milli-Q^{TM} water purification system (Millipore Corporation, Milford, MA, USA) was used to prepare Milli-Q water.

5.3.2. Preparation of a lentil protein isolate

Lentil protein isolate was produced at POS Bio-Sciences pilot scale facility using their commercial alkaline extraction and isoelectric precipitation process. In brief, flour was dispersed in water at a 1:10 (w/w) flour to water ratio and pH adjusted to 9.5 using 1.0 M NaOH for 1 h at room temperature. Insolubles were recovered via centrifugation, whereas the supernatant was subsequently adjusted to pH 4.5 using 0.1 M HCl to induce the precipitation of proteins. Proteins were then neutralized followed by spray drying.

5.3.3. Microcapsules preparation

The microcapsules were prepared using a wall material comprised of LPI, maltodextrin, and sodium alginate, but with different core materials (e.g., canola, fish, and flaxseed oils). All capsules were produced using a two-step process: emulsion preparation followed by spray drying, as described by Chang et al. (2016). In brief, LPI (2% w/w in the

emulsion, the concentration was corrected for protein level within the powder) was dissolved in Milli-Q water and adjusted to pH 3.0 with 2.0 M HCl or 2.0 M NaOH. Afterwards, the protein solution was stirring at 500 rpm for overnight at 4 °C, followed by re-adjustment of pH to 3.0. The aqueous solutions of other wall materials [e.g., maltodextrin (17% w/w in the emulsion) and sodium alginate (1% w/w in the emulsion)] were prepared by dispersing them in Milli-Q water for 3 h at room temperature (22-23 °C). The primary oil-in-water emulsion was prepared by homogenizing the oil (5% w/w in the emulsion) with the LPI solution using a Polytron PT 2100 Homogenizer (Kinematica AG, Lucerne, Switzerland) equipped with a 12 mm PT-DA 2112/2EC generating probe at 15,000 rpm for 5 min. Subsequently, the sodium alginate solution was mixed with the primary emulsion for 10 min at 500 rpm, followed by the homogenization at 15,000 rpm for 3 min to produce the secondary emulsion. The tertiary emulsion was prepared in the same manner as described in the preparation of secondary emulsion by using the mixture of maltodextrin solution and the secondary emulsion.

The microcapsules (1:4 of core/wall material ratio) were produced by spray drying the tertiary emulsion using a laboratory-scale Buchi Advanced Mini Spray Drier B-290 (Buchi Labortechnik AG, Flawil, Switzerland) equipped with an atomizing nozzle (0.7 mm diameter). The inlet temperature was adjusted to 180 °C, and the outlet temperature was stabilized at 85 ± 1 °C by controlling the sample flow rate (5-20%). The spray dryer had 35 m^3/h of air flow rate and 5 bars of compressed air pressure. The emulsion was continuously stirring at 300 rpm to maintain homogeneity and avoid destabilization when pumping into the sample chamber (65 × 110 × 70 cm). The production of microcapsules was performed in triplicate.

5.3.4. Physical properties

For moisture content measurements, 0.5 g of microcapsules were dried in a convection oven (APTLine ED, Binder GmbH, Tuttlingen, Germany) at 105 °C for ~12 h. The dried microcapsules were then weighted using an analytical balance (Sartorius, USA) with precision of 0.0001 g to determine the moisture content based on weight difference. An AquaLab 4TX water activity meter (Decagon Devices, Inc., Pullman, WA, USA) was used to assess the water activity of microcapsules at 22 °C. For color measurements, a ColorFlex EZ 45/0 Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to determine the color of microcapsules based on CIE tristimulus value system, in which the color of microcapsules was expressed as *L* (lightness), *a* (red – green), and *b* (blue – yellow).

For particle size measurements, 1 g of microcapsules were suspended under agitation to determine the particle size using a laser diffraction-based particle analyzer (LS 13 320 SW Particle Size Analyzer, Beckman Coulter, Inc., Brea, CA, USA). A Universal Liquid Module was selected as a sample module and particle size was recorded as volume-mass diameter ($d_{4,3}$) (Chang et al., 2016).

For wettability measurements, 1 g of microcapsules were transferred into 80 mL of Milli-Q water through a sieve [#12 (1.70 mm) Tyler mesh filter, Mentor, OH, USA]. The dissolving behavior of microcapsules was observed for 30 min, followed by stirring at 500 rpm for 1 min, to determine the wettability of microcapsules into 4 levels [e.g., excellent (the microcapsules completely wet as contacting with water, and dissolve after 30 min), good (the microcapsules partly wet as contacting with water, and precipitate after 30 min), fair (the microcapsules slightly wet as contacting with water, and partly float on the surface of water after 30 min), and poor (the microcapsules hardly wet as contacting with water, and mostly float on the surface of water after 30 min)] (Balmaceda et al., 1976).

Surface oil of microcapsules was determined according to the method of Chang et al. (2016). In brief, 15 mL of hexane was added to 1 g of microcapsules in a 50 mL beaker and mixed well for 15 s to extract the surface oil. The solvent mixture was filtered through #3 Whatman filter paper (Whatman International Ltd., Maidstone, UK), followed by the evaporation of organic solvent in a 50 mL beaker under a fume hood for ~12 h. Finally, the residual organic solvent was dried in a convection oven (APTLine ED, Binder GmbH, Tuttlingen, Germany) at 105 °C for 30 min, and the surface oil of microcapsules was calculated gravimetrically. The entrapment efficiency (EE) was calculated by the method given by Anwar and Kunz (2011) as follows:

$$EE = (\text{Total oil} - \text{Surface oil}) / \text{Total oil} \times 100\% \qquad [5.1.]$$

where total oil is 20% (w/w) oil in the microcapsules.

Data for all physical tests was reported as the mean ± one standard deviation from triplicate microcapsule preparations (n = 3).

5.3.5. *Stability test*

Oil extraction

For monitoring storage stability, the microcapsules (8 g) and the free oils (4 g) were stored in sealed glass bottles at room temperature (22-23 ºC) in the absence of light for 1

month. The hydrolytic stability [e.g., free fatty acid (FFA) content] and the oxidative stability [e.g., peroxide value (PV) and 2-thiobarbituric acid reactive substances (TBARS)] of the encapsulated and free oils were investigated on every 5 days during storage. The encapsulated oil was extracted prior to the measurement according to the methods of Can Karaca et al. (2013a) and Klinkesorn et al. (2005) with some modifications. Briefly, Milli-Q water (60 mL) was added to 8 g of microcapsules and stirred at 500 rpm for 5 min. The resulting solution was then extracted with 100 mL of hexane/isopropanol (3:1, v/v) under stirring for 15 min and centrifuged (Sorvall RC Plus Superspeed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) at 4,193 × *g* for 10 min. The clear organic phase was collected. After filtration (#1 Whatman filter paper, Whatman International Ltd., Maidstone, England) through anhydrous Na_2SO_4, the solvent was evaporated under a stream of nitrogen in the fume hood to collect the encapsulated oil for further experiments.

Free fatty acid (FFA)

The FFA contents in the encapsulated and free oils were determined using the direct titration method according to AOCS (2000). In brief, the oil sample (1 g) was dissolved into ethyl alcohol (25 mL), followed by the addition of phenolphthalein indicator (50 μL). The resulting solution was then titrated with 0.01 N NaOH until the faint permanent pink color appeared. FFA contents of the encapsulated and free oils were calculated using the following formula:

$$\text{FFA (\%)} = [(V \times N \times 282.46) / W] \times 100 \qquad [5.2.]$$

where V is the volume (L) of NaOH solution used for titration, N is the normality of NaOH solution, and W is the oil weight (g). Data was reported as the mean ± one standard deviation from triplicate microcapsule preparations (n = 3).

Peroxide value (PV)

The PV of the encapsulated and free oils was determined by an iodometric titration method to measure the iodine produced from the reaction between potassium iodide and the peroxides present in the oil samples (Kolanowski et al., 2004). In brief, 0.2 g of the oil sample was dissolved into 30 mL of acetic acid/chloroform (3:2, v/v) mixture, followed by the addition of 0.5 mL of saturated potassium iodide. After that, the mixture was occasionally shaken for 1 min to liberate iodine. 30 mL of Milli-Q water and 0.5 mL of starch indicator

(1%, w/v) were then added to stop the reaction and produce the violet color, respectively. Finally, the liberated iodine was titrated with 0.001N sodium thiosulfate ($Na_2S_2O_3$) until the solution became colorless. A blank sample was carried out through all the steps. PV of the oil sample was calculated as (Pegg, 2005):

$$PV = (S - B) \times N \times 1000 / W \quad [5.3.]$$

where S is the volume (mL) of $Na_2S_2O_3$ solution used for the titration of oil samples, B is the volume (mL) of $Na_2S_2O_3$ solution used for the titration of the blank (without oil samples), N is the normality of $Na_2S_2O_3$ solution, and W is the oil sample weight (g). Data was reported as the mean ± one standard deviation from triplicate microcapsule preparations (n = 3).

2-Thiobarbituric acid reactive substances (TBARS)

The TBARS values of the encapsulated and free oils were measured based on the reaction of 2-thiobarbituric acid (TBA, a chromogenic reagent) with malondialdehyde (MDA, a compound resulting from secondary lipid oxidation) to produce a pink chromophore with absorbance maximum at 532 nm. In brief, the sample was prepared by dissolving the encapsulated or free oil (40 mg) in 2-butanol in a 10 mL of volumetric flask. 1,1,1,3,3-tetraethoxypropane (TEP, 100 μM) was diluted in 2-butanol in a 10 mL of volumetric flask to prepare MDA standards (1.25-50.00 μM). In a 2.0 mL of Eppendorf tube, 200 μL of the sample, the MDA standard, or 2-butanol (presented as a blank) was mixed with 50 μL of 8.1% (w/v) sodium dodecyl sulfate (SDS), 375 μL of 20% (v/v) acetic acid (at pH 3.5), 375 μL of 0.8% (w/v) TBA, and 8.25 μL of 0.02% (w/v) butylated hydroxytoluene [BHT, in dimethyl sulfoxide (DMSO)]. Afterwards, the reaction was induced by heating the mixture at 95 °C for 1 h in a water bath, in order to accelerate the reaction to reach the maximum color development. After cooling in cold water, 0.9 mL of 2-butanol/pyridine (15:1, v/v) was added into the Eppendorf tube and vigorously mixed for 30 s to extract the chromophore, followed by the centrifugation (Eppendorf Centrifuge 5424, Hamburg, Germany) at 4,000 × *g* for 10 min. The absorbance of the upper organic layer at 532 nm was recorded against a 2-butanol blank. Finally, the TBARS value of the sample was reported as MDA content (nmol)/sample oil weight (mg), which was calculated using the standard curve (obtained from MDA standards) and the absorbance of the sample (Akhlaghi and Bandy, 2010; Pegg, 2005). Data was reported as the mean ± one standard deviation from triplicate microcapsule preparations (n = 3).

Oxidative stability index (OSI)

The OSI of the encapsulated and free oils with and without antioxidants [e.g., butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ)] was estimated right after the microcapsules' preparation using a Rancimat (Model 679, Metrohm, Herisau, Switzerland). In brief, synthetic antioxidants (0.02% w/w) were dissolved into the oil and stirring for 1 h in a dark cooling room (4 ^{o}C) before the Rancimat test. 3 g of microcapsules or oils (e.g., free oils, oils with BHT, and oils with TBHQ) were exposed to a stream of air (20 L/h of flow rate) at 100 ^{o}C to accelerate the oxidative reaction. The volatile oxidation products were then collected and dissolved into distilled water to increase the conductivity. Finally, the induction period, which is defined as the time taken to reach an inflection point on the curve of conductivity versus time (h), was recorded and expressed as the OSI (AOCS, 1994). Data was reported as the mean ± one standard deviation from triplicate microcapsule preparations (n = 3).

5.3.6. In vitro release behavior

In vitro release behavior of the encapsulated oil under a simulated gastrointestinal model was studied according to the method of Burgar et al. (2009) with some modifications. Simulated gastric fluid (SGF) was prepared by dissolving 3.2 g of pepsin in 1000 mL of the salt solution (at pH 1.2), which included 2 g of NaCl, 7 mL of 36% (v/v) HCl, and Milli-Q water (to make up the volume to 1000 mL). Simulated intestinal fluid (SIF) was prepared by dissolving 6.8 g of monobasic potassium phosphate (KH_2PO_4) and 77 mL of 0.2 N NaOH in 750 mL Milli-Q water, followed by the addition of 10 g of pancreatin. The mixture was then adjusted to pH 6.8 using 1.0 M NaOH, and the final volume was made up to 1000 mL with Milli-Q water. Both of SGF and SIF were stored at 4 °C for further use.

For exposure to SGF, 3 g of microcapsules was mixed with 30 mL of SGF and incubated in a water bath at 37 °C and 100 rpm for 2 h. The solid particles were then removed by the filtration of the solution through #1 Whatman filter paper (Whatman International Ltd., Maidstone, England). Subsequently, the resulting solution was mixed with hexane at 1:1 (v/v) ratio for 15 min, followed by the centrifugation (Sorvall RC Plus Superspeed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) at 9,100 × *g* for 10 min. The organic phase was collected and the aqueous phase was re-extracted by hexane at 1:1 (v/v) ratio. Finally, the organic phase was filtered through #1 Whatman filter paper (Whatman International Ltd., Maidstone, England) with anhydrous Na_2SO_4, and the organic solvent was evaporated under a stream of nitrogen in a fume hood. The amount of released

oil was determined gravimetrically. For exposure to SGF and SIF in sequence, 3 g of microcapsules was dissolved in 30 mL of SGF and incubated under same conditions for 2 h, followed by the pH adjustment to 6.8 using 1.0 M NaOH. The addition of SIF (30 mL) was then applied, and incubating the mixture under same condition for 3 h. The amount of released oil was determined gravimetrically as described above. Data was reported as the mean ± one standard deviation from triplicate microcapsule preparations (n = 3).

5.3.7. Fatty acid composition

To determine changes to the fatty acid profiles of the free oils, oils directly after encapsulation (extracted as described previously in section 5.3.5.), and those released (from SGF and SGF + SIF treatments), the content of fatty acid methyl esters (FAMEs) was measured using a gas chromatography (model 7890A, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a 30 m cis/trans FAME column (DB-23, Agilent Technologies, Inc., Santa Clara, CA, USA) and detected by a hydrogen ionization detector (model 6850, Agilent Technologies, Inc., Santa Clara, CA, USA). In brief, FAMEs of the oil samples were obtained by trans-methanolation treatment of 0.008 g of the oil sample in 2 mL of methanolysis reagent [H_2SO_4:methanol = 1:99 (v/v)] at 100 °C in a gravity convection oven (APTLine ED, Binder GmbH, Tuttlingen, Germany) for 30 min. The chromatographic column was initially warmed at 160 °C for 30 min and then increased to 240 °C. All the studied FAMEs were adequately separated in 30 min under these conditions. The fatty acid composition was identified by comparing the retention time with the standard (PUFA-2, purchased from Sigma-Aldrich). Data was reported as the mean ± one standard deviation from triplicate microcapsule preparations (n = 3).

5.3.8. Statistics

The results were expressed as the mean ± one standard deviation of three independent microcapsule preparations. One-way analysis of variance (ANOVA) was performed to evaluate the effect of oil type, treatments (e.g., free oils, oils after spray drying, released oils under SGF, and released oils under SGF + SIF) and antioxidants on the physical properties, fatty acid composition, and OSI, respectively. Two-way ANOVA with Tukey Post Hoc test was completed to determine the effect of oil type and storage time/treatments on the stability properties (e.g., FFA, PV, and TBARS) and *in vitro* release behavior. Statistical analysis was carried out by using the software Systat v10 (San Jose, CA, USA) at 95% confidence interval.

5.4. Results and discussion

5.4.1. Physical properties of microcapsules

The physical properties of microcapsules prepared with different oil-types are shown in Table 5.1. An analysis of variance indicated that the physical properties (e.g., moisture content, water activity, wettability, particle size, surface oil, and entrapment efficiency) of microcapsules were not affected by oil-type ($p > 0.05$) with the exception of color ($p < 0.05$). Overall, microcapsules prepared with different oils had similar physical properties (moisture content: ~3.5%; water activity: ~0.35; wettability: fair; particle size: ~8.9 μm; surface oil: ~2.4%; and entrapment efficiency: ~87.8%) (Table 5.1). In general, lipid oxidation is decelerated when water activity is in the range of 0.2-0.4 (Velasco et al., 2003), and the industrial moisture standard is in the range of 3-4% for shelf stable dried powder (Klinkersorn et al., 2005). It was observed that microcapsules containing flaxseed oil were more dark yellow in color than those containing fish oil, followed by those with canola oil, where its *L* value (~90.53) and *b* value (~10.28) were much lower and higher than other microcapsules, respectively (Table 5.1). This was most likely caused by visible differences in the color of the oil itself. Nykter et al. (2006) suggested that the color of oil can be affected by the amount of chlorophyll present, the microbial treatment used, and the deodorization process. Specifically, oil containing less chlorophyll (e.g., below 1 mg/kg of oil) is lighter yellow in color; the deodorization step during processing can decrease the yellow and red pigments in the oil; and fungal treatments can greatly increase the yellow and red pigments during the storage (Nykter et al., 2006).

Particle size is an important parameter, since it affects flowability, compressibility, bulk density, wettability and stability of the microcapsules (Koc et al., 2011). Koc and co-workers (2011) reported smaller particles (< 40 μm) are beneficial to decrease the oxidation level. In the current study, the particle size (~8.9 μm) was smaller relative to those reported in other studies (~12.9 μm) (Table 5.1), which maybe because of the lower total solid contents in the initial emulsions. Turchiuli et al. (2005) found that the particle size of a vegetable oil microcapsule prepared with maltodextrin and acacia gum was greatly increased from 18 μm to 85 μm as the total solid content increased from 30% to 50%.

Table 5.1. Physical properties of the microcapsules prepared in this study in comparison with other proteins-based capsules to deliver canola oil, fish oil, and flaxseed oil. Different small letters in the same column indicate a significant difference ($p \leq 0.05$) in the current study. Abbreviations include: lentil protein isolate (LPI), maltodextrin (MD), sodium alginate (SA), whey protein isolate (WPI), whey protein concentrate (WPC), sodium caseinate (SC), chickpea protein isolate (ChPI), and skipjack roe protein hydrolysate (SRPH).

Wall materials	Core Oil	Moisture (%)	Water activity	Color			Wettability	Particle size (μm)	Surface oil (%)	Entrapment efficiency (%)
				L	*a*	*b*				
A) Current study										
LPI, SA, and MD	Canola	3.50 ± 0.09[a]	0.36 ± 0.01[a]	92.43 ± 0.31[a]	0.12 ± 0.03[a]	5.57 ± 0.25[a]	Fair	9.04 ± 0.11[a]	2.43 ± 0.04[a]	87.85 ± 0.18[a]
	Fish	3.47 ± 0.10[a]	0.34 ± 0.05[a]	91.11 ± 0.30[b]	0.11 ± 0.02[a]	7.30 ± 0.16[b]	Fair	8.57 ± 0.42[a]	2.38 ± 0.42[a]	88.12 ± 2.10[a]
	Flax-seed	3.53 ± 0.46[a]	0.34 ± 0.03[a]	90.53 ± 0.23[c]	0.21 ± 0.04[b]	10.28 ± 0.12[c]	Fair	8.99 ± 0.36[a]	2.52 ± 0.40[a]	87.38 ± 2.00[a]
B) Literature works										
WPI[1]	Fish	1.48 ± 0.04	-	-	-	-	-	3.10 ± 0.00	-	75.65 ± 1.19
SRPH[2]	Fish	1.66 ± 0.09	-	-	-	-	-	17.07 ± 0.57	-	13.00
WPC and SC[2]	Fish	1.13 ± 0.11	-	-	-	-	-	10.23 ± 0.10	-	70.00
ChPI and MD[3]	Flax-seed	3.71 ± 0.46	0.06 ± 0.00	-	-	-	-	24.00	2.64 ± 0.04	83.62 ± 0.40
WPC and lactose[4]	Flax-seed	3.98 ± 0.13	0.36 ± 0.02	-	-	-	-	-	4.73 ± 0.16	86.77 ± 0.51
SC and lactose[4]	Flax-seed	3.88 ± 0.09	0.35 ± 0.01	-	-	-	-	10.01 ± 0.58	5.57 ± 0.09	84.51 ± 0.25

References: [1]Aghbashlo et al. (2013), [2]Intarasirisawat et al., (2015), [3]Can Karaca et al. (2013a), and [4]Goyal et al. (2015).

Surface oil can negatively impact the oxidative stability, wettability and dissolubility by resulting in the aggregation of microcapsules (Can Karaca et al., 2013a). The lower surface oil (~2.4%) and higher entrapment efficiency (~87.8%) observed in the current study relative to other works (surface oil of ~4.3% and entrapment efficiency of ~68.9%) (Table 5.1) can be attributed to the formation of condensed and strong wall structure by LPI, sodium alginate and maltodextrin. It is reported that the electrostatic complex coating on the oil droplet was produced by the negatively charged sodium alginate and positively charged LPI, and maltodextrin improved drying properties of microcapsules (Chang et al., 2016).

Moreover, the fatty acid compositions of the free and encapsulated oils are shown in Table 5.2. It was proposed that high temperatures used during spray drying may lead to an increased oxidation of unsaturated fatty acid and alteration of fatty acid composition (Ng et al., 2013). However, in the current study, an analysis of variance indicated the fatty acid compositions of oils before and after spray drying were not significantly changed ($p > 0.05$). Overall, saturated fatty acids (SAFA) were detected as ~4.0%, ~28.0%, and ~2.6% in the microcapsules containing canola, fish and flaxseed oils, respectively; while unsaturated fatty acids were detected as ~96.0%, ~72.0%, and ~97.4% in the microcapsules containing canola, fish and flaxseed oils, respectively (Table 5.2). Because the oils were rapidly encapsulated within the protein-carbohydrate wall matrix, the alteration of fatty acids in oils was suppressed (Reineccius, 2004b). In addition, the condensed wall structures prevented heat transfer and keep the core materials' (e.g., oils) temperature below 100 °C during the dehydration (Reineccius, 2004b). Therefore, the microcapsules design with LPI, sodium alginate, and maltodextrin to encapsulate oils using spray drying exhibited representative physical properties in comparison with other studies.

Table 5.2. Fatty acid profiles of free oils, oils extracted from microcapsules after spray drying, and released oils under simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) treatments from microcapsules with canola oil (a), fish oil (b), and flaxseed oil (c). Different small letters in the same row indicate a significant difference ($p \leq 0.05$) in the current study. Abbreviations include: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).

a. Canola oil microcapsules

Fatty acid	***Free oil***	***After spray drying***	***SGF***	***SGF + SIF***
C14:0	-	-	-	-
C16:0	1.50 ± 0.00	1.52 ± 0.02	1.57 ± 0.02	1.55 ± 0.02
C16:1 (n9)	1.18 ± 0.01	1.24 ± 0.04	1.25 ± 0.01	1.16 ± 0.02
C18:0	1.36 ± 0.03	1.33 ± 0.01	1.43 ± 0.07	1.32 ± 0.08
C18:1 (n9)	22.11 ± 0.16	21.79 ± 0.08	22.78 ± 0.53	20.86 ± 0.18
C18:1 (n7)	10.62 ± 0.12	10.63 ± 0.09	11.42 ± 0.63	10.23 ± 0.34
C18:2 (n6)	14.35 ± 0.03	14.39 ± 0.01	14.20 ± 0.16	14.74 ± 0.09
C18:3 (n6)	2.65 ± 0.04	2.63 ± 0.01	2.56 ± 0.17	2.65 ± 0.04
C18:3 (n3)	35.68 ± 0.24	36.01 ± 0.18	33.91 ± 1.21	37.17 ± 0.53
C20:0	1.21 ± 0.03	1.18 ± 0.01	1.33 ± 0.06	1.12 ± 0.01
C20:1 (n9)	9.33 ± 0.10	9.29 ± 0.09	9.57 ± 0.01	9.18 ± 0.05
C20:4 (n6)	-	-	-	-
C20:5 (n3)	-	-	-	-
C22:4 (n6)	-	-	-	-
C22:6 (n3)	-	-	-	-
SAFA	4.07 ± 0.05^{a}	4.04 ± 0.03^{a}	4.32 ± 0.16^{b}	4.00 ± 0.10^{a}
MUFA	43.25 ± 0.22^{a}	42.94 ± 0.16^{a}	45.01 ± 1.18^{b}	41.44 ± 0.47^{c}
PUFA	52.68 ± 0.12^{a}	53.02 ± 0.19^{ac}	50.67 ± 1.34^{b}	54.56 ± 0.58^{c}

Table 5.2. (continued).

b. Fish oil microcapsules

Fatty acid	***Free oil***	***After spray drying***	***SGF***	***SGF + SIF***
C14:0	25.60 ± 0.26	24.80 ± 0.21	23.99 ± 0.05	24.03 ± 0.52
C16:0	2.25 ± 0.02	2.20 ± 0.02	2.16 ± 0.01	2.16 ± 0.03
C16:1 (n9)	18.97 ± 0.18	18.45 ± 0.11	17.95 ± 0.07	17.94 ± 0.27
C18:0	0.90 ± 0.01	0.89 ± 0.02	0.88 ± 0.01	0.87 ± 0.01
C18:1 (n9)	1.18 ± 0.01	1.20 ± 0.01	1.26 ± 0.01	1.24 ± 0.03
C18:1 (n7)	4.65 ± 0.04	4.60 ± 0.05	4.54 ± 0.05	4.48 ± 0.04
C18:2 (n6)	0.31 ± 0.00	0.35 ± 0.00	0.39 ± 0.00	0.53 ± 0.03
C18:3 (n6)	0.29 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00
C18:3 (n3)	0.93 ± 0.00	1.21 ± 0.02	1.21 ± 0.02	1.41 ± 0.03
C20:0	0.14 ± 0.01	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00
C20:1 (n9)	0.75 ± 0.38	0.54 ± 0.01	0.54 ± 0.00	0.53 ± 0.00
C20:4 (n6)	0.27 ± 0.00	0.27 ± 0.00	0.27 ± 0.00	0.26 ± 0.00
C20:5 (n3)	30.57 ± 0.51	31.24 ± 0.16	31.68 ± 0.02	31.48 ± 0.43
C22:4 (n6)	0.44 ± 0.02	0.46 ± 0.01	0.50 ± 0.00	0.49 ± 0.02
C22:6 (n3)	12.74 ± 0.37	13.38 ± 0.15	14.22 ± 0.13	14.18 ± 0.48
SAFA	28.90 ± 0.31^{a}	28.03 ± 0.20^{b}	27.16 ± 0.04^{bc}	27.20 ± 0.56^{c}
MUFA	25.56 ± 0.59^{a}	24.79 ± 0.16^{ab}	24.30 ± 0.12^{b}	24.19 ± 0.33^{b}
PUFA	45.54 ± 0.89^{a}	47.18 ± 0.26^{ab}	48.54 ± 0.13^{b}	48.61 ± 0.89^{b}

Table 5.2. (continued).

c. Flaxseed oil microcapsules				
Fatty acid	***Free oil***	***After spray drying***	***SGF***	***SGF + SIF***
C14:0	-	-	-	-
C16:0	0.97 ± 0.02	0.95 ± 0.01	0.95 ± 0.00	0.97 ± 0.01
C16:1 (n9)	-	-	-	-
C18:0	1.37 ± 0.03	1.33 ± 0.01	1.35 ± 0.02	1.26 ± 0.04
C18:1 (n9)	3.31 ± 0.12	3.21 ± 0.02	3.29 ± 0.06	3.08 ± 0.11
C18:1 (n7)	1.86 ± 0.14	1.75 ± 0.01	1.74 ± 0.10	1.50 ± 0.15
C18:2 (n6)	5.07 ± 0.03	5.06 ± 0.01	5.11 ± 0.02	5.16 ± 0.06
C18:3 (n6)	-	-	-	-
C18:3 (n3)	84.44 ± 0.69	85.24 ± 0.11	85.04 ± 0.24	86.22 ± 0.79
C20:0	0.44 ± 0.05	0.36 ± 0.01	0.39 ± 0.02	0.28 ± 0.05
C20:1 (n9)	2.54 ± 0.30	2.11 ± 0.06	2.13 ± 0.04	1.55 ± 0.37
C20:4 (n6)	-	-	-	-
C20:5 (n3)	-	-	-	-
C22:4 (n6)	-	-	-	-
C22:6 (n3)	-	-	-	-
SAFA	2.78 ± 0.10^{a}	2.64 ± 0.02^{ab}	2.69 ± 0.03^{ab}	2.50 ± 0.11^{b}
MUFA	7.71 ± 0.56^{a}	7.06 ± 0.09^{ab}	7.17 ± 0.19^{ab}	6.13 ± 0.63^{b}
PUFA	89.51 ± 0.65^{a}	90.30 ± 0.10^{ab}	90.15 ± 0.22^{ab}	91.37 ± 0.73^{b}

5.4.2. Storage stability of free and encapsulated oils

Hydrolytic stability

FFAs are the result of hydrolytic rancidity of oils, and give an indication of the hydrolytic stability during processing and storage. FFAs have both hydrophilic and hydrophobic groups, which then can concentrate at the surface of oils to increase the diffusion of oxygen, and as such, is considered as a proxidant to accelerate lipid oxidation (Choe and Min, 2006; O'Connor et al., 2007). The FFA contents of the free and encapsulated canola, fish and flaxseed oils over 30 d of storage are determined in Figure 5.1. An analysis of variance indicated that both of microencapsulation and storage time were significant ($p < 0.05$) factors governing the FFA content, with the exception of canola oil ($p > 0.05$). The FFA contents in the free and encapsulated canola oil were similar before (~0.0038%) and after 1 month of storage (~0.0041%). Most likely, the FFAs were removed by a refining step during the production of the commercial canola oil (Rycebosch et al., 2013). Therefore, it was shown little hydrolysis occurred, in order to generate new FFAs during the storage of canola oil and microcapsules [Figure 5.1 (A)]. In contrast, chemical or enzymatic spoilage might be happened to generate FFAs in the fish oil and flaxseed oil (de Koning, 2001), which may not be refined during their production. In the present study, the FFAs in the free fish oil started to be generated at day 0 (~0.0028%) and then increased relatively at a constant rate until day 30 (~0.0055%) [Figure 5.1 (B)]. FFAs generation within the entrapped fish oil was slowed significantly, ending with a final concentration of ~0.0042% on day 30 [Figure 5.1 (B)]. In the case of flaxseed oil, generation of new FFAs in the free and entrapped oils was absent (~0.0046%) until day 15. Afterwards, the FFA content increased at a much greater rate in the free oil than the entrapped oil, rising to ~0.0063% and ~0.0051% at day 30, respectively [Figure 5.1 (C)]. The rates of FFA increase for the free and encapsulated oils were not stable (Figure 5.1), because hydrophilic groups in FFA attached to water molecules to decrease the sensitivity of color detection in the aqueous solution during titration to determine FFA content (O'Connor et al., 2007). However, in terms of FFA, all of the free and encapsulated oils in this study had acceptable quality (FFA < 0.15%) after 1 month storage (Sun-Waterhouse et al., 2011).

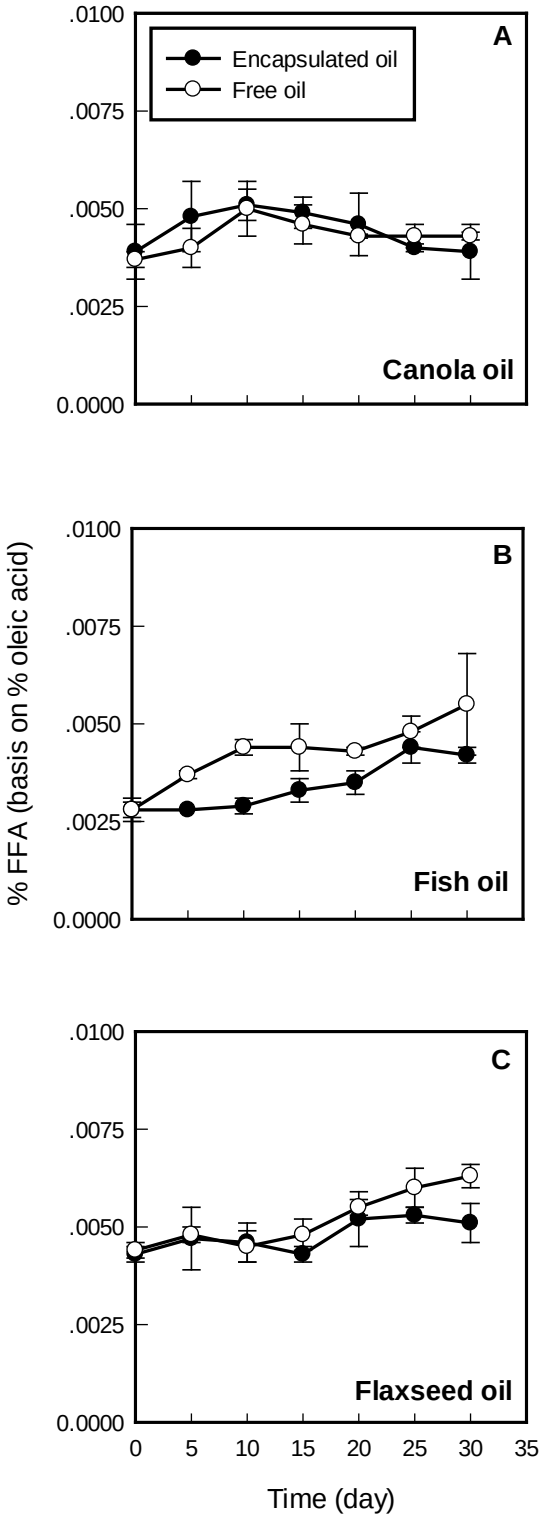


Figure 5.1. Changes in free fatty acid (FFA) content for the free and encapsulated canola oil (A), fish oil (B), and flaxseed oil (C) over 30 days of storage. Data represent the mean ± one standard deviation (n = 3).

Oxidative stability

a. Peroxide and 2-thiobarbituric acid reactive substances values

The PV is used to measure primary oxidative products (e.g., hydroperoxides) within the initial stage of lipid deterioration, whereas the TBARS test measures secondary oxidative products (e.g., aldehydes, ketones, and carbonyl compounds) from the decomposition of hydroperoxides (Pegg, 2005). PV and TBARS value of the free and encapsulated oils during storage are presented in Figure 5.2. An analysis of variance indicated that both PV and TBARS values of free and encapsulated oils were affected by both microencapsulation and storage time, along with their interaction ($p < 0.05$). Overall, the free and encapsulated canola oil experienced better oxidative stability than the free and encapsulated fish and flaxseed oils. Microencapsulation exhibited greater oxidative protection effect in fish oil and flaxseed oil than in canola oil. In case of canola oil, the encapsulated oil [PV: ~5.02 meq active O_2/kg; TBARS: ~0.820 MDA eq. (nmol/mg oil)] had slight lower PV and TBARS value than the free oil [PV: ~8.09 meq active O_2/kg; TBARS: ~0.983 MDA eq. (nmol/mg oil)] after 1 month storage, whereas the encapsulated fish oil [PV: ~5.34 meq active O_2/kg; TBARS: ~1.320 MDA eq. (nmol/mg oil)] and flaxseed oil [PV: ~9.59 meq active O_2/kg; TBARS: ~1.154 MDA eq. (nmol/mg oil)] showed much lower PV and TBARS values than the free fish oil [PV: ~13.04 meq active O_2/kg; TBARS: ~3.013 MDA eq. (nmol/mg oil)] and flaxseed oil [PV: ~18.73 meq active O_2/kg; TBARS: ~1.473 MDA eq. (nmol/mg oil)] after storage (Figure 5.2). In the comparison between fish oil samples and flaxseed oil samples, it is clear to see the combination of LPI, sodium alginate and maltodextrin provided better protection for fish oil to against the production of hydroperoxides. However, in terms of secondary oxidation products, the flaxseed oil samples showed a gentle increase on TBARS values in contrast with a dramatically rise for fish oil samples (Figure 5.2).

Several reasons could be contributed to the results. (1) Although microencapsulation can depress the oxygen diffusion into capsules, the changes of oxygen concentration were not stable, due to the complexity of wall structure (Imagi et al., 1992). (2) FFAs have amphiphilic properties to increase oxygen solubility in the fatty acids (Choe & Min, 2006), so, more oxygen would be dissolved into the free and encapsulated fish oil and flaxseed oil with higher amounts of FFAs (Figure 5.1) to result in oxidation. (3) The oils with more unsaturated fatty acids are more susceptible to oxidation (Choe and Min, 2006), so, it was proposed the encapsulated fish oil with higher amount of SAFA (~28.0%) should be more oxidative stable than others (Table 5.2). However, Sun-Waterhouse et al. (2011) detected the changes of fatty

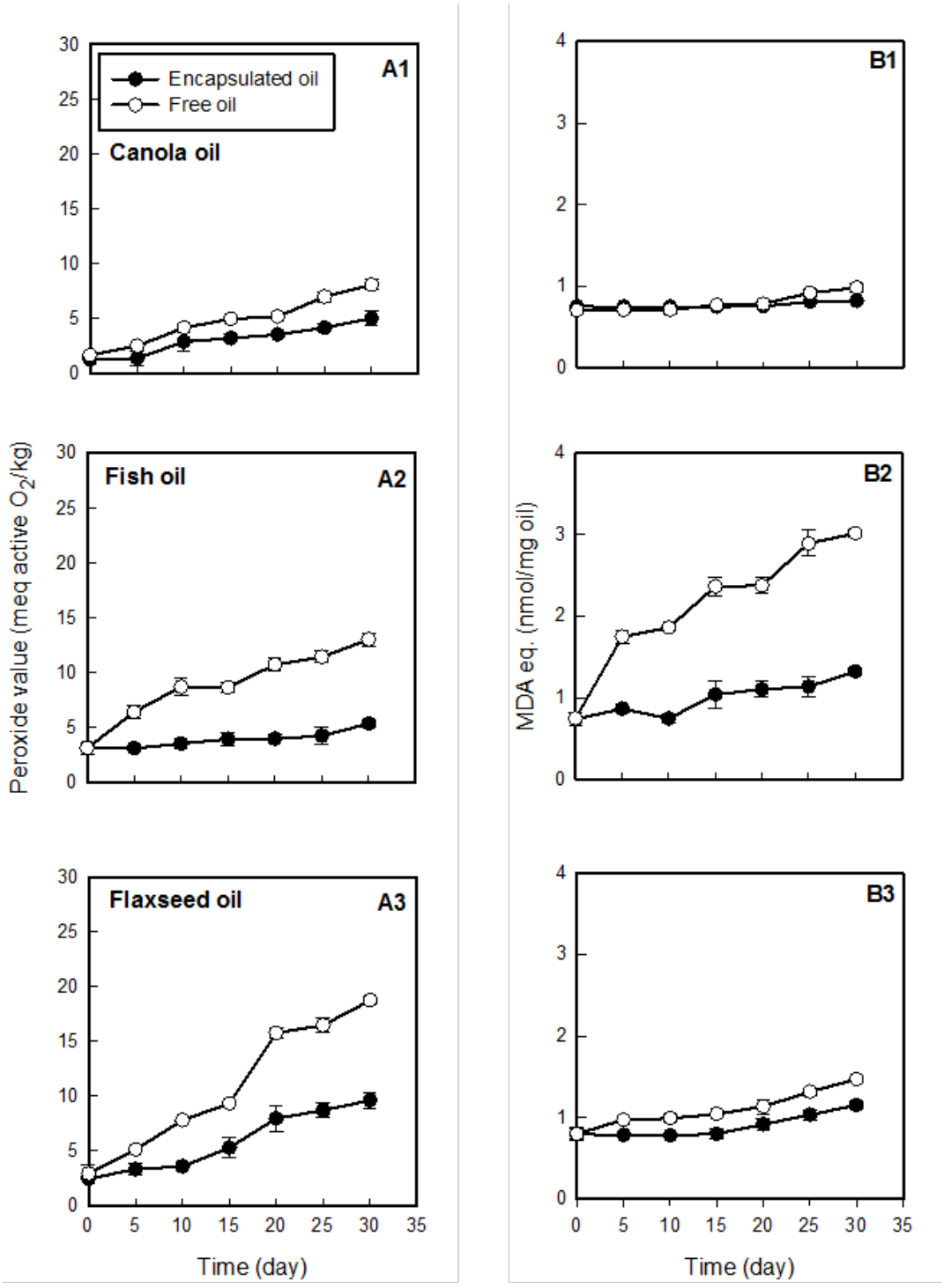


Figure 5.2. Changes in (A) peroxide value (PV) and (B) 2-thiobarbituric acid reactive substances (TBARS) for the free and encapsulated canola oil (1), fish oil (2), and flaxseed oil (3) over 30 days of storage. Data represent the mean ± one standard deviation (n = 3).

acid composition among the surface and encapsulated oils over 30 days of storage, and found more polyunsaturated fatty acids (PUFA), especially the PUFA with longer chain and more double bonds, were released to the surface of microcapsules during storage. Therefore, the encapsulated fish oil [with ~31.2% of EPA (C20:5, n3) and ~13.4% of DHA (C22:6, n3)] [Table 5.2 (b)] and flaxseed oil [with ~85.2% of ALA (C18:3, n3)] [Table 5.2(c)] would migrate to the surface of capsules to raise the oxidation rate. (4) Fatty acids with more double bonds have more sites for hydrogen abstraction and generate more free radicals to produce more hydroperoxides, which will be further decomposed to aldehydes or ketones (Choe and Min, 2006). Therefore, the fish oil samples (with EPA and DHA) exhibited higher TBARS values than the flaxseed oil samples (with ALA). (5) In the current study, the free and encapsulated canola oil had relatively low level of PV and TBARS values than other samples (Figure 5.2), due to the presence of tocopherols (e.g., α-, β-, and γ-tocopherol) to prevent oxidation in the canola oil (Pelser et al., 2007). However, starting on day 20, because of the degradation of tocopherols resulting from the oil degradation or oxidation, the TBARS values of free and encapsulated canola oils gently increased [Figure 5.2 (B1)], which was also demonstrated in the kenaf seed oil microcapsules with sodium caseinate, maltodextrin and lecithin (Ng et al., 2013). In the present study, the PVs of free and encapsulated oils (excepting the free flaxseed oil on day 30) were still fallen into the industrial acceptable level (PV < 18 meq active O_2/kg) for oil quality (Sun-Waterhouse et al., 2011) after 1 month storage.

In all cases, the storage time significantly increased PV and TBARS values in the free and encapsulated oils ($p < 0.05$), because physical and chemical changes of microcapsules and oil diffusion through the wall materials could be happened to release more oils to be prone to oxidation during storage (Aghbashlo et al., 2013). The permeation of oxygen through the wall to the inside of microcapsules is also another reason contributing to the increased oxidation during storage, which was also demonstrated in fish oil microcapsules prepared with whey protein isolate (Aghbashlo et al., 2013).

b. Oxidative stability index

Accelerated oxidative test using the Rancimat has been used to predict shelf-life of lipid foods during storage and evaluate the efficiency of antioxidants in a short time. During the test, oils are oxidized to short-chain volatile acids (e.g., carboxylic acids) that are then collected in distilled water to increase the electric conductivity, and the time (also known as OSI) required to induce a sharp increase of conductivity is recorded to indirectly measure the

oxidative stability. Therefore, the higher OSI indicates better stability under accelerated storage conditions (Gallardo et al., 2013). The OSI of the encapsulated and free oils with/without antioxidants (e.g., BHT and TBHQ) is shown in Figure 5.3. An analysis of variance indicated that both the addition of antioxidants and microencapsulation significantly improved the oxidative stability of the oils ($p < 0.05$), with the exception of BHT in fish oil ($p > 0.05$). Overall, the OSI increased in the following order: free oil < oil with BHT < encapsulated oil < oil with TBHQ. Interestingly, the encapsulated fish oil (OSI: 6.3 h) exhibited better oxidative stability than the oil with TBHQ (OSI: 5.0 h), because of the lower solubility of TBHQ in the oils with higher amount of saturated fatty acids and higher opportunity to generate free radicals from fatty acids with more double bonds (Martin-Polvillo et al., 2004; Hossain et al., 2010). Hossain and co-workers (2010) compared the antioxidative capacity between BHT and TBHQ in fish oil with tetradecane, and they found TBHQ (OSI: 39.9 h) displayed much lower antioxidative capacity than BHT (OSI: > 48 h) in the fish oil with tetradecane, due to the extremely low solubility of TBHQ in tetradecane (24.6%). In the present study, the fish oil had higher amount of SAFA (~28.9%) than canola oil (~4.1%) and flaxseed oil (~2.8%), which led to poorer solubility of TBHQ (Table 5.2). Moreover, due to the presence of EPA and DHA in the fish oil [Table 5.2 (b)], higher amount of free radicals were accumulated at the end of induction period (Martin-Polvillo et al., 2004). Therefore, TBHQ showed weaker antioxidative capacity in the fish oil than in canola oil and flaxseed oil. In theory, the porosity of the wall structure on the microcapsules affects the oxygen permeability to further determine the oxidative stability of the encapsulated oil (Imagi et al., 1992). Therefore, due to the exceptional OSIs obtained from the encapsulated oils, it is proved that the microencapsulation using LPI with maltodextrin and sodium alginate produced a highly compacted and strong wall matrix to protect oils from deteriorative oxidation, which expressed the comparative antioxidative capacity as synthetic antioxidants.

In general, synthetic antioxidants (e.g., BHT and TBHQ) are added to protect the oil quality and reduce the deterioration during the processing and storage. In the current study, the oils with BHT (canola oil: 18.6 h, fish oil: 1.9 h, flaxseed oil: 3.8 h) had slightly higher OSI than the free oils (canola oil: 17.8 h, fish oil: 1.7 h, flaxseed oil: 2.6 h), whereas the addition of TBHQ greatly improved the oil stability 3-5 fold (Figure 5.3). The efficiency of phenolic antioxidants is closely related to the number of hydroxyl groups on the aromatic ring, as well as the ability to provide hydrogen to peroxide radical to interrupt the propagation, and their polarity (McClements and Decker, 2007, Hossain et al., 2010). There

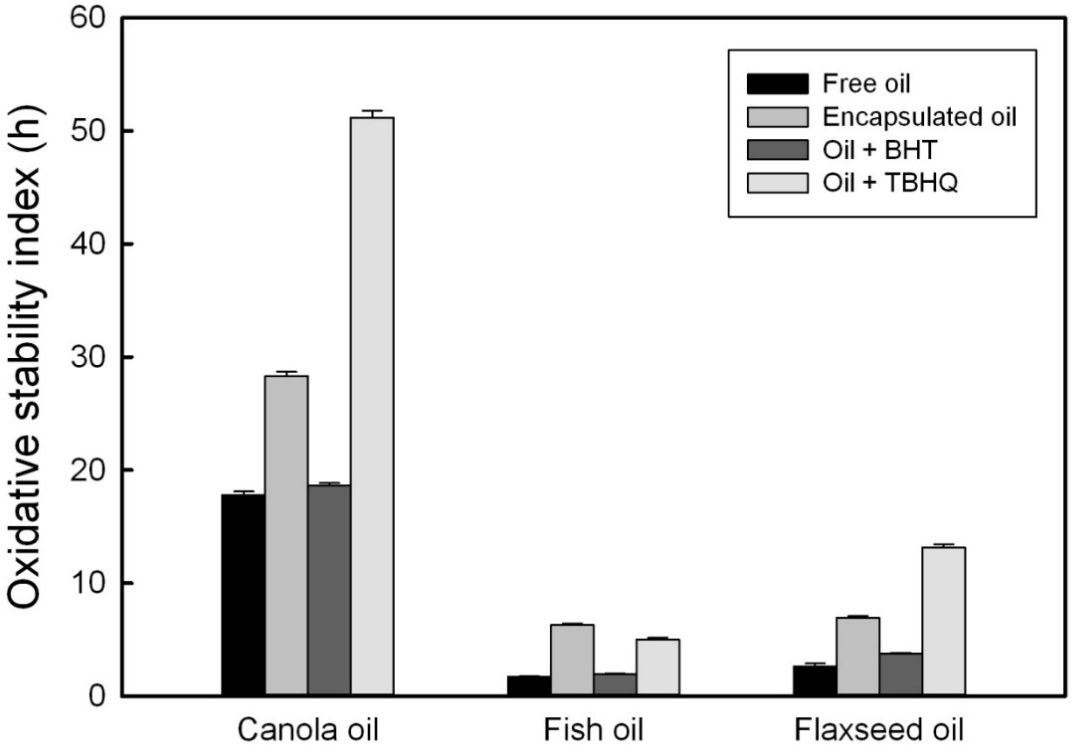


Figure 5.3. Oxidative stability index (h) of the encapsulated and free canola oil, fish oil and flaxseed oil with/without butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ). Data represent the mean ± one standard deviation (n = 3).

is only one hydroxyl group that is adjacent to the two *tert*-butyl groups on BHT to result in steric hindrance, so, BHT slowly reacts with peroxide radicals. However, two hydroxyl groups present on 1, 4 position to the aromatic ring on TBHQ, which easily donate hydrogens to peroxide radicals and finally produce stable quinone type compounds (McClements and Decker, 2007; Hossain et al., 2010). Moreover, TBHQ can be easily accumulated at the air-oil interface to prevent oxidation, due to its higher polarity (McClements and Decker, 2007). Therefore, TBHQ is a more effective antioxidant than BHT to protect oil quality.

5.4.3. In vitro release behavior of encapsulated oils

The release characteristics of encapsulated oils under simulated gastrointestinal tract conditions can have a big impact on the adsorption and accumulation of heart healthy fatty acids (e.g., ALA, EPA, and DHA) (Fathi et al., 2014). The amount of released oils (e.g., derived from canola, fish and flaxseed) from the microcapsules exposed to SGF and SIF is shown in Figure 5.4. An analysis of variance indicated that all of oil-type and digestive conditions, along with their interaction, significantly affect the oil release ($p < 0.05$). Overall, a much higher percent of released oil was observed in sequential exposure under SGF and SIF conditions (~66.6%), as compared to SGF condition alone (~5.2%) (Figure 5.4). The longer digestion process resulting in greater degradation of the microcapsules under SGF + SIF conditions could be attributed to this result, because pepsin (in SGF) and pancreatin (including amylase and trypsin, in SIF) can hydrolyze both proteins (e.g., LPI) and carbohydrates (e.g., sodium alginate and maltodextrin) to change the microcapsules' wall structure and produce more pores to release the oils (Goyal et al., 2015). However, due to the resistance of protein to peptic hydrolysis, only maltodextrin was hydrolyzed by the strong pH and ionic changes under SGF condition to release a small amount of oils (Fathi et al., 2014). In addition, because of the presence of surface oil, particle aggregation was happened to decrease the digestibility of microcapsules under SGF condition, whereas a longer period of mechanical stress applied on the microcapsules was helpful to break the hydrophobic interactions between particles to release more oils under SGF + SIF conditions.

In the current study, the microcapsules released a significant ($p < 0.05$) higher amount of canola oil (~8.9%) in comparison with fish oil (~3.2%) and flaxseed oil (~3.4%) under SGF condition, whereas more fish oil (~73.4%) was released from the microcapsules under SGF + SIF conditions (Figure 5.4). Different fatty acids compositions could be a reason for the results. It was observed that the released canola oil contained a significant ($p < 0.05$) higher amount of SAFA and monounsaturated fatty acids (MUFA) and lower amount of

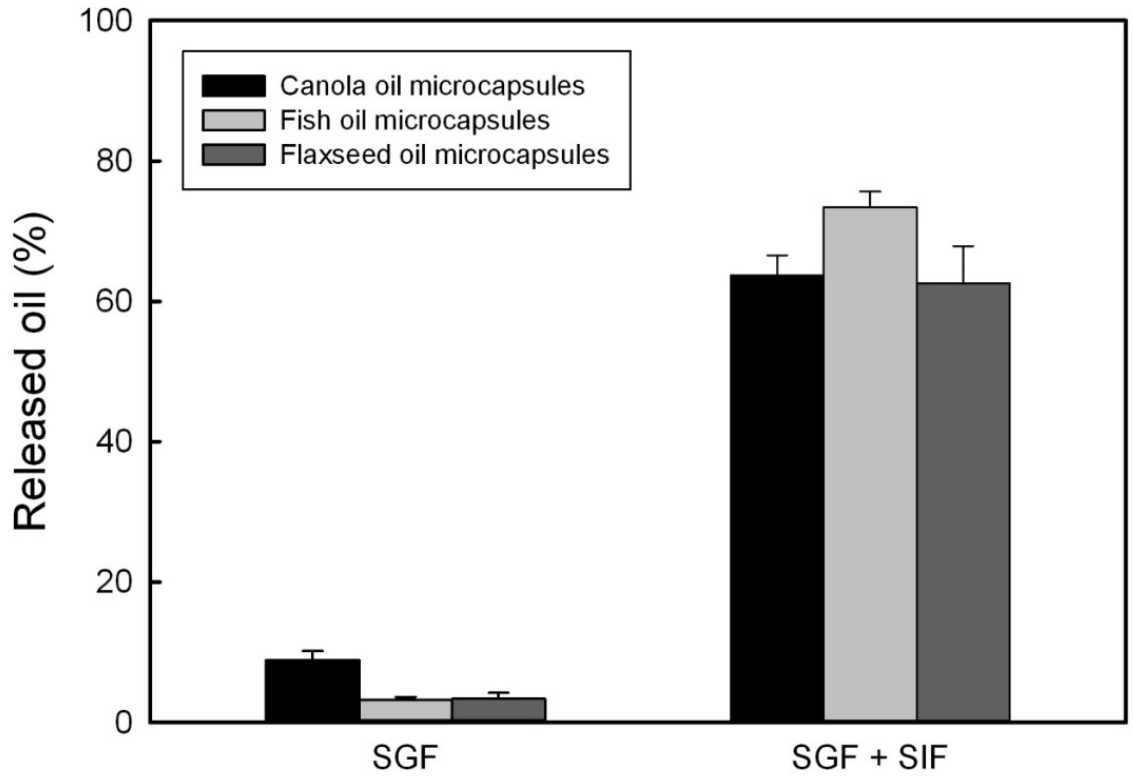


Figure 5.4. *In vitro* release behavior of canola oil, fish oil, and flaxseed oil from microcapsules under simulated gastric fluid (SGF) and sequential exposure to simulated gastric fluid and simulated intestinal fluid (SGF + SIF). Data represent the mean ± one standard deviation (n = 3).

polyunsaturated fatty acids (PUFA) under SGF condition comparing with the encapsulated canola oil, but there was no significant ($p > 0.05$) changes on fatty acids compositions in the released fish oil and flaxseed oil (Table 5.2). In theory, fatty acids with lower hydrophobicity (with shorter chain length and less double bond) exhibit better ability to penetrate through the cell membrane and higher rates of absorption and metabolism (Matsuno and Adachi, 1993), so, canola oil was more easily released under SGF condition. Furthermore, Pourashouri et al. (2014) demonstrated that more SAFA [e.g., palmitic acid (C16:0) and stearic acid (C18:0)] stayed in the surface oils, whereas PUFA [e.g., EPA (C20:5, n3) and DHA (C22:6, n3)] proportion was greatly higher in the encapsulated oils, and no significant difference was detected for MUFA [e.g., sapienic acid (C16:1, n9) and oleic acid (C18:1, n9)] when comparing the surface oils with the encapsulated oils. They found PUFA was hardly to attain in the surface oil at the extremely high temperature (180 °C) during spray drying and closely bound with wall materials of microcapsules. Therefore, more encapsulated fish oil (with EPA and DHA) was released after a longer digestive treatment (with SGF and SIF).

The amounts of released oils under SGF condition are in agreement with the findings from Shen et al. (2011) and Goyal et al. (2015) working on the fish oil microcapsules with sodium caseinate, glucose monohydrate, and corn starch and the flaxseed oil microcapsules with whey protein concentrate and lactose, respectively. However, Goyal et al. (2015) reported only ~23.1% of released flaxseed oil under SGF + SIF conditions, which was much lower as compared with ~62.6% in the current study, because the globular conformation of whey protein concentrate was highly resistant to enzymatic hydrolysis during digestion. On the other hand, Can Karaca et al. (2013a) reported very high amounts of flaxseed oil were released under SGF (~37%) and SGF + SIF (~84%) conditions from microcapsules prepared with chickpea protein/lentil protein and maltodextrin. This large difference could be caused by the different wall materials, in which sodium alginate and LPI produced a much stronger complex matrix in the current study.

5.5. Conclusions

In the present study, canola, fish and flaxseed oils (containing relative high amount of unsaturated fatty acids) were stabilized through microencapsulation developed by the combination of LPI, sodium alginate and maltodextrin. Spray drying was demonstrated to be a good microencapsulation technique for these oils, due to the negligible effect on fatty acids profiles, and gave microcapsules with good physical properties (e.g., moisture content, water activity, wettability, particle size, surface oil, and entrapment efficiency). The combination of

LPI, sodium alginate and maltodextrin exhibited great protection to susceptible oils (e.g., canola, fish, and flaxseed oils) from hydrolytic rancidity and oxidative degradation over 30 days of storage, and offered greater antioxidative capacity than synthetic antioxidants in the case of fish oil. However, due to differences on oil processing steps, fatty acid composition, FFA content and oxygen diffusion, different oils had various rates of hydrolysis and oxidation. *In vitro* release test showed that the amount of released oils was higher under sequential exposure of SGF + SIF conditions than that of SGF condition, in which more encapsulated canola oil was released under SGF condition, whereas the addition of SIF stimulated more fish oil to be released from microcapsules. Therefore, the oils had non-negligible impacts on the storage stability and *in vitro* release behavior. The microcapsules formulated in the current study could be potentially used as a universal platform to fortify high value omega fatty acids-rich oils in commercial food and supplementary products.

6. GENERAL DISCUSSIONS

Omega fatty acids-rich oils (e.g., canola oil, fish oil, and flaxseed oil) are gaining increasing interest in the food industry because of their numerous health benefits (e.g., improving cardiovascular and mental health, preventing immune response disorders, providing anti-inflammatory effects, and playing a positive role in early childhood development). However, the major hurdles associated with the incorporation of the omega fatty acids-rich oils into food products are their insolubility in water and susceptibility to oxidative deterioration (especially when exposed to oxygen, light, moisture, and heat), which decreases shelf-life, produces off-flavors, and negatively affects consumers' acceptability of developed products (Velasco et al., 2003; Bakry et al., 2016). Therefore, microencapsulation, which involves coating bioactive particles or droplets (e.g., omega fatty acids-rich oils) within a biopolymer matrix to produce microcapsules, is considered as a viable method to protect those high value oils and maintain their biological and functional properties (Gharsallaoui et al., 2007; Bakry et al., 2016). Proteins extracted from animals (e.g., whey proteins, gelatin, and casein) and from plants (e.g., soy proteins, pulse proteins, and canola proteins) are widely used for the microencapsulation of omega fatty acids-rich oils, because of their biocompatibility, biodegradability, functional properties, and emulsifying capacity (Nesterenko et al., 2013). Over the past decades, the application of plant proteins as an alternative to animal-derived proteins in food products has become an increasingly interesting area for research, due to increased perceived safety concerns of animal-derived products, religious preference, and economic benefits. Therefore, the preparation of microcapsules has been turning towards plant proteins as preferred wall materials of the future.

A prerequisite to effectively encapsulate omega fatty acids-rich oils is to create a stable emulsion, in which oil droplets are fully covered by protein films to produce microcapsules with maximum entrapment efficiency and better oxidative stability. In theory, during emulsion formation, proteins migrate to the interface and re-orient to expose both hydrophobic and hydrophilic moieties, allowing them to become integrated with the oil-water interface to effectively reduce interfacial tension. Subsequently, a viscoelastic protein film is formed to partially cover the droplet surface to further prevent droplet aggregation and

coalescence, so as to stabilize the emulsion (McClements, 2005; Morris and Gunning, 2008). Stabilization typically occurs through electrostatic repulsion at pHs away from the protein's isoelectric point or steric hindrance (Morris and Gunning, 2008).

In the present research, the physicochemical, interfacial, and emulsifying properties of pea protein isolate (PPI), soy protein isolate (SPI), lentil protein isolate (LPI) and canola protein isolate (CPI) at pH 3.0, 5.0, and 7.0 were investigated to produce a stable emulsion, in which the emulsifying and interfacial properties of proteins were found to be dependent on their surface charge, surface hydrophobicity and solubility. Surface charge was found to change from positive to negative as pH increased from 3.0 to 7.0, reflecting the solvent pH being below and above the protein's isoelectric point, respectively. Surface hydrophobicity influences the ability of a protein to adsorb to the oil-water interface (McClement, 2005). For most of protein isolates (except PPI), hydrophobicity at pH 3.0 was higher than at pH 5.0, followed by at pH 7.0, in which LPI showed greater hydrophobicity than others at pH 3.0. Solubility determines the amount of protein that is available to migrate to the oil-water interface to stabilize the emulsion (Liang and Tang, 2013). Regardless of protein type, solubility was found to be similar at pH 3.0 and 7.0 which was higher than at pH 5.0. CPI (~90.4%) had the highest solubility at pH 3.0, followed by SPI (~67.8%), LPI (~56.2%) and PPI (~13.6%). In general, proteins are solubilized in the aqueous phase and accumulate at the oil-water interface to lower the interfacial tension during emulsion formation (Damodaran, 1996). In the current research, the addition of all protein isolates greatly lowered the interfacial tension at all pHs, in which the ability for all proteins to lower the interfacial tension was similar at pH 3.0 and 5.0 (~14 mN/m), however was significantly improved at pH 7.0 (~10 mN/m), due to the formation of the viscoelastic layers at the interface via hydrophobic interaction at pH 3.0 and aggregation of the relatively neutral proteins at pH 5.0 (Tcholakova et al., 2006). Therefore, all soluble and insoluble protein isolates played a role to lower the interfacial tension. This was also the case for β-lactoglobulin at pH 3.0 and 5.0 with large differences on the physicochemical properties (Lam and Nickerson, 2014). The long-term stability of emulsions was significantly affected by the interfacial rheology, which is determined by the formation of a viscoelastic protein film at the interface (Bos and Van Vliet, 2001). For the time sweep test, the interfacial storage modulus (G_i') was higher than the interfacial loss modulus (G_i'') at pH 3.0 and 5.0 (except PPI at pH 5.0) indicating the formation of a viscoelastic protein film through protein-protein interactions and the rearrangement of the protein's tertiary structure. In contrast, no interfacial film was formed at pH 7.0 because $G_i' < G_i''$. In the frequency sweep test, CPI showed a better ability to form a

stronger interfacial film than LPI, followed by PPI and SPI at pH 3.0 and 5.0. The stronger intermolecular interaction between adsorbed proteins with higher hydrophobicity at the interface could contribute to this result (Lucassen-Reynders et al., 1975). This was also demonstrated on the interfacial properties of β-casein and β-lactoglobulin (Seta et al., 2014). Finally, emulsion stability was found to be similar in magnitude at pH 3.0 and 7.0 for PPI, SPI, and LPI, due to the similar droplet size at pH 3.0 and 5.0. However, all of them were found to be unstable at pH 5.0, which was close to the pI of the legume proteins. In contrast, CPI only created a stable emulsion at pH 3.0, whereas the emulsions stabilized by CPI at pH 5.0 and 7.0 were unstable, because the lower surface charge resulted in droplet flocculation during the gravitational creaming experiment. Overall, due to the relative high surface charge, solubility and hydrophobicity, LPI at pH 3.0 was selected as a proper emulsifier to create stable emulsions for encapsulation purposes.

The selection of appropriate wall materials is the next fundamental step to develop stable microcapsules with high entrapment efficiency. The wall materials play an important role in protecting the core materials (e.g., omega fatty acids-rich oils) against oxidative deterioration, control the release of bioactive ingredients, and improve the storage stability under environmental stresses. Nesterenko and co-workers (2013) demonstrated that the combination of proteins and polysaccharides used as wall materials offered desirable characteristics to develop microcapsules, such as good emulsifying properties to stabilize high value oils and lower viscosity under high concentrations. Therefore, LPI (2-8% w/w in initial emulsions) at pH 3.0 combined with other wall materials (e.g., maltodextrin, sodium alginate, and lecithin) was initially studied to encapsulate canola oil (20-30% w/w in final microcapsules) using spray drying, which is the most commonly used drying technology for microencapsulation. Maltodextrin (DE: 9.0-12.0) was used as a processing aid during microencapsulation (Madene et al., 2006); sodium alginate increased the viscosity of the continuous phase, and formed the electrostatic complex with LPI at pH 3.0 as a second wall barrier (Guzey and McClements, 2006; Zhang et al., 2015a; Chang et al., 2016); lecithin was thought to improve emulsion stability, due to its amphiphilic nature (Carvalho et al., 2014). Both the emulsion properties (e.g., emulsion stability, viscosity, and droplet size) and microcapsules' properties (e.g., surface oil and entrapment efficiency) were analyzed to determine the best capsule design. As LPI and oil concentrations increased, droplet size, viscosity, and surface oil increased, whereas entrapment efficiency decreased. The addition of lecithin negatively affected the microcapsules' properties, which was thought to be due to competition with LPI molecules at the oil-water interface, which resulted in the depletion of

one of them. Therefore, the combination of LPI (2% w/w in the initial emulsion), maltodextrin, and sodium alginate was determined as the best capsule design to encapsulate canola oil (20% w/w in final microcapsules). Since physical properties (e.g., moisture content, water activity, color, wettability, and particle size) and oxidative stability are important for industrial application, the LPI-MD-SA microcapsules were compared with the LPI-MD microcapsules (presented as a control). It was found LPI-MD-SA microcapsules had a lower moisture content, water activity and smaller particle size, which are beneficial to prolong the shelf-life and depress the possibility of oxidation. Oxidative stability test was performed on peroxide value (PV, an indicator of primary lipid oxidation) and 2-thiobarbituric acid reactive substances (TBARS, an indicator of secondary lipid oxidation). The encapsulated canola oil in the LPI-MD microcapsules showed similar oxidative stability as the free canola oil, which were less stable than the oil encapsulated in the LPI-MD-SA microcapsules. A number of reasons can contribute to this result: (1) the LPI-MD-SA emulsions had larger droplet size to offer less surface area for air diffusion (Heinzelmann and Franke, 1999); (2) the combination of LPI, maltodextrin, and sodium alginate effectively covered oil droplets to produce microcapsules with less exposed surface oil and higher entrapment efficiency; and (3) the LPI-MD-SA microcapsules had a less porous and more complex wall structure to protect the core material. Therefore, the LPI-MD-SA system was selected as the most effective capsule design to encapsulate other omega fatty acids-rich oils.

It is well known that omega fatty acids (e.g., omega-3, -6, and -9 fatty acids) have demonstrated beneficial effects towards cardiovascular diseases, hypertension, and visual and brain development. The consumption of omega fatty acids-rich oils (e.g., canola oil, fish oil, flaxseed oil) has increased over the last decades. Canola and flaxseed oils are good sources of oleic acid, linoleic acid, and α-linolenic acid (ALA), whereas fish oil represents the most widely used source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential fatty acids to maintain cardiovascular, mental, and immune system health (Ruxton et al., 2007; Jordan, 2010). However, due to their polyunsaturated structure, they are chemically and enzymatically unstable during storage. In the present research, these oils were encapsulated within the LPI, sodium alginate, and maltodextrin wall matrix. The oil-type did not significantly affect the physical properties of microcapsules, with the exception of color, which was determined by the original oil color. Fortunately, the moisture content (~3.5%), water activity (~0.35), and particle size (~8.9 μm) of the microcapsules reached the industrial standards for a shelf stable dried powder. Storage stability (including hydrolytic stability and oxidative stability) of the encapsulated oils over 30 days storage period was investigated at

room temperature. The encapsulated canola oil exhibited better storage stability, because of the refining step employed in commercial production (free fatty acids removed) and lower degree of unsaturation. However, due to the presence of multiple sites of unsaturation (e.g., EPA and DHA), the encapsulated fish oil had significantly higher TBARS values than the encapsulated flaxseed oil during storage. In order to evaluate the antioxidative efficiency of microencapsulation, their oxidative stability index (OSI) was measured using a Rancimat for the encapsulated and free oils with/without antioxidants [e.g., butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ)]. Mostly, the OSI had the following order: free oil < oil with BHT < encapsulated oil < oil with TBHQ. Interestingly, the encapsulated fish oil displayed a higher OSI than the oil with TBHQ, which indicated that microencapsulation using LPI, maltodextrin, and sodium alginate exhibited more efficient protection to lipid oxidation than the synthetic antioxidants (e.g., BHT and TBHQ) for fish oil.

The release characteristics of the encapsulated oils were studied under simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), in which ~67% of the encapsulated oils were released under the sequential exposure of SGF and SIF conditions, as compared to SGF condition alone (~5%), because of greater degradation of the microcapsules under longer digestive process and the breakage of electrostatic bonding between LPI and sodium alginate by the alternation of solution pH (6.8). In addition, higher amount of canola oil was released under SGF conditions than others, since fatty acids with shorter chain length and less double bond were more easily penetrated through the wall materials (Matsuno and Adachi, 1993), whereas more fish oil was released under SGF + SIF conditions, because polyunsaturated fatty acids hardly stay at the surface of microcapsules and need a longer time to be released (Pourashouri et al., 2014).

It is very necessary to study the bioavailability of the encapsulated oils in specific regions within the digestive system and to investigate the interactions between proteins and encapsulated oils in the future. However, the present research demonstrated that LPI can be used as a promising emulsifier to produce a stable emulsion. The combination of LPI, maltodextrin, and sodium alginate can effectively encapsulate omega fatty acids-rich oils, in order to protect those high value oils against oxidative deterioration and prolong the shelf-life.

7. GENERAL CONCLUSIONS

The overall goal of this research was to improve the oxidative stability of omega fatty acid-rich oils (e.g., canola, fish, and flaxseed oils) during storage by encapsulation technique using plant protein-based matrices. Specifically, a pre-encapsulation screening process was performed to select a protein isolate (from pea, soy, lentil and canola protein isolates) at a proper pH (e.g., pH 3.0, 5.0 and 7.0) to effectively stabilize the oil-in-water emulsion. Then, the selected protein isolate was used as a fundamental wall material to develop stable microcapsules with other wall materials (e.g., maltodextrin, lecithin, and sodium alginate), in which emulsion properties (e.g., droplet size, viscosity, and emulsion stability), microcapsules' properties (e.g., moisture content, water activity, color, wettability, particle size, surface oil, and entrapment efficiency), and oxidative stability (e.g., PV and TBARS) were investigated to determine the best capsule design. Finally, the best capsule design was used to entrap different omega fatty acid-rich oils (e.g., canola, fish, and flaxseed oils) to study their storage stability and release characteristics.

In the first stage of this research, the effect of pH on the physicochemical, interfacial, and emulsifying properties of pea, soy, lentil and canola protein isolates was studied, in order to select one protein/pH to produce a stable emulsion. Findings suggested that for a protein to be a good emulsifier should have the following attributes: (1) it should have high surface charge to be easily solubilized and provide repulsive force once coated on the oil droplets; (2) it should have good solubility to be readily adsorbed to the interface; and (3) it should have high surface hydrophobicity to form a stronger viscoelastic protein film via intermolecular interactions. Overall, proteins with high surface charge and low hydrophobicity had better ability to lower interfacial tension, whereas proteins with high surface charge and high hydrophobicity can form stronger viscoelastic films at the interface. Therefore, the selection of an effective plant protein emulsifier really entails finding a balance between properties needed to associate at the oil-water interface with those needed to develop a strong interfacial film. LPI was selected as a promising emulsifier to produce a stable emulsion, because of its high surface charge, solubility, and hydrophobicity at pH 3.0.

In the second stage of this research, a mixture of LPI and maltodextrin with/without lecithin and/or sodium alginate was studied to develop stable microcapsules using spray drying, in order to offer good physical properties and provide protection against oxidation. Initially, canola oil (20-30% w/w in final microcapsules) was entrapped using the mixture of LPI (2-8% w/w) and maltodextrin (9.5-18% w/w). Emulsion and microcapsules' properties were analyzed to select the proper capsule design. Due to the advanced emulsion properties and the highest entrapment efficiency, microcapsules prepared using 20% oil, 2% LPI and 18% maltodextrin was selected for further re-design to improve the entrapment efficiency using different preparation conditions and wall materials. Finally, the microcapsule with 2% LPI, 17% maltodextrin and 1% sodium alginate was determined as the best capsule design to offer good physical properties and effective protection against oxidation, which indicated the addition of negative charged polysaccharide is necessary to strengthen the wall structure of microcapsules by the electrostatic complexation with the positive charged protein.

In the third stage of this research, different omega fatty acids-rich oils (e.g., canola, fish, and flaxseed oils) were encapsulated using the combination of LPI, maltodextrin, and sodium alginate, followed by the assessment of physical properties, storage stability and *in vitro* release behavior of the encapsulated oils. Overall, all microcapsules displayed similar physical properties (except the color). The microencapsulation offered great protection to the oils against hydrolysis and oxidation over the 30 d of storage at room temperature. The combination of LPI, maltodextrin, and sodium alginate even provided greater antioxidative capacity than the synthetic antioxidants (e.g., BHT and TBHQ) to protect fish oil. However, because of the difference on the fatty acid composition, processing steps, free fatty acid content, and air diffusion, different oils exhibited various rates of hydrolysis and oxidation. *In vitro* release tests showed that only minor amounts of oils (~5%) were released under SGF condition, whereas the majority of oils (~67%) with higher concentrations of polyunsaturated fatty acids were released under sequential exposure of SGF and SIF. Therefore, the storage stability and release characteristics were significantly influenced by the oils.

Based on the great physicochemical and functional properties, LPI represented as an effective emulsifier that is alternative to soy and animal-derived proteins and produced a stable oil-in-water emulsion for microencapsulation. The combination of LPI, maltodextrin and sodium alginate can be applied as appropriate wall materials to encapsulate omega fatty acids-rich oils to further provide excellent physical properties and antioxidative capacity, in order to be potentially used as a universal platform in commercial food and supplement products to enhance the delivery of healthy oils.

8. FUTURE STUDIES

Omega fatty acids are generally recognized for their ability to maintain human health and reduce the risk of diseases, so, food products fortified by omega fatty acids-rich oils have been attracted more attention. However, due to their susceptibility to oxidation and insolubility in water, incorporation of those oils in food products is particularly challenging. Microencapsulation is considered as an effective way to solve these problems. In the current research, 20% of omega fatty acids-rich oils (e.g., canola, fish, and flaxseed oils) were encapsulated using the mixture of LPI, maltodextrin, and sodium alginate to produce microcapsules with relative higher entrapment efficiency (~88%) in comparison with other studies (Aghbashlo et al., 2013; Can Karaca et al., 2013a; Goyal et al., 2015; Intarasirisawat et al., 2015). Ideally, the food industry targets to entrap 30-70% of oils to produce microcapsules with < 2% surface oil and > 98% entrapment efficiency (Drusch and Berg, 2008; Nickerson et al., 2014). It has been reported that lower amounts of oil (5-50%) could be encapsulated using spray drying comparing with complex coacervation (40-90%) (Desai and Park, 2005; Ray et al., 2016). Therefore, further work is needed to increase the entrapped oil content with desirable entrapment efficiency. Gharsallaoui and co-workers (2007) stated that wall materials play an important role in the determination of the emulsion properties to further significantly influence the efficiency of oil encapsulation. Modification could be explored as a way to improve the emulsifying properties of LPI to further improve the properties of developed microcapsules. For instance, Zhang et al. (2015b) developed fish oil microcapsules using the Maillard reaction products (HSPI-MD) of partially hydrolyzed SPI (using Neutrase at 54 °C) and maltodextrin (DE 8-10) by freeze drying. They found that HSPI-MD conjugates-based microcapsules had much higher entrapment efficiency (87%) than the microcapsules prepared with native SPI and maltodextrin (66%) and microcapsules prepared with partially hydrolyzed SPI and maltodextrin (57%), because the limited hydrolysis improved the emulsifying properties of SPI by breaking SPI into short chain length, which were interact with maltodextrin to produce a strong structural matrix to encapsulate fish oil. Xiao et al. (2011) applied complex coacervation between SPI (after ultrasonic treatment to increase solubility) and gum Arabic (at 1:1 ratio, pH 4) to encapsulate

sweet orange oil (10-70%) with relative high entrapment efficiency (70-85%). In the work by Tamjidi et al. (2013), fish oil (33% w/w in the final microcapsules) was encapsulated within the gelatin-acacia gum coacervates using complex coacervation and reached ~92% entrapment efficiency. Moreover, Tamm et al. (2016) studied antioxidant property of β-lactoglobulin hydrolysates in spray dried fish oil microcapsules. They found enzymatic hydrolysis using trypsin greatly improved antioxidant property of β-lactoglobulin (degree of hydrolysis was 6%), because the hydrolysis enhanced the effectiveness of peptides to adsorb at the oil-water interface to increase the accessibility of antioxidant amino acids (e.g., cysteine, methionine, and glutathione), and small peptides (< 1 kDa) in hydrolyzed β-lactoglobulin exhibited increased iron chelating activity (O'Loughlin et al., 2015). Therefore, the combination of chemical reaction (e.g., Maillard reaction and electrostatic complexation), modification of LPI (e.g., physical, chemical, and enzymatic) and different microencapsulation techniques could be tried to encapsulate a higher amount of omega fatty acid-rich oils to produce microcapsules with greater entrapment efficiency and oxidative stability.

The controlled release of omega fatty acids-rich oils are one the most important aspects of microencapsulation. It was observed that only ~67% of encapsulated oils were released under simulated gastrointestinal fluids in the current research, so, part of the oils were complexed with wall materials within microcapsules as the waste. Due to the differences in solubility, strength of wall structure, and wettability, the choice of wall materials exhibits a great impact on the release of encapsulated oils during *in vitro* digestion (Bakry et al., 2016). Binsi et al. (2017) produced microcapsules using fish roe with/without gum Arabic by spray drying. They found the addition of gum Arabic not only greatly increased entrapment efficiency from 72% to 97%, but it also improved oil release (from 87% to 96%) under simulated gastrointestinal fluids, because protein aggregation was happened within the microcapsules without gum Arabic to entrap oil globules inside of the aggregated protein mass during spray drying, whereas the protein aggregation was minimized in the microcapsules with gum Arabic to increase the exposure of proteolytic enzymatic degradation. In the work by Can Karaca et al. (2013a), about 84% of encapsulated flaxseed oil was released from microcapsules (with ~84% entrapment efficiency) prepared by only chickpea or lentil protein isolates with maltodextrin under *in vitro* digestive process. Thereby, the adjustment of wall materials (e.g., addition or removement of polysaccharides) could be investigated to stimulate the release of encapsulated oils under gastrointestinal fluids, but remain/improve the entrapment efficiency. Furthermore, the reaction between encapsulated

oils and proteins may have relevant incidence to depress the release of encapsulated oils and lead to the loss of nutritional value (e.g., loss of essential amino acids). As early as last century, a number of studies have demonstrated the reaction of oils with amino acids to result in the loss of methionine, tryptophan, histidine, and lysine, but the interaction was subjected under high relative humidity (≥ 80%) at high temperature (e.g., 50 °C) (Gardner, 1979; Matoba et al., 1984). Very little information is available to understand how the reaction proceeds at low water activity (0.2-0.4) with room temperature, and how the reaction affects the bioavailability of encapsulated oils from microcapsules. Overall, much effort through research is still needed to develop wall materials and identify appropriate encapsulation techniques, in order to improve the bioavailability of encapsulated omega fatty acid-rich oils and optimize the microencapsulation with suitable oil content and entrapment efficiency.

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