Inulin-monoglycerides emulsion gel as potential fat replacer and effect of inulin to delay lipid oxidation

by

Feijie Li

A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements for the degree of

Master of Science

in

Chemistry
(Food Science and Nutrition)

Carleton University
Ottawa, Ontario

Abstract

Inulin (oligo/polysaccharide) has gained increased interest due to its functionality and health benefits. This research was aimed to investigate potential applications of emulsion gel incorporating Jerusalem artichoke (JA) inulin to replace/reduce fat in food products as well as effect of JA inulin on lipid oxidation. Physical characteristics of emulsion gels with purified JA inulin (1%) were characterized by both light and cryo-environmental scanning electron microscopes. Incorporation of JA inulin developed a smaller fat globule size compared to the control. JA inulin could inhibit oxidation of aqueous dispersion of linoleic acid with active concentration range of 0.08-0.33 mg/ml. It was concluded that JA inulin had a positive effect on emulsion gel, which could potentially act as a fat replacement and could delay lipid oxidation.

Acknowledgements

First of all, I would like to give my deepest gratitude to my supervisor, Dr. Farah Hosseinian for her patient and professional guidance during my research. I appreciate being able to work together with her, who has passion for research and teaching which makes me interested in research. Each time after we discussed project or some questions that confused me, I always could get some new ideas and solutions from her professional knowledge and experience. My project could not have been completed smoothly without her academic support and encouragement.

I would also like to thank all the members in our lab which is like a big family that gives me a warm and love environment during my study life. I wish to thank Dr. Aynur Gunenc for her experienced research guidance and valuable advice which facilitates my research. Thanks to Nasim Meshginfar for her help in my research and life. Thanks to Hengguang Xu for his help with HPLC and freeze dryer.

I am deeply grateful for help from many faculty and staff members in our Chemistry Department. Thanks to Dr. Robert Crutchley for his advice and encouragement in my seminar presentations. Thanks to Ms. Chantelle Gravelle for her kindness and help all the time during my graduate study. Thanks to Dr. Jianqun Wang for his knowledge and skill with scanning electron microscope. Thanks to Peter Mosher and Shari Levac for their help during my research. Thanks to Jim Logan for his kindness and technical support.

Thanks to my friends, Ramak Esfandi, Achini Japahuge, Mallory Waters and Wenyu Zhang for their help in my study and life.

I would also like to thank for funding from Natural Science and Engineering Research Council of Canada.

Lastly but not least, my deepest gratitude is dedicated to my family. Thanks to my dear husband, Lingheng Meng, for your love, encouragement and support at all times. Thanks to my parents and parents in law for their understanding, support and encouragement all the time, which makes me focused on my study. Thanks to my brother and sister who provide help and support when I need it.

Table of Contents

Abstract ii

Acknowledgements iii

Table of Contents v

List of Tables ix

List of Figures x

List of Appendices xiii

List of Abbreviation xiv

Chapter 1: Literature review 1

1.1 Jerusalem artichoke 1

1.2 Chemical structure, physiochemical property and functionality of inulin 3

1.2.1 Basic property of inulin 3

1.2.2 Functionality of inulin 5

1.2.2.1 Fat replacement 5

1.2.2.2 Prebiotics 6

1.2.2.3 Drug delivery carrier 7

1.3 Emulsions 9

1.3.1 Emulsifiers 9

1.3.2 Hydrophilic-lipophilic balance (HLB) 11

1.3.3 Factors influencing the stability of food emulsions 13

1.3.4 Emulsion applications 13

1.4 Gels 13

1.5 Lipid oxidation 14

1.6 Oxidative stress and antioxidants **16**

1.6.1 Oxidative stress and diseases **16**

1.6.2 Antioxidants and mechanisms **16**

1.7 Artificial cell membranes and applications. **17**

1.7.1 Biological membrane modeling **17**

1.7.2 Lecithin composition **17**

1.8 Hypothesis and objectives **18**

1.8.1 Hypothesis **18**

1.8.2 Objectives **18**

Chapter 2: Effect of JA inulin on emulsion gel acting as potential fat replacement **19**

2.1 Introduction **19**

2.2 Materials and methods **20**

2.2.1 Chemicals and reagents **20**

2.2.2 Extraction of inulin from Jerusalem artichoke tuber **20**

2.2.2.1 JA powder preparation 20

2.2.2.2 Inulin extraction 21

2.2.3 Characterization of JA inulin by HPLC **21**

2.2.4 Gel formation **22**

2.2.4.1 Thermal-induced gel formation of inulin 22

2.2.4.2 Thermal-induced gel formation of monoglycerides-oil 22

2.2.4.3 Microscopic observation of inulin gel and oleogel 22

2.2.5 Temperature effect of water phase on emulsion formation **22**

2.2.6 Different formulations of emulsion **23**

2.2.7 Optimization of emulsion formulations **24**

2.2.8 Incorporation of different concentrations of PIE in emulsion gel..... 25

2.2.9 Incorporation of 1% JA PIE in emulsion gel 26

2.2.10 Microscopic observation of emulsion gel 26

2.2.11 Determination of melting point..... 27

2.3 Results and discussion 27

2.3.1 HPLC 27

2.3.2 Thermal-induced inulin gel and oleogel..... 31

2.3.3 Microstructures of inulin gel and oleogel 33

2.3.4 Temperature effect..... 34

2.3.5 Emulsions with different formulations 35

2.3.6 Optimization of emulsion formulations 35

2.3.7 Effect of different concentrations of PIE on emulsion gel..... 36

2.3.8 Effect of inulin on emulsion gel..... 36

2.3.9 Microstructure of emulsion gel..... 37

2.3.9.1 Polarized light microscope..... 37

2.3.9.2 Cryo-environmental scanning electron microscope..... 39

2.3.10 Melting point analysis..... 40

2.4 Conclusion 41

Chapter 3: Effect of JA inulin on lipid oxidation and its potential application on pharmaceuticals 43

3.1 Introduction..... 43

3.2 Materials and methods 44

3.2.1 Chemicals and reagents..... 44

3.2.2 Linoleic acid oxidation in an aqueous phase 44

3.2.2.1 Preparations of ULVs 44
3.2.2.2 Antioxidant assay 45
3.2.2.3 Statistics analysis 45
3.2.3 Interaction of inulin with artificial cell membrane 46
3.2.3.1 Artificial cell membrane preparation 46
3.2.3.2 SEM analysis 46
3.3 Results and discussion 46
3.3.1 Antioxidant activity 46
3.3.1.1 Effect of different samples 46
3.3.1.2 Effect of different concentrations of CIE 49
3.3.1.3 Effect of different concentrations of PIE 50
3.3.2 Membrane incorporation and inulin interaction 51
3.4 Conclusion 53
Chapter 4: General conclusion and future study 55
Appendices 57
Appendix A 57
A.1 Statistics analysis of different samples in Figure 3-2 57
A.2 Statistics analysis of different concentrations of CIE in Figure 3-4 ... 58
A.3 Statistics analysis of different concentrations of PIE in Figure 3-5 ... 59
References 60

List of Tables

Table 1-1 Major components and its content in fresh JA tuber (Bhagia et al., 2018) 2

Table 1-2 Composition of dry bagasse of JA tuber (Bhagia et al., 2018)..... 2

Table 1-3 Small molecules emulsifiers commonly used in food emulsion, adopted from (McClements, 2015) 10

Table 2-1 Different formulations emulsion properties for screening the most stable emulsions 24

Table 2-2 Optimization of formulation 4 and 6 by different percentages of emulsifiers . 25

Table 2-3 Different concentrations of purified inulin extract incorporated in emulsion.. 26

Table 2-4 Formulations A, B, C and D of emulsions 26

Table 2-5 Retention time and %area of each peak in JA purified inulin extract analyzed by HPLC 29

Table 2-6 Melting point of CI, PIE and emulsion gel formulation C and D, each result was average of two measurements..... 41

List of Figures

Figure 1-1 (A) JA tubers and (B) JA flowers (GardenMaine, 2012)..... 2

Figure 1-2 Chemical structure of inulin, drawn by BIOVIA draw..... 4

Figure 1-3 HLB scale and the approximate ranges into which solubilising agents, detergents, emulsifiers and antifoaming agents fall, based on the method devised by (Griffin, 1949)..... 12

Figure 1-4 Equation of blended HLB calculation..... 12

Figure 2-1 Definition of volumetric gel index (Kim et al., 2001) 22

Figure 2-2 HPLC chromatograms of (A) 0.25 mg/ml commercial inulin from chicory root; (B) 1 mg/ml purified inulin extract (PIE) from JA, deproteinization and decolorization were performed by salt and charcoal (stage 1 purification); (C) 1 mg/ml further ethanol precipitated inulin from PIE (stage 2 purification). 30

Figure 2-3 All inulin (CI, PIE, CIE) samples were prepared in freeze dried powders (first row), dissolved in 80℃ distilled water solution (20%, w/v, second row) and formed gel (third row). 31

Figure 2-4 Monoglycerides structured olive oil (5%, w/v) and formed oleogel 32

Figure 2-5 Polarized light microscope images (magnification: 200x) of (A) CI gel; (B) PIE gel; (C) CIE gel; (D) oleogel, corresponding to gels in Figure 2-3 and Figure 2-4... 33

Figure 2-6 Emulsion gel formation influenced by temperature (A) 80℃ water phase; (B) room temperature (23 ℃) water phase 34

Figure 2-7 Emulsions with different formulations (see Table 2-1) prepared after one week 35

Figure 2-8 Emulsion gel with different percentages of purified inulin extract (PIE)..... 36

Figure 2-9 The pictures of emulsions (A) without purified inulin extract (PIE); (B) with 1% PIE, in the absence of Tween 80: See Table 2-4 for recipes of formulations 37
Figure 2-10 Images of emulsion gel (1) formulation C: without 1% purified inulin extract (PIE); formulation D: with 1% PIE, in the presence of Tween 80, see recipes in Table 2-4; (2) polarized light microscope (magnification: 200x) images of formulations C and D. 39
Figure 2-11 Cryo-ESEM micrograph images of emulsions (C) without 1% purified inulin extract (PIE); (D) with 1% PIE, in the presence of Tween 80: see recipes in Table 2-4.. 39
Figure 3-1 Conjugated dienes formation from linoleic acid oxidation, modified from (Min & Boff, 2002). 43
Figure 3-2 Effect of various samples on AAPH-induced linoleic acid oxidation, as tested by absorbance of produced conjugated diene during 120 min, each test was performed in triplicates (individually), data in each curve were the average of triplicate results..... 47
Figure 3-3 Delayed time of lipid oxidation by CIE compared to control, each test was performed in triplicates (individually), data in each curve were the average of triplicate results. 48
Figure 3-4 Effect of different concentration of CIE on AAPH-induced linoleic acid oxidation, as tested by absorbance of produced conjugated diene during 120 min, each test was performed in triplicates (individually), data in each curve were the average of triplicate results. 49
Figure 3-5 Effect of different concentration of PIE on AAPH-induced linoleic acid oxidation, as tested by absorbance of produced conjugated diene during 120 min, each

test was performed in triplicates (individually), data in each curve were the average of triplicate results. 50
Figure 3-6 Schematic model of porous polycarbonate membrane coated with lecithin and interacted with inulin 52
Figure 3-7 SEM micrograph image of 200 nm porous (A) polycarbonate membrane without any treatment; (B) treated by lecithin; (C) polycarbonate membrane treated by lecithin without 1% PIE; (D) with 1% PIE 53

List of Appendices

Appendix A……. …….……58

A.1 Statistics analysis of different samples in Figure 3-2…….58

A.2 Statistics analysis of different concentrations of CIE in Figure 3-4…….59

A.3 Statistics analysis of different concentrations of PIE in Figure 3-5…….60

List of Abbreviation

JA	Jerusalem artichoke
DP	Degree of polymerization
CI	Commercial inulin from chicory root
PIE	Purified inulin extract from JA
CIE	Crude inulin extract from JA
HLB	Hydrophilic-lipophilic balance
PLM	Polarized light microscope
SEM	Scanning electron microscope
ULVs	Unilamellar liposome vesicles
HPLC	High performance liquid chromatography
AAPH	2, 2'-azobis (2-amidino-propane) dihydrochloride
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
ANCOVA	Analysis of covariance
Cryo-ESEM	Cryo-environmental scanning electron microscope

Chapter 1: Literature review

1.1 Jerusalem artichoke

Jerusalem artichoke (JA) (*Helianthus tuberosus, L.*) is a plant that is a member of the sunflower family (*Asteraceae*) and it is native to North America (Li et al., 2015). JA can grow well in a relative wide range of environment conditions due to its resistance to environment stress, such as non-fertile land, frost, drought and plant diseases. Nowadays, JA is mainly cultivated in North America, Northern Europe, China, Australia as well as New Zealand.

JA tuber is rich in inulin (oligosaccharide/polysaccharides), which is a good resource for human food, animal feed and bioenergy products. JA has many advantages in terms of inulin content, yield of biomass production, improvement of soil and water reservation (Li, Li, Wang, Du, & Qin, 2013) comparing to several other plants such as chicory root (*Cichorium intybus*), dandelion (*Taraxacum officinale*), onion (*Allium cepa*), among others (Singh, Singh, & Larroche, 2019). Commercial inulin is often produced from chicory root in powdered form.

According to the US Salinity Laboratory, JA is regarded as moderately salt tolerant crop and can grow in non-fertile land including marginal, saline and alkaline land, which reduces cost for sugar production. What is more, inulin content in JA tuber accounts for 50%~60% (dry weight) and is not affected by salt stress. In the study of Bhagia et al. (2018), fresh JA tuber is hydraulic pressed to obtain juice and wet bagasse, in which composition is analyzed by HPLC and result is shown in **Table 1-1**. The components of dry bagasse are shown in **Table 1-2** (Bhagia et al., 2018).

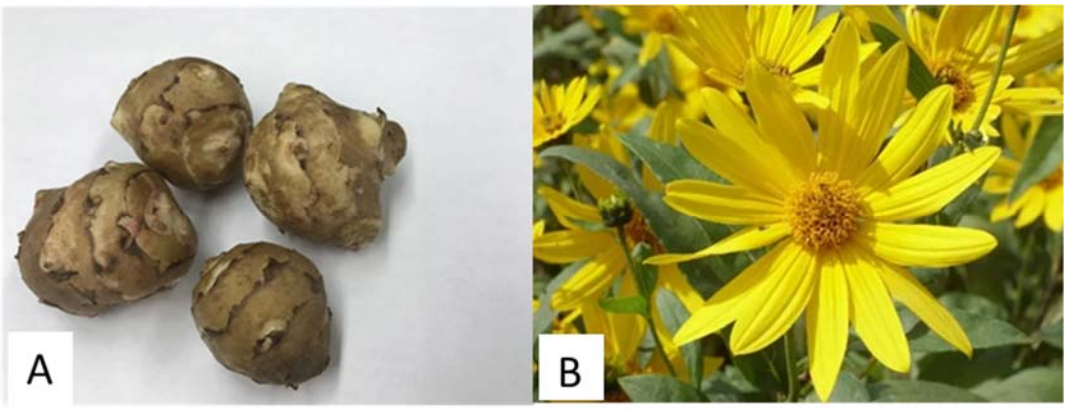


Figure 1-1 (A) JA tubers and (B) JA flowers (GardenMaine, 2012).

Table 1-1 Major components and its content in fresh JA tuber (Bhagia et al., 2018).

Components	Percentage (%)
Water	65.03
Inulin, glucose, fructose, sucrose	15.5
Dry bagasse	12.63
Loss in hydraulic press	6.77

Table 1-2 Composition of dry bagasse of JA tuber (Bhagia et al., 2018).

Composition	Percentage (%)
Glucan	17.2~25.9
Arabinan	12.8~16.0
Galactan	4.6~6.0
Xylan, rhamnan, mannan	3.9~6.1
Klason ligin	11~13

It has been known that browning in JA tubers is partially due to the presence of polyphenol oxidase and polyphenols. Enzymatic browning reactions occur in fruits and vegetables during food preservation and processing, which cause undesirable browning, unpleasant flavor and losses of nutrient quality (Tchoné, Bärwald, & Meier, 2005). This mainly involves two reactions catalyzed by polyphenol oxidase in the presence of molecular oxygen: (1) monophenols are converted to o-diphenols (2) o-diphenols are oxidized subsequently to o-quinones, which polymerize into red, brown or black pigment spontaneously (Ziyan & Pekyardimci, 2003). One of strategies inhibiting the browning reaction is inactivating polyphenol oxidase activity. Normally, sulphite is used to inactivate PPO activity in food industries, lemon juice, ascorbic acid and citric acid can also act as natural inhibitors (Tchoné et al., 2005).

1.2 Chemical structure, physiochemical property and functionality of inulin

1.2.1 Basic property of inulin

Inulin is a dietary fiber (polysaccharide) in which D-fructose is linked by β(2→1) linkage with end of glucose residue (Causey, Feirtag, Gallaher, Tungland, & Slavin, 2000). The chemical structure of inulin is shown in **Figure 1-2**. The degree of polymerization (DP) of inulin varies from 2 to 60, which depends on plant resources, harvest time, storage and process conditions (Saengthongpinit & Sajjaanantakul, 2005). In the study of Li et al. (2015), inulin content and its DP that obtained from Jerusalem Artichoke were evaluated during the period of growth from 10 days before blossom to 80 days after blossom. It revealed that their changing trends were almost the same. Inulin content reached maximum of 12.21% at 40 days after blossom and its maximum DP is up to 18 at 50 days after blossom (Li et al., 2015).

Different DP determines its biological and physical properties (Rubel et al., 2018). Short-chain inulin is more water soluble than long-chain inulin. While high DP inulin, often labeled as high performance, has good ability of forming gel. In the study of Kim et al. (2001), certain concentration of inulin solution became gel when it was heated and cooled to room temperature for 1 day. The minimum concentration and temperature that could form a gel were 15% and 40℃, respectively (Kim, Faqih, & Wang, 2001).

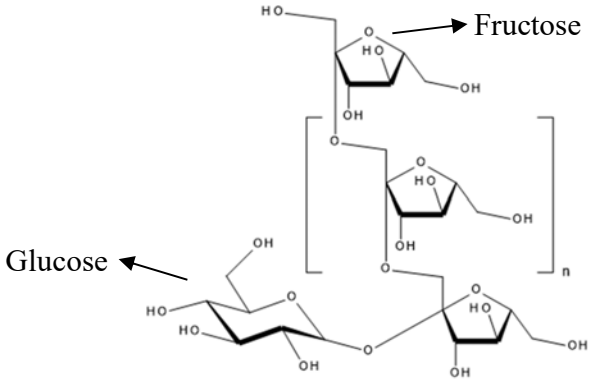


Figure 1-2 Chemical structure of inulin, drawn by BIOVIA draw.

The gelation of inulin is affected by temperature, heating time and pH. Glibowski & Wasko (2008) investigated the effect of some factors on the rheological properties of inulin and its gelling property. These factors include heating temperature, heating time and pH. Their results revealed that the inulin gel hardness, shear stress and microstructure was dramatically affected by heating temperature, heating time and pH. The gel became softer and microstructure became porous due to increase of heating temperature, elongation of heating time and pH decrease. In addition, acidic condition caused hydrolysis of inulin that was not affected by heating temperature and heating time. It was supposed that gelation of both high polymerized and low polymerized inulin solution needs inulin crystallites which act as seeding crystals. At higher temperature (for example, above 80℃), gelation could

be inhibited after inulin crystallites dissolve. At lower temperature, inulin crystallites partially dissolve in water, which is easier to form gel with higher hardness (Glibowski & Wasko, 2008).

Water solubility of inulin increases with temperature increases. Based on the data from (Inulin, DrugBank,), the solubility of inulin in water reaches 28 g/100 ml at 80℃. This database also shows the melting point of inulin from Jerusalem Artichoke is 178℃ when its degree of polymerization is 38.

1.2.2 Functionality of inulin

Based on its structure and property, inulin has many functions and applications.

1.2.2.1 Fat replacement

Inulin can act as fat replacement. Traditionally, solid fat is derived from saturated animal or vegetable fat, or from oil that has been hydrogenated, converting cis unsaturated fatty acids to their trans and saturated forms, which associates with the increase risk of coronary heart disease. With increased health concern, people prefer to choose low-fat or reduced-fat food. On the other hand, fat has a significant effect on the physical properties of dairy products (Dave, 2012). Low-fat or reduced-fat foods have less organoleptic properties than full fat foods (Hamilton, Knox, Hill, & Parr, 2000). For example, texture of fat replacement food products is often grainy and undesirable.

One of the promising strategies is using fat replacement which has similar physical and organoleptic properties as fat. Inulin can be used as fat replacement in food, since it can form gel in aqueous phase. Several studies have demonstrated that inulin is used as fat replacer in food products, such as bakery products including cake (Majzoobi, Mohammadi, Mesbahi, & Farahnaky, 2018), muffin (Zahn, Pepke, & Rohm, 2010) etc., dairy products

(Meyer, Bayarri, Tárrega, & Costell, 2011) including ice cream, cheese, yogurt, kefir, custard etc., improving their mouthfeel and creaminess. Researchers claimed that the functional properties of cheese analogue were improved by partially replacing milk fat with inulin, which generally increased its meltability, density, cohesiveness and viscosity (Sołowiej et al., 2014). It also improves mouth-feel and texture of food. A study showed that incorporation of inulin can reduce syneresis in spread due to its amazing water binding capacity (Fadaei, Poursharif, Daneshi, & Honarvar, 2012). The effect of inulin on food products not only depends on its concentration but also on its DP.

1.2.2.2 Prebiotics

Inulin is a dietary fiber which benefits some intestinal microflora. Dietary fiber that has positive effects on gut microbial composition and activity is also named prebiotics (Health Canada, 2018). These beneficial microorganisms ferment dietary fiber into short-chain fatty acids, such as acetic acid, propionic acid and butyric acid (van der Beek et al., 2018). These short chain fatty acids inhibit colon cancer development. Inulin can promote production of glucagon-like peptide-1 hormone which suppresses appetites and also stimulates insulin secretion. This helps to reduce obesity (Barclay, Ginic-markovic, Cooper, & Petrovsky, 2010). Since there is no inulinase enzymes which can break down β(2→1) glycosidic bonds of inulin in humans, this makes it indigestible in human digestive systems. While it is digestible by some beneficial microorganisms in large intestine such as bifidobacteria.

The length of inulin chain affects its prebiotic effects. One study showed lower degree of polymerization (DP) inulin has better activity in terms of stimulating the growth of probiotics in yogurt according to optical density of cultures measured at 37℃ after 32h

incubation (Li et al., 2015). Whereas another study revealed long chain inulin (DP 3-60) had more pronounced prebiotic potency towards *in vitro* microbial communities derived from proximal and distal colon than short chain oligofructose (DP 2-20) (Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2007). This conclusion was based on the results that inulin had inhibitory effect on opportunistic pathogens (*faecal coliforms and staphylococci*) and higher beneficial effects towards *bifidobacteria* as analyzed by real-time polymerase chain reaction technique. One factor caused different conclusion between the above studies is measurement time. It was known that inulin (oligofructose) with short chain length are typically more biodegradable than longer chain length (Roberfroid, Loo, & Gibson, 1998). Therefore, it needed a longer time for long chain inulin fermented, which prolonged the period for growth of probiotics and production of short chain fatty acids. This is also in agreement with the finding that more carbohydrate breakdown appeared in the distal region of colon (Wiele et al., 2007). Other studies claimed that a combination of long chain and short chain fructan has better prebiotics effect than individual fractions (Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2004).

1.2.2.3 Drug delivery carrier

Inulin is a potential material that acts as a drug or bioactive compound delivery carrier combined with other natural polymers in pharmaceutical and food applications due to its biocompatible, nontoxic properties (Mandracchia et al., 2018). Studies show that inulin or modified inulin potentially can be used as in colon-targeted drug delivery systems (Mandracchia et al., 2018; Walz, Hagemann, Trentzsch, Weber, & Henle, 2018). This is

because it cannot be digested in stomach and small intestine while can be degraded in colon by beneficial microorganisms (Barclay et al., 2010).

Inulin combined with alginate can act as an oral protein drug delivery carrier. Alginate-inulin hydrogels containing bovine serum albumin were developed using ionotropic gelation technique where calcium chloride was used as a cross-linker. These hydrogels could potentially acts as oral protein drug delivery system (Norudin, Mohamed, & Yahya, 2018). In that study, hydrogels beads could be well formed when the concentration of sodium alginate was fixed at 2% (w/v) and the concentration of inulin ranged from 5% to 15% (w/v). It revealed that protein encapsulation efficiency ranged from 48.3% to 68.9% and increased with the concentration of inulin increased. The swelling behavior of hydrogels was pH sensitive and the swell index was lower in acidic medium and higher in alkaline medium. The swelling behavior was in agreement with the protein release profile. In simulated gastric fluid (pH=1.2), only minimal protein was released within 2 hours comparing to 100% protein was released in simulated intestinal fluid (pH=7.4) within 1.5 hours. This indicated that alginate-inulin hydrogels ideally protected BSA from release in gastric environment and released all drug at targeted area (small intestine) as potential oral drug carriers.

For food applications, inulin can be used as coating material that encapsulating bioactive compounds, enhancing nutritional profile. Nano complexes of whey protein isolate and inulin were developed to encapsulate resveratrol, a polyphenolic hydrophobic

bioactive compound, for potential dairy food applications. Meanwhile, these complexes can be used as potential prebiotic source (Ha et al., 2016).

1.3 Emulsions

Emulsions are basically composed of dispersed phase, emulsifiers and continuous phase. The dispersed phase (water/oil) suspends in the continuous phase (oil/water) in the form of small droplets in the presence of emulsifiers which reduce the surface tension and prevents them from separating (Sivapratha & Sarkar, 2018). Commercial food emulsions typically contain various components, including oils, emulsifiers, thickening agents, gelling agents, preservatives, antioxidants, sweeteners, colorants, flavors, etc (McClements, 2015). Composition of ingredients determines physicochemical and nutritional properties of an emulsion.

1.3.1 Emulsifiers

Emulsifiers are described as ingredients that helps to form and stabilize emulsions (Hasenhuettl & Hartel, 2008). It may be expanded to help blending of mutual insoluble phases, such as stabilizing foams (gas in liquid or solid) and dispersions (solids in liquids or other solids).

An emulsifier is essentially composed of amphiphilic compound which consists of hydrophilic “head” group (water-loving) and lipophilic “tail” group (oil-loving). It is mainly used to reduce the surface tension between dispersed phase and continuous phase, improving emulsion formation and its stability. The characteristics of an emulsifier are determined by the nature of its head group and tail group. Emulsifiers can be divided into several classes including anionic (e.g., fatty acid salts), cationic (e.g., lauric arginate),

zwitterionic (e.g. lecithin) and nonionic (e.g., monoglycerides, Tweens, Spans). A wide range of emulsifiers commonly used in food products are listed in **Table 1-3**.

Table 1-3 Small molecules emulsifiers commonly used in food emulsion, adopted from (McClements, 2015).

Emulsifiers	Solubility	E number	U.S.FDA	ADI (mg/kg)
Ionic				
Lecithin	Oil/water	E322	184.1400	NL
Fatty acid salts	Oil/water	E470	172.863	NL
Sodium stearoyl lactylate	Water	E481	172.846	0-20
Calcium stearoyl lactylate	Oil	E482	172.844	0-20
Citric acid esters of MG	Water	E472c	172.832	NL
Diacetyl tartaric acid esters of MG	Water	E472e	184.1101	0-50
Nonionic				
Monoglycerides	Oil	E471	184.1505	NL
Acetic acid esters of MG	Oil	E472a	172.828	NL
Lactic acid esters of MG	Oil	E472b	172.852	NL
Succinic acid esters of MG		----	172.830	----
Polyglycerol esters of FA	Water	E475	172.854	0-25
Propylene glycerol esters of FA	Oil	E477	172.856	0-25
Sucrose esters of FA	Oil/water	E473	172.859	0-10
Sorbitan monostearate	Water	E491	172.842	0-25
Sorbitan tristearate	Oil	E492	----	0-15
Polysorbate 60	Water	E435	172.836	0-25
Polysorbate 65	Water	E436	172.838	0-25
Polysorbate 80	Water	E433	172.840	0-25

Note: MG=monoglycerides; FA=fatty acid salts; E numbers and U.S.FDA are codes for substances that are permitted to be used as food additives for use within in the European Union and United states, respectively. ADI= acceptable daily intake; NL=not limited.

1.3.2 Hydrophilic-lipophilic balance (HLB)

Hydrophilic-lipophilic balance (HLB) is used to describe the hydrophilic or lipophilic property of a substance and it is mainly used to classify emulsifiers/surfactants. HLB is described by a value which has a range of 0-20 to indicate these properties of surfactants. Each of surfactant is assigned an HLB value that depends on its chemical structure. If a surfactant has a high HLB value (10-18) (**Figure 1-3**), it is predominantly hydrophilic and tends to dissolve in aqueous phase, forming oil-in-water emulsion. Whereas a surfactant with a low HLB value (3-6) (**Figure 1-3**) is mostly lipophilic and preferentially dissolves in oil phase, forming water-in-oil emulsion (McClements, 2015).

HLB value of a surfactant provides a useful information whether it is hydrophilic or lipophilic and can also predict what type of emulsion will be formed. However, some surfactant is influenced by temperature and experimental conditions significantly. In addition, different types of oil have their individually required HLB value (Pasquali, Taurozzi, & Bregni, 2008). Therefore, in order to make a stable emulsion, it is important to consider these factors when selecting a surfactant rather than only concerning the HLB value.

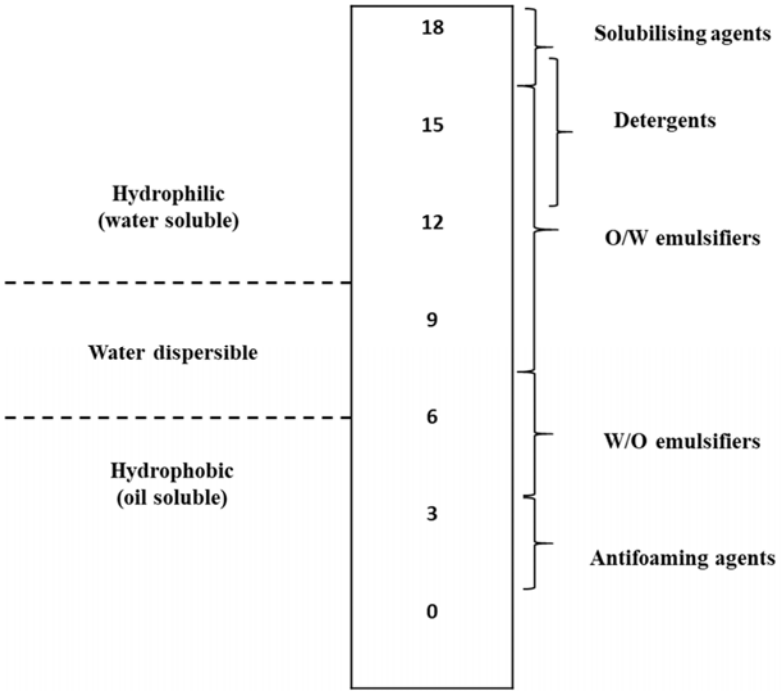


Figure 1-3 HLB scale and the approximate ranges into which solubilizing agents, detergents, emulsifiers and antifoaming agents fall, based on the method devised by (Griffin, 1949).

Normally, a blend of two or more surfactants give an emulsion better stability by adjusting different ratios of them. For non-ionic emulsifiers, HLB of two blended emulsifiers is calculated by the following equations (Griffin, 1954):

$$\%(\mathrm{A})=\frac{100(X-HLB_{(B)})}{HLB_{(A)}-HLB_{(B)}}$$

$$\%(\mathrm{B})=\%100\text{-}\%(\mathrm{A})$$

Figure 1-4 Equation of blended HLB calculation

Where A and B are two kind of emulsifiers. X is the blended HLB. Ingredients (mainly oil phase) in emulsion have their "required HLB value", which can be obtained by amount of experiments. By searching the required HLB value of certain oil phase, the ratio of emulsifiers is achieved by the equation shown in **Figure 1-4**.

1.3.3 Factors influencing the stability of food emulsions

The stability of food emulsions is influenced by many factors, such as composition, temperature, pH, ion concentration of salt and impurity. Additionally, droplet size of dispersed phase plays an important role in the stability of emulsion. Smaller droplet size is more stable than large size. The droplet size is related to the agitate technique during process. Generally, by using various of technique, the droplet size increases in the order of ultrasound technique, homogenizer, vortex and magnetic stir. Of course, the speed and power in these techniques are also the factors that need to be considered.

1.3.4 Emulsion applications

Emulsions are widely used in food, cosmetic and pharmaceutical products. The familiar food emulsions in our daily life includes milk, butter, mayonnaise, ice cream, cheese, etc. Nano emulsions have potential application in functional food products. In particular, encapsulation of functional compounds, such as bioactive compounds, micronutrients, flavorings, antioxidants in food products to improve products value is highly desired (Donsì, 2018). Emulsions are also widely used to prepare drug delivery vehicles. Many microemulsions have been developed for different delivery routes including oral, transdermal, buccal, parenteral, rectal routes and so on, with some advantages in terms of thermodynamic stability, ease of fabrication, high absorption rates, etc., comparing to solvent without surfactant system (Jadhav, Shaikh, Ambade, & Kadam, 2006).

1.4 Gels

Gel is an insoluble semi-rigid network in which liquid dispersed in solid network. The main ingredients to make food gels are biopolymers, such as carbohydrate and proteins.

Clark (1996) revealed that gels can be divided into associative or particulate gels based on the mechanism of networking between polymer molecules. In the former case, polysaccharides changes from coils shape to helix shape during gelation, which helps polymer chains to form a network structure between molecules. In the latter case, particulate gel is formed by large and random aggregation between polymer chains (Clark, 1996).

Oleogel can be defined as a lipophilic liquid and solid mixtures, of which oleogelators (<10wt.%) structure bulk oil and form lipid network as reviewed by Dassanayake et al. (2011). The oleogelators can be divided into two groups: self-assembly system and crystal particles system. The former includes monoglycerides, sorbitan monostearate, etc. The latter covers fatty acids, wax esters, rice bran wax, etc (Dassanayake, Kodali, & Ueno, 2011). Oleogelation is a transformation process of oil into thermo-reversible and three-dimensional gel network (Mert & Demirkesen, 2016).

1.5 Lipid oxidation

In research, lipid oxidation is normally caused by ROO• that generated by 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) (He et al., 2013). AAPH is a water-soluble azo compound which is a free radical generator. For convenience, AAPH is expressed as R-N=N-R based on its chemical structure. Decomposition of AAPH produces one molecule nitrogen gas and two molecules of carbon-centered radicals R • spontaneously (reaction 1) at 37℃. R• radicals react with oxygen and form ROO• which causes lipid oxidation (reaction 3-5) (Peyrat-Maillard, Cuvelier, & Berset, 2003) .

$$R\text{-}N{=}N\text{-}R \rightarrow (1\text{-}e)\ R\text{-}R + 2e\ R\bullet + N_2 \quad (1)$$

$$R\bullet + O_2 \rightarrow RO_2\bullet \quad (2)$$

$$RO_2\bullet + LH \rightarrow ROOH + L\bullet \quad (3)$$

$$L\bullet + O2 \rightarrow LO_2\bullet \quad (4)$$

$$LO_2\bullet + LH \rightarrow LOOH + H\bullet \quad (5)$$

Where R-N=N-R is AAPH (radical generator), LH is linoleic acid, L• is linoleic radical, LO2• is peroxy radical, and e is the efficiency of free radical production. A free radical reaction has three key steps: initiation, propagation and termination (Martins, Fernández, & Camara, 2018). Reaction 1-3 is initiation step, and reaction 4-5 is propagation step. Reaction 5 shows LOO• abstracts hydrogen radical from another linoleic acid molecule, causing an autocatalytic chain reaction. If an antioxidant is added, it reacts with active L• and LOO•, which can stabilize these radicals and delay lipid oxidation (Peyrat-Maillard et al., 2003). In termination stage, radicals react with each other and form stable products.

It is important to choose a simple, reliable and economical measuring methods for determination of antioxidant capacity. The commonly used measuring methods includes oxygen radical absorbance capacity assay, 2,2-diphenyl-1-picrylhydrazyl assay, Total phenol content assay, among others (Karadag, Ozcelik, & Saner, 2009). There seems no standard method to determine antioxidant capacity of a compound because every method has limitation. In addition, for an antioxidant, the antioxidant capacity may be different for different radicals or oxidant sources. Therefore, it is necessary to conduct multiple assays based on experimental objectives.

1.6 Oxidative stress and antioxidants

1.6.1 Oxidative stress and diseases

Many diseases are related to oxidative stress caused by toxic reactive oxygen species. Oxidative stress including oxidation of lipids, proteins and DNA is induced by an imbalance between pro-oxidant and antioxidant homeostasis. This imbalance leads to production of ROS, such as highly reactive hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2) and superoxide radical ($\bullet O_2^-$). It also causes dysfunction of cells and production of other toxic substances including aldehydes, ketones and cholesterol oxide (Barnham, Masters, & Bush, 2004). Oxidative stress is related to occurrence of neurodegenerative diseases, for example, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Also, atherosclerosis and certain types of cancer are initiated by oxidative stress (Karadag et al., 2009).

1.6.2 Antioxidants and mechanisms

Antioxidant reactions have different mechanisms in which antioxidants can either act as physical barriers (e.g., cell membranes), metal chelators (e.g., catechins to prevent generation of reactive oxygen species) or serve as chemical traps (e.g., carotenoids), catalytic system (e.g., superoxide dismutase) and chain-breaker (e.g., ascorbic acid) to scavenge reactive oxygen species (Karadag et al., 2009). Dietary antioxidants widely act as transition metal chelators, radical chain reaction inhibitors and oxidative enzyme inhibitors (Huang, Ou, & Prior, 2005).

1.7 Artificial cell membranes and applications

1.7.1 Biological membrane modeling

Biological membrane modeling can be employed in a number of biological studies including targeted drug delivery, cell-cell interactions, nanoparticles-membrane interaction, lipid mobility, biomolecule sensing and surface functionalization (Hartman, Kim, Kim, & Nam, 2015). Supported lipid bilayers have been extensively used as a model for cell-cell interaction. Supported lipid bilayers are single lipid bilayer self-assembled on a solid substrate, such as silica, which is similar to live cell membrane (Lin, Yu, Triffo, & Groves, 2010). In artificial skin models research, simple homogenous polymer materials such as silicone membrane can be used to study the basic mechanisms controlling passive transport through a membrane (Ng, Rouse, Sanderson, & Eccleston, 2012). Lipid-based parallel artificial membrane permeability assay membranes are useful for screening of passive transport (Kansy, Senner, & Gubernator, 1998). Blood brain barrier model is another example. Sivandzade and Cucullo (2018) reviewed several modern in-vitro blood barrier model modelling, including co-culture, transwell, microfluidic platforms and microfluidics (fabricated via 3D printing) (Sivandzade & Cucullo, 2018). Each modelling has advantages and disadvantages depending on what is the objectives of experiments that will be performed.

1.7.2 Lecithin composition

Commercial soy lecithin which is made from degumming vegetable oils is a complicated mixture which mainly consists of 52-73% phospholipids which comprise phosphatidylcholine, phosphatidylethanolamine and inositol phosphatides, 33-35%

soybean oil and small amounts of other substances including sterols, sterol glycosides, carbohydrates, pigments, traces of tocopherol, among others (Scholfield, 1981).

The main component in cell membrane is phospholipids. Therefore, soy lecithin can be used as one of ingredients to make artificial cell membrane model.

1.8 Hypothesis and objectives

1.8.1 Hypothesis

It is hypothesized that:

(1) If Jerusalem artichoke (JA) inulin is incorporated in emulsion, the texture and stability of food emulsion gels will be improved.

(2) If JA inulin is incorporated in biological liposomal model (lipid), lipid oxidation will be delayed.

1.8.2 Objectives

The objectives of this study were to:

(1) Extract and characterize inulin from JA by HPLC.

(2) Incorporate JA inulin in emulsions (water in oil) and observe the texture and microstructure by polarized light microscope and cryo-environmental scanning electron microscope.

(3) Incorporate JA inulin in delaying AAPH-induced lipid oxidation using liposomes.

(4) Investigate how JA inulin interact and distribute in artificial cell membrane.

Chapter 2: Effect of JA inulin on emulsion gel acting as potential fat replacement

2.1 Introduction

Traditionally, solid fat is derived from saturated animal or vegetable fat (Hofberger, 2018), or from oil that has been partially hydrogenated converting cis unsaturated fatty acids to their trans and saturated forms, which might be associated with an increased risk of coronary heart disease (Mozaffarian, Katan, Ascherio, Stampfer, & Willett., 2006). With increased health concerns, people prefer to choose low-fat or reduced-fat food. However, fat has a significant effect on the physical properties of food products (Dave, 2012). Low-fat or reduced-fat foods with grainy and undesirable texture have less organoleptic properties than full fat foods (Hamilton et al., 2000). One of the promising strategies is using fat replacement that has similar physical and organoleptic properties as fat. For instance, succinyl chitosan in cake formulations (Rios, Garzón, Lannes, & Rosell, 2018), hydroxypropyl methylcellulose in muffins (Oh & Lee, 2018), soy protein hydrolysate/xanthan gum in low-fat ice cream (Liu, Wang, Liu, Wu, & Zhang, 2018), and chia and oat emulsion gel in low-fat sausages (Pintado, Herrero, Colmenero, Cavalheiro, & Capillas, 2018) were used as fat replacements.

Inulin-monoglycerides emulsion-based gel is a potential fat replacement. Inulin could form gel in aqueous phase at a higher concentration (>15%) though heating and cooling process (Kim et al., 2001). In addition, monoglycerides and vegetable oils could form semi-solid aggregated network structure when cooling from heating (Ojijo, Neeman, Eger, & Shimoni, 2004). Studies reported that long-chain saturated monoglycerides have property of forming highly hydrated lamellar Lα phase in the presence of anionic co-

surfactant and water when temperature is above its Krafft point (Marangoni et al., 2007). When it cools below Krafft point, the material forms α gel and finally forms an anhydrous crystalline phase. Crystallization behaviour of monoglycerides is largely similar in both lipophilic solvent (oil) and hydrophilic solvent (water) (Verstringe, Moens, Clercq, & Dewettinck, 2015).

According to the above information, this chapter was aimed to incorporate JA inulin and to see its effect on emulsion gel, which acts as potential fat replacement.

2.2 Materials and methods

2.2.1 Chemicals and reagents

Monoglycerides (Alphadim® 90 SBK) were kindly provided by Caravan Ingredients, Inc. (Lenexa, Kansas, USA). Extra virgin olive oil was purchased from Amazon.ca. Tween 80 (polyoxyethylene-20-sorbitan monooleate), sucrose (ultrapure) were from Fisher Scientific (CA). Activated charcoal (powder, 100 particle size (mesh)) and inulin from chicory (commercial inulin) were from Sigma-Aldrich (St Louis, MO, USA). $CaCl_2$ was from Sigma chemical Co. (St. Louis, MO, USA). Na_2CO_3 was from VWR International Co. (Mississauga, ON, CA). Glucose (45% w/v H2O solution) was from Sigma-Aldrich (UK). Fructose (D-fructose) was from BioShop Canada Inc.

2.2.2 Extraction of inulin from Jerusalem artichoke tuber

2.2.2.1 JA powder preparation

Jerusalem artichoke (JA) tubers were sliced, freeze dried and milled by coffee blender (KitchenAid, St. Joseph, Michigan, USA) to obtain powdered tubers. It was stored in desiccators at room temperatures till further analysis (Srinameb, Nuchadomrong, Jogloy, Patanothai, & Srijaranai, 2015).

2.2.2.2 Inulin extraction

Crude inulin extract (CIE): JA powder was extracted with distilled water (1:20 w/v) at 80℃ for 2 hours. After cooling to room temperature, the mixture was filtered and centrifuged (Thermo Fisher Scientific, Langenselbold, Germany) at a speed of 4500 g at 23℃ for 15 min. Supernatant was freeze dried (Labconco, Kansas, USA).

Purified inulin extract (PIE): in order to purify inulin, deproteinization (Huang, Chen, & Wang, 2011) and decoloration (Hongxin, 2008) were performed.

***Deproteinization*:** $CaCl_2$ (0.1%, w/v) was added into CIE solution and mixed until flocci were no longer produced. Then, Na_2CO_3 was added to precipitate redundant $CaCl_2$.

***Decoloration*:** color was removed by adding 2% (w/v) activated charcoal and the mixture was kept at 80℃ in a water bath for 15 min, filtrated and freeze dried to obtain PIE.

Ethanol precipitated inulin (EPI): 4 parts of ethanol were added to 1 part of PIE solution (5%) to get ethanol precipitated inulin (Ku, Jansen, Oles, Lazar, & Rader, 2003).

2.2.3 Characterization of JA inulin by HPLC

Chromatographic separation was carried out on high-performance liquid chromatography (HPLC, Waters e2695, Waters Corporation, Milford, USA) with refractive index (RI) detector (Waters 2414, Waters Corporation, Milford, USA). Commercial inulin from chicory root (CI, 0.25 mg/ml), purified inulin extract (PIE, 1 mg/ml) and ethanol precipitated inulin (EPI, 1 mg/ml) were separated by Sugar-Pak^{TM} I column (Waters, USA) at 60℃ with 10 µL injection volume. Milli-Q water (Millipore)

was used as mobile phase with a flow rate of 0.6 ml/min. Glucose, fructose and sucrose were used as standards.

2.2.4 Gel formation

2.2.4.1 Thermal-induced gel formation of inulin

Commercial inulin (CI) from chicory root, purified inulin extract (PIE) and crude inulin extract (CIE) were dissolved in distilled water with a concentration of 20% at 80℃ using a water bath, respectively. These solutions were heated for 5 min and cooled to room temperature (23℃) for 24 hours under gravity to set gel structure. In order to compare gel formation degree, definition of volumetric gel index must be introduced, which is equal to the ratio of volume of gel to total volume (**Figure 2-1**).

$$\text{Volumetric gel index} = \frac{volume\ of\ gel}{total\ volume} \times 100\%$$

Figure 2-1 Definition of volumetric gel index (Kim et al., 2001)

2.2.4.2 Thermal-induced gel formation of monoglycerides-oil

Fine granular monoglycerides were added with a concentration of 5% to olive oil and melted at 80℃ using a water bath. Once all the monoglycerides melted, it was cooled at room temperature (23℃).

2.2.4.3 Microscopic observation of inulin gel and oleogel

Inulin gel formed by CI, PIE, CIE with distilled water and oleogel formed by monoglycerides with olive oil were observed under polarized light microscope (Axioplan 2 imaging and Axiophot 2 universal microscope) to see their microstructures.

2.2.5 Temperature effect of water phase on emulsion formation

Temperature was investigated to see its effect on emulsion formation. The oil phase containing olive oil and monoglycerides was heated at 80℃ with a water bath until

monoglycerides melted completely. The treated water phase was heated at the same temperature (80℃), and the control was placed at room temperature (23℃). Oil phase and water phase were simply mixed to see effect of temperature.

2.2.6 Different formulations of emulsion

As it was introduced previously, combination of high HLB emulsifier and low HLB emulsifier increases the stability of emulsion. In present work, monoglycerides with lower HLB (around 3.8, based on the value of glyceryl stearate) and Tween 80 with higher HLB (15) were chosen as two emulsifiers. The required HLB value of olive oil was 7 (SaffireBlue, 2018). Based on the above information and equation in **Figure 1-4**. The ratio of monoglycerides to Tween 80 was calculated to get 5:2.

Emulsions with different percentage of ingredients (9 formulations) were prepared as listed in **Table 2-1**. The ingredients consist of olive oil, distilled water, monoglycerides (as an emulsifier) and Tween 80 (as a co-emulsifier).

First monoglycerides dissolved in oil completely (melting point of monoglycerides was 72℃), and then water phase including Tween 80 (at 80℃) were added to the oil phase (at 80℃), followed by strong vortexing (Mini-vortexer VM-3000; VWR, Radnor, USA) until a desired consistency was achieved. Then, they were cooled to room temperature (23℃) until further analysis.

Table 2-1 Different formulations emulsion properties for screening the most stable emulsions.

Formulation	Oil (%)	Monoglycerides (%)	Tween 80 (%)	Water (%)
1	9.5	0.5	0.2	89.8
2	19.0	1.0	0.4	79.6
3	28.5	1.5	0.6	69.4
4	38.0	2.0	0.8	59.2
5	47.5	2.5	1.0	49.0
6	57.0	3.0	1.2	38.8
7	66.5	3.5	1.4	28.6
8	76.0	4.0	1.6	18.4
9	85.5	4.5	1.8	8.2

Note: Oil=olive oil; Monoglycerides=commercial emulsifiers (Alphadim® 90 SBK); Tween 80=Polyoxyethylene-20-sorbitan monooleate; Water=distilled water

2.2.7 Optimization of emulsion formulations

According to the results of nine formulations, formulation 4 and 6 were chosen for further optimization. Taking safety and stability into consideration, content of Tween 80 was reduced and percentage of monoglycerides was increased. Preparation method was the same as described in “section **2.2.6**”.

Table 2-2 Optimization of formulation 4 and 6 by different percentages of emulsifiers.

Formulation	Oil (%)	Monoglycerides (%)	Tween 80 (%)	Water (%)
4a	36.0	4.0	0.4	59.6
4b	38.0	2.0	0.4	59.6
4c	38.0	2.0	0.2	59.8
6a	57.0	3.0	0.5	39.5
6b	56.0	4.0	0.5	39.5
6c	55.0	5.0	0.5	39.5

Note: Oil=olive oil; Monoglycerides=commercial emulsifiers (Alphadim® 90 SBK); Tween 80=Polyoxyethylene-20-sorbitan monooleate; Water=distilled water

2.2.8 Incorporation of different concentrations of PIE in emulsion gel

Purified inulin extract (PIE) as a functional ingredient was incorporated in emulsion. To investigate suitable PIE concentration, emulsion with 0%, 1%, 5%, 10% PIE were prepared. Incorporation of 10% commercial inulin (CI) from chicory root acted as a comparison. The percentages of other ingredients were prepared as shown in **Table 2-3** based on formulation 6. In preparation, PIE was dissolved in water phase, specific method was the same as described in "section **2.2.6**".

Table 2-3 Different concentrations of purified inulin extract incorporated in emulsion.

Oil (%)	Monoglycerides (%)	Tween 80 (%)	Water (%)	PIE (%)
57.0	3.0	0.5	39.5	0
57.0	3.0	0.5	38.5	1
57.0	3.0	0.5	34.5	5
57.0	3.0	0.5	29.5	10

Note: Oil=olive oil; Monoglycerides=commercial emulsifiers (Alphadim® 90 SBK); Tween 80=Polyoxyethylene-20-sorbitan monooleate; Water=distilled water; PIE=purified inulin extract from Jerusalem artichoke.

2.2.9 Incorporation of 1% JA PIE in emulsion gel

According to preliminary experimental results, emulsions with 1% purified inulin extract (PIE) and their controls were prepared as listed in **Table 2-4**. Formulation B and D had PIE (1%) but formulation A and C. Formulation C and D had Tween 80 but formulation A and B. Formulation A and B was aimed to see effect of 1% PIE in the absence of Tween 80.

Table 2-4 Formulations A, B, C and D of emulsions.

Formulation	Oil (%)	Monoglycerides (%)	Tween 80 (%)	Water (%)	PIE (%)
A	57	3	0	40	0
B	57	3	0	39	1
C	58	3	0.8	38.2	0
D	58	3	0.8	37.2	1

Note: Oil=olive oil; Monoglycerides=commercial emulsifiers (Alphadim® 90 SBK); Tween 80=Polyoxyethylene-20-sorbitan monooleate; Water=distilled water; PIE=purified inulin extract from Jerusalem artichoke.

2.2.10 Microscopic observation of emulsion gel

Formulation C and D were observed under polarized light microscope and cryo-Environmental scanning electron microscope (Nano Imaging Facility Laboratory of Carleton University, Ottawa, ON) to see differences between their morphological characteristics, so that the effect of 1% PIE on microstructure of emulsion gel was achieved.

2.2.11 Determination of melting point

Melting point of commercial inulin (CI) from chicory root, purified inulin extract (PIE), emulsion formulation C and D were measured by the conventional capillary tube method using melting point apparatus and capillary tube. For CI and PIE, each sample was loaded and packed in a capillary tube with a height of 1~2 mm and tested in the melting point apparatus (Electrothermal IA9100, UK). When measuring melting point of emulsion gel (formulation C and D), a capillary with two open ends was used. One end dipped into the emulsion gel in a height of around 1mm and then measured in the same way as CI and PIE. The apparatus was heated at maximum heating rate to a temperature 20℃ below expected temperature. Ramp rate was set at 10℃/min. The first temperature of melting range was recorded when first drop of liquid was seen. The second temperature of melting range was recorded once sample completely melted or sublimated. Between each sample analysis, apparatus should be cooled to at least 20℃ below the expected temperature. Each sample was performed in duplicate.

2.3 Results and discussion

2.3.1 HPLC

HPLC chromatograms of commercial inulin (CI) from chicory root, purified inulin extract (PIE) and ethanol precipitated inulin (EPI) were shown in **Figure 2-2**A, B, and C,

respectively. CI showed one peak (**Figure 2-2A**), which could be inulin with a range of degree of polymerization (DP). In the study of Bohm et al. (2005), High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) revealed commercial chicory inulin (from Sigma-Aldrich) had an average DP between 13 and 30 (Böhm, Kaiser, Trebstein, & Henle, 2005).

PIE (**Figure 2-2C**) mainly showed five peaks; "b" had the same retention time as the ones in CI (**Figure 2-2A**), but it had an extended peak width, which probably represented that the DP had wider distribution (Böhm et al., 2005). In order to figure out other peaks, simple standard sugars (fructose, glucose and sucrose) were analyzed, and compared with their corresponding retention times. It turned out that peaks e was sucrose, with peak area of 17.92% (**Table 2-5**).

According to Beirão-da-costa et al. (2005), that analyzed chicory root and salsify root inulins by HPLC-RI with Sugar pak I column (the same column and detector with present work), inulin with higher degree of polymerization (DP) came out from column earlier than lower DP, fructose and glucose came out at last (Beirao-da-costa, Maria Luisa Januario, Maria Isabel Nunes Simao, Filipa Maria Sequeira Leitao, 2005). Therefore, other peaks were inulin with higher DP. These DPs were possibly in the range of 3-50 according to the study of Srinam et al. (2015). In that study, DP of inulin from JA tuber ranged from 3-50; DPs 3-20 had the highest content of 70% approximately. DP<3 (simple sugars) only accounted for around 4% (Srinameb et al., 2015). However, DP varies if extraction conditions are different, which includes extracted solvent (Ku et al., 2003), temperature, time (Srinameb et al., 2015), and pH (Glibowski & Wasko, 2008)

Table 2-5 Retention time and %area of each peak in JA purified inulin extract analyzed by HPLC.

Name	Retention time (Rt)	% Area
a	4.196	15.72
b	4.503	45.48
c	5.206	9.94
d	5.755	10.94
e	6.648	17.92

Interestingly, EPI (**Figure 2-2C**) had almost the same chromatograms with CI (**Figure 2-2A**). This indicated addition of ethanol to PIE solution allowed higher DP precipitated, which agreed with literature (Temkov, Petkova, Denev, & Krastanov, 2015). Also, Ku et al. (2003) revealed that higher ratio of ethanol and inulin solution precipitated more inulin and precipitated sequence was from high DP to low DP. For example, when 2 parts of ethanol were added to 1 part of inulin solution, only high DP inulin started to precipitate. When 4 parts of ethanol were added to 1 part of inulin solution, most of high DP inulin precipitated and lower DP inulin began to precipitate (Ku et al., 2003).

In general, peaks of PIE from JA had a wider distribution than CI (commercial inulin from chicory root). Addition of ethanol helped to precipitate inulin with higher DP inulin. According to peak areas, peak a-d (higher DP inulin, DP>2) constituted 80.28%, of which peak b accounted for 45.48%, sucrose took up 17.92% in PIE from JA (**Table 2-5**).

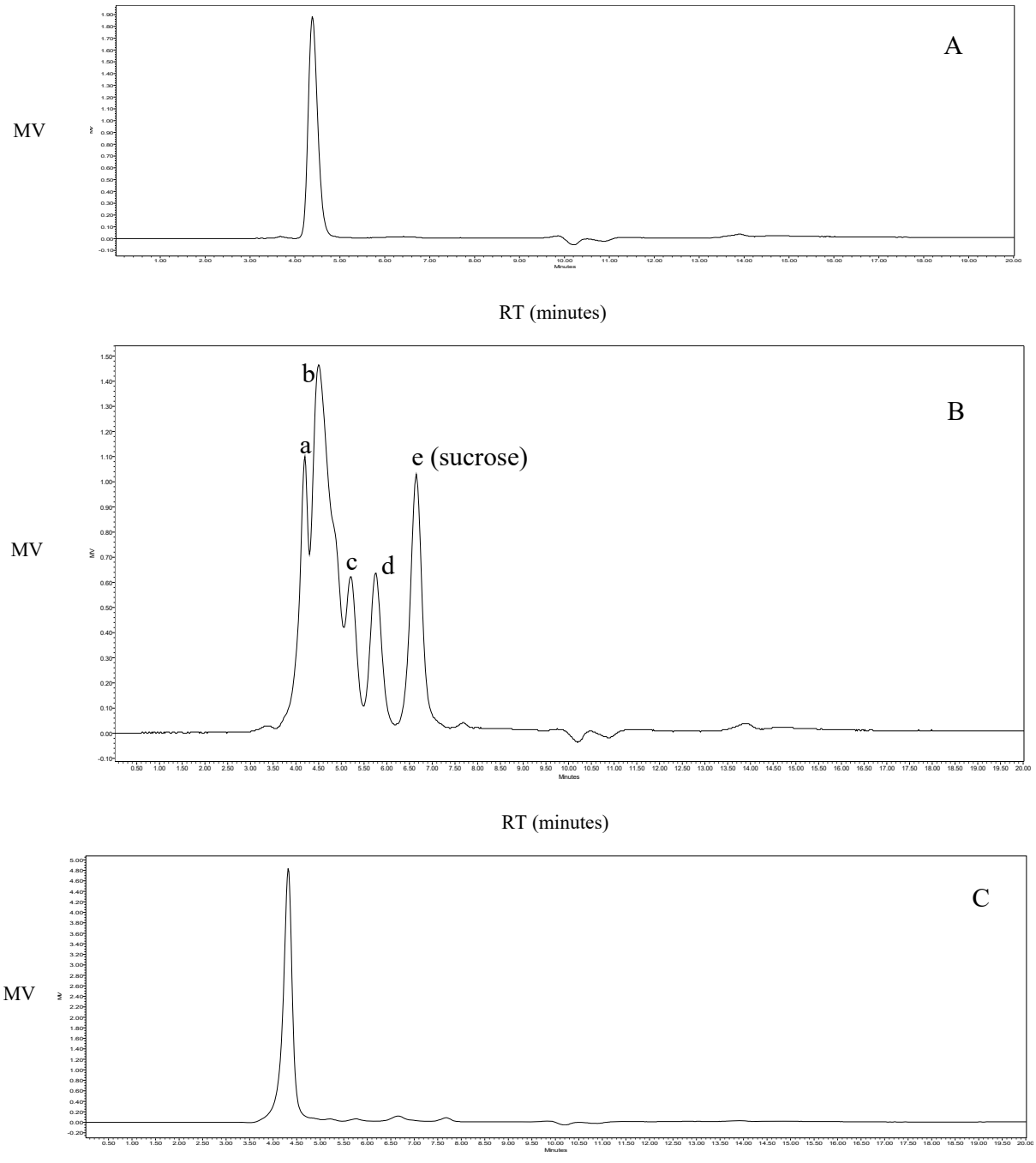


Figure 2-2 HPLC chromatograms of (A) 0.25 mg/ml commercial inulin from chicory root; (B) 1 mg/ml purified inulin extract (PIE) from JA, deproteinization and decolorization were performed by salt and charcoal (stage 1 purification); (C) 1 mg/ml further ethanol precipitated inulin from PIE (stage 2 purification).

2.3.2 Thermal-induced inulin gel and oleogel

Figure 2-3 showed pictures before and after inulin-gel formation. The first row in **Figure 2-3** displayed powders of commercial inulin (CI) from chicory root, purified inulin extract (PIE) and crude inulin extract (CIE) from JA, respectively. Colors of PIE and CIE were darker than CI, possibly due to remaining of inherent color or products of enzymatic browning reactions in the presence of polyphenoloxidases (PPO) and polyphenols in JA tuber (Tchoné et al., 2005).

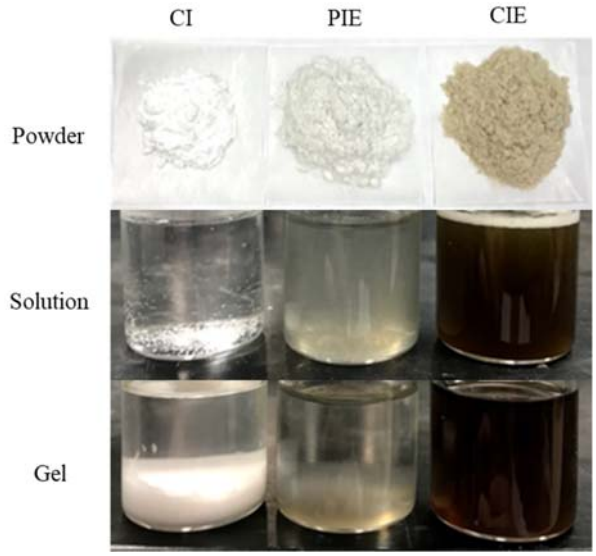


Figure 2-3 All inulin (CI, PIE, CIE) samples were prepared in freeze dried powders (first row), dissolved in 80°C distilled water solution (20%, w/v, second row) and formed gel (third row).

Note:
CI=commercial inulin from chicory; PIE=purified inulin extract from JA;
CIE=crude inulin extract from JA.

CI dissolved in distilled water and formed a white gel after cooling for 1 day as seen in the third row of **Figure 2-3**, whereas PIE gel presented greyish color and CIE gel appeared brown color. As it was mentioned earlier, degree of gel formation was expressed as volumetric gel index. CI gel has higher volumetric gel index (26%) than PIE gel (22%)

and CIE gel (18%), which was likely due to different DP of these inulin samples. Volumetric gel index was affected by inulin concentration, heating temperature, pH, shearing and solvent added (Kim et al., 2001).

Inulin solution started to form a gel slowly as temperature decreased. This displayed a typical sol-gel transition. Soluble polymer gradually became insoluble and formed a semi-solid structure (gel) in a polymer solution (sol) under gravity (Kim et al., 2001), due to association of polymer molecules (Funami, Funami, Yada, & Nakao, 1999).

Figure 2-4 showed pictures before and after monoglycerides-structured oil gel (oleogel). Monoglycerides melted in olive oil at 80℃, which was higher than its melting point (72℃). As temperature decreased, monoglycerides structured oil and formed gel-like material. This kind of gel could be termed oleogel or organogel. In this process, oil transfers into thermo-reversible three-dimensional gel network in the presence of oleogelators, which was called oleogelation/organogelation (Mert & Demirkesen, 2016).

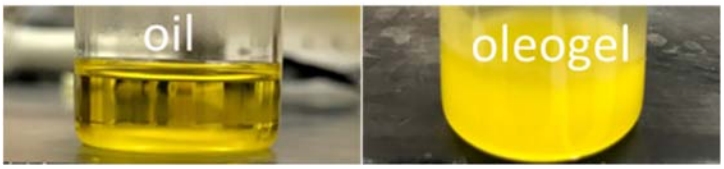


Figure 2-4 Monoglycerides structured olive oil (5%, w/v) and formed oleogel.

In present work, formulations of emulsion were combination of ingredients in inulin gel and oleogel.

2.3.3 Microstructures of inulin gel and oleogel

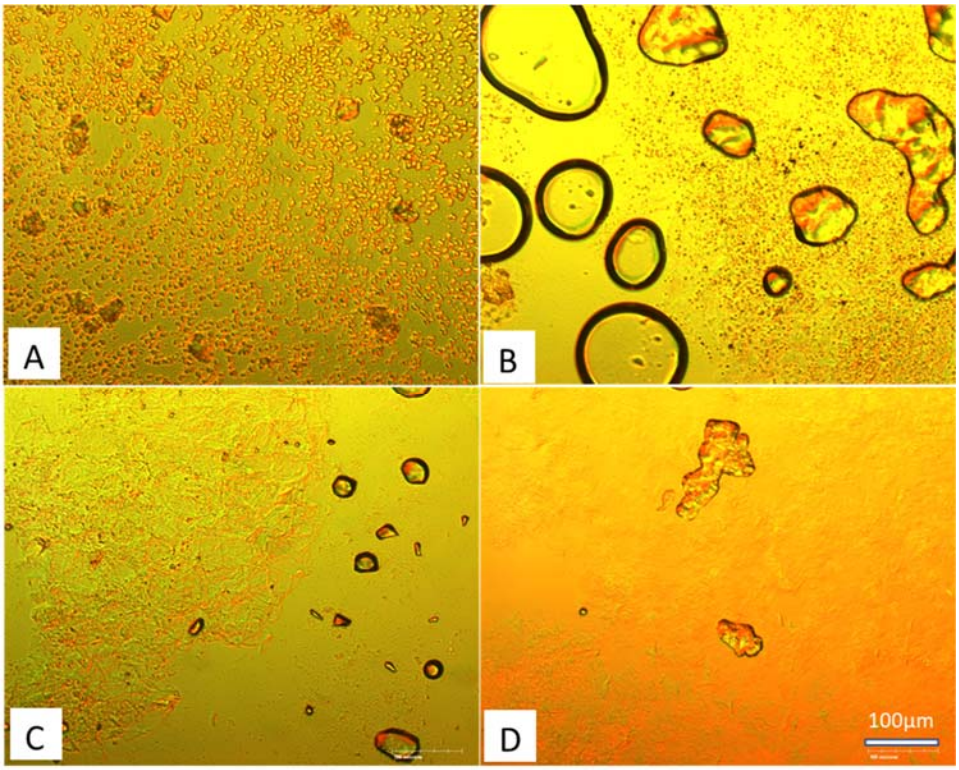


Figure 2-5 Polarized light microscope images (magnification: 200x) of (A) CI gel; (B) PIE gel; (C) CIE gel; (D) oleogel, corresponding to gels in Figure 2-3 and Figure 2-4.

Note: CI=commercial inulin from chicory root; PIE=purified inulin extract from JA; CIE=crude inulin extract from JA

Figure 2-5 (**A-C**) showed microscopic images of particles in CI gel, PIE gel, CIE gel suspended in water. **Figure 2-5D** showed structured olive oil (oleogel). All these gels had two kinds of gel particles in terms of size and forms. Small size particles accounted for the most, and large size particles were in minority. **Figure 2-5A** showed microstructure of CI gel, the average particle size was approximate 10µm which was two times larger than the particle size of PIE gel (**Figure 2-5B**). It could be noted that there were some large air bubbles inside PIE gel that gave it a porous texture. In the previous HPLC analysis, it was known that PIE had a wider DP distribution, which meant both long-chain polymers and

short-chain molecules existed in PIE. Different sizes of molecules dispersed and packed efficiently in spatial arrangement. This might be one reason for several bulky crystals appeared in the image of PIE gel (**Figure 2-5B**). Interestingly, unlike CI and PIE, CIE gel (**Figure 2-5C**) had large pieces of crystals similar to the appearance of oleogel (**Figure 2-5D**). This was likely due to connection of some other compounds inside CIE (such as proteins) with different DPs of inulin, which made it form large pieces of crystals.

2.3.4 Temperature effect

Effect of temperature on emulsion gel formation was shown in **Figure 2-6**. When room temperature water was added to oil phase, bulk gel produced immediately which could not disperse well in water (**Figure 2-6B**). Whereas the treated one formed liquid mixture of oil and water (**Figure 2-6A**). Monoglycerides with high melting point (72℃) could structure oil and form oleogel at low temperature. This system was temperature sensitive. Oil phase and water phase should be prepared at the same temperature to avoid forming bulk gel immediately. Therefore, temperature is an important factor in the preparation of emulsion.

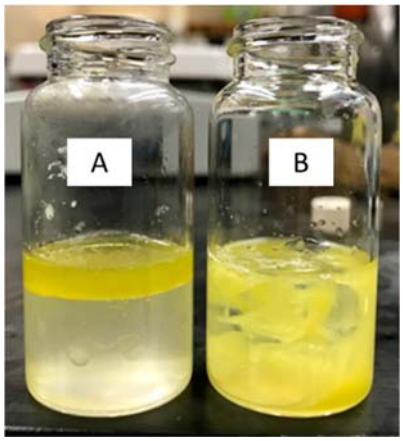


Figure 2-6 Emulsion gel formation influenced by temperature (A) 80℃ water phase; (B) room temperature (23 ℃) water phase.

2.3.5 Emulsions with different formulations

Figure 2-7 showed that nine formulations of emulsions prepared after one week. Formulations 4~8 were all one phase and stable. Of which, oil phase contents ranged from 38.0%~76.0%. Formulation 4 was liquid and creamy, while formulation 5~8 emulsions were gel-like solid. Formulation 4 (oil-in-water) and 6 (water-in-oil) were chosen for optimization.

This result is almost in agreement with the study of Marangoni et al. (2007). In that study, when oil ratio was in the range of 27%~66% and minimum content of monoglyceride was 4%, stable gel emulsions were formed. The co-surfactant used in that study was anionic (sodium stearoyl Lactylate) SSL, which was necessary to keep the stability of gel emulsion (Marangoni et al., 2007). In present work, Tween 80 acted as a co-surfactant, could also help to make stable emulsions.

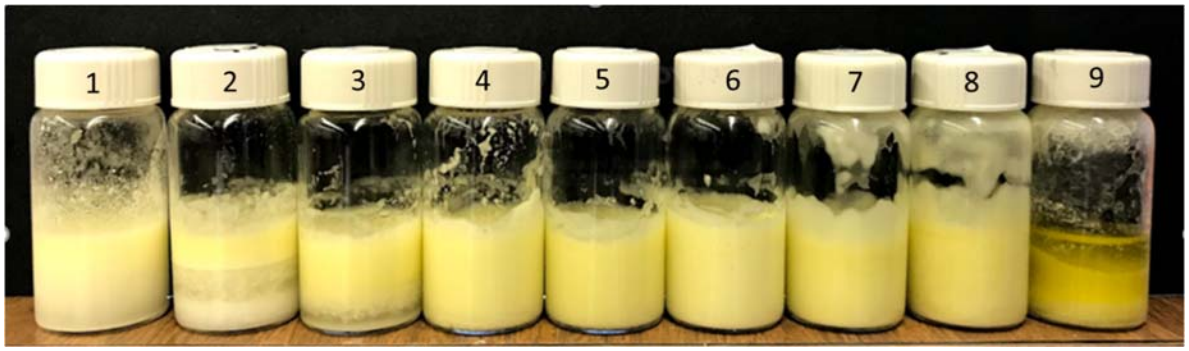


Figure 2-7 Emulsions with different formulations (see Table 2-1) prepared after one week.

2.3.6 Optimization of emulsion formulations

Table 2-2 showed optimized formulations of formulation 4 and 6. For formulation 4, only formulation 4b remained in one phase and stable after 24 hours. Formulation 4a and 4c separated. For formulation 6, all three formulations remained in one phase and were semisolid. After 24 hours, they were still one phase and their physical states changed from

liquid to solid. To conserve the amount of emulsifiers and make solid emulsion, formulation 6a is the best choice for further study. For convenience, this gel-like emulsion is called an emulsion gel.

2.3.7 Effect of different concentrations of PIE on emulsion gel

Figure 2-8 showed different concentrations of PIE in emulsion gels. Incorporation of 1% PIE in emulsion gel improved its appearance compared to emulsion gels without PIE. As PIE concentration increased, texture of emulsion became coarse and color became darker. Emulsion gel containing 10% PIE separated. This might be due to PIE containing impurities, making it less soluble in water, which affected the whole emulsion appearance. If higher concentration of inulin is required, ethanol precipitated inulin (PEI) is a better choice. As was analyzed in HPLC previously, EPI was purer and had almost the same characteristics as CI.

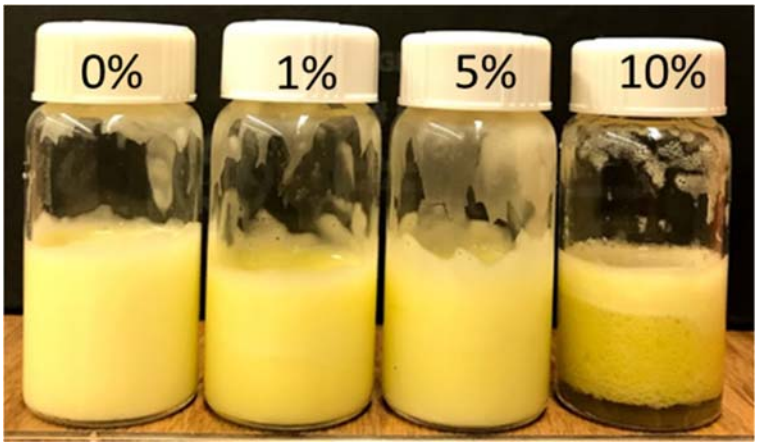


Figure 2-8 Emulsion gel with different percentages of purified inulin extract (PIE).

2.3.8 Effect of inulin on emulsion gel

The pictures of emulsion gels (A, B) that prepared according to **Table 2-4** recipes were shown in **Figure 2-9**. Emulsion A showed syneresis whereas B was homogenous and had lighter color. This indicated 1% PIE improved the homogeneity of emulsion gel. The

appearance of both A and B became worse after 24 hours. Therefore, co-surfactant Tween 80 is necessary as an ingredient. It was assumed that incorporation of inulin (B) could result in stronger, denser network, which prevented droplets from exudation. The physico-chemical properties of emulsion gel need to be further investigated.

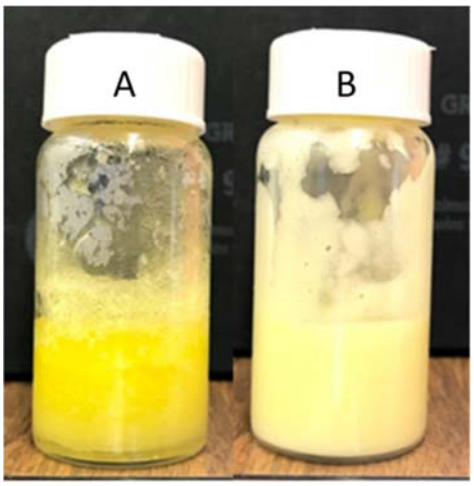


Figure 2-9 The pictures of emulsions (A) without purified inulin extract (PIE); (B) with 1% PIE, in the absence of Tween 80: See Table 2-4 for recipes of formulations.

Note: PIE=purified inulin extract from JA; Tween 80= Polyoxyethylene-20-sorbitan monooleate

2.3.9 Microstructure of emulsion gel

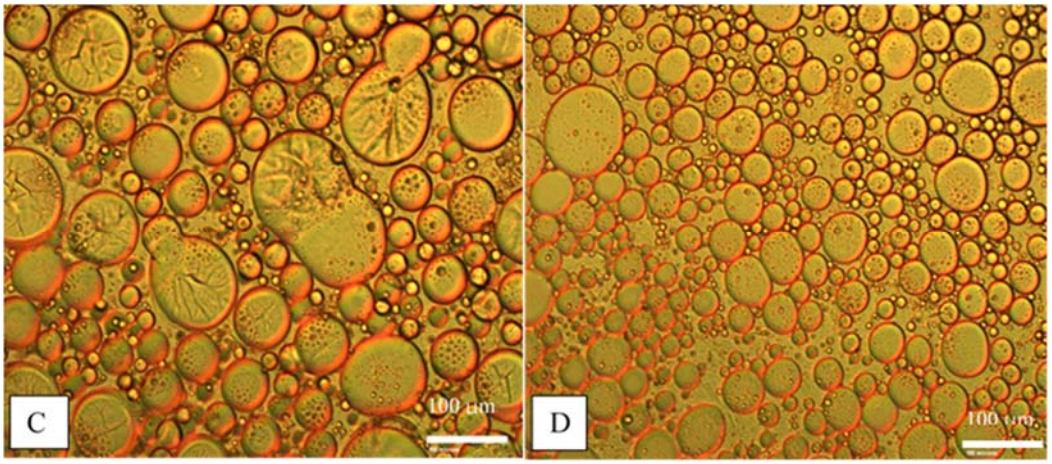
To investigate difference between microstructures of emulsion gels caused by 1% PIE, C and D were analyzed by polarized light microscope and Cryo-environmental scanning electron microscope. The emulsions C and D including Tween 80 were shown in **Figure 2-10(1)**, they had better appearance and texture than A and B.

2.3.9.1 Polarized light microscope

Microstructures of formulation C and D visualized under polarized light microscope were shown in **Figure 2-10(2)**. Droplets were trapped in shells (wall material) consisted of monoglycerides, Tween 80 and inulin, dispersed in continuous phase. It was noted that

most of droplets in formulation D (emulsion with 1% PIE) were smaller and more homogeneous than control. In addition, droplets were full in granule while the control showed big droplets that crumpled. This phenomenon indicated addition of 1% PIE could prevent emulsion from syneresis and made it more stable. This was corresponding with observation on a macro level: emulsion with 1% PIE had better appearance and more fluid than the control, which was attributed to excellent water binding ability of inulin as discussed by literature (Fadaei et el, 2012).

(1)



(2)

Figure 2-10 Images of emulsion gel (1) formulation C: without 1% purified inulin extract (PIE); formulation D: with 1% PIE, in the presence of Tween 80, see recipes in Table 2-4; (2) polarized light microscope (magnification: 200x) images of formulations C and D.

2.3.9.2 Cryo-environmental scanning electron microscope

Morphological characteristics of emulsion gels (C and D) were also determined by cryo-environmental scanning electron microscope (cryo-ESEM) (**Figure 2-11**). Control emulsion gel (**Figure 2-11C**) exhibited hazy texture with large pore sizes whereas emulsion incorporating 1% PIE (**Figure 2-11D**) displayed porous network with smaller pore sizes. This was in agreement with images visualized by polarized light microscope (**Figure 2-10(2)**). The morphological characteristics of emulsion with 1% PIE probably contributed to its creamy and smooth texture.

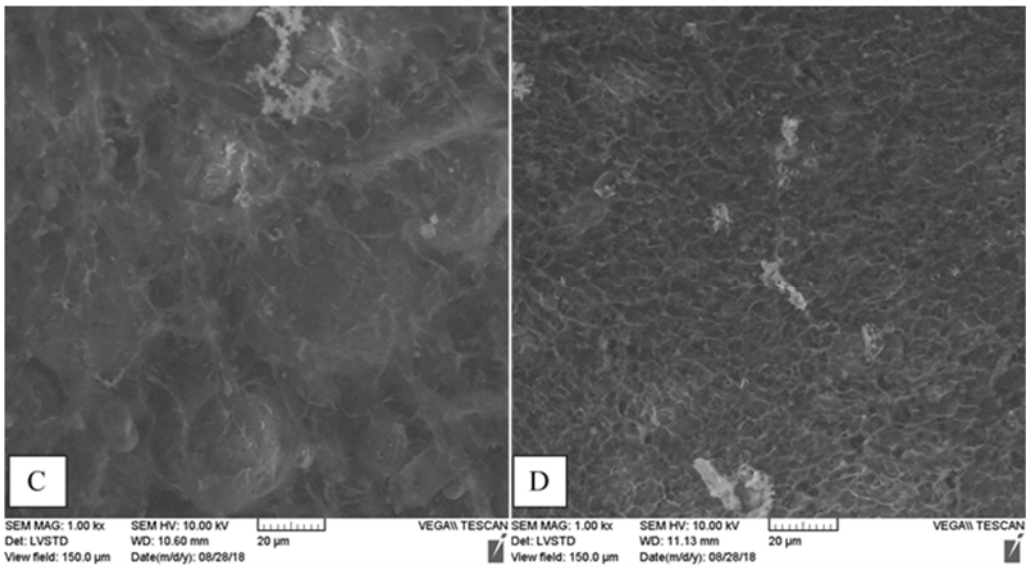


Figure 2-11 Cryo-ESEM micrograph images of emulsions (C) without 1% purified inulin extract (PIE); (D) with 1% PIE, in the presence of Tween 80: see recipes in Table 2-4.

Note: cryo-ESEM=cryo-Environmental scanning electron microscope

When emulsions were observed by cryo-ESEM, constant temperature and pressure were -65℃ and 18~30 Pa, respectively. With temperature and pressure decreased, water

underwent frozen and partial sublimation according to typical pressure-temperature phase diagram of water in physical chemistry. Fat (solid oil) might also undergo partial sublimation at this condition since its melting point (around 6℃) is close to water. However, sublimation percentages of ice (frozen water) and fat (solid oil) were different. In this case, it was assumed that remaining matrix was a cross-link network mainly formed by structured water (gel) and oil (fat) contributed by inulin, monoglyceride and Tween 80 after partial sublimation of fat and ice. If ice crystals remained, incorporation of 1% PIE (**Figure 2-11D**) made ice crystals sizes smaller than the control (**Figure 2-11C**). This indicated inulin could inhibit ice from forming large crystals, which has a potential application on ice cream (Aleong, Frochot, & Goff, 2008).

2.3.10 Melting point analysis

Melting point range of commercial inulin from chicory root (CI), purified inulin extract (PIE) from JA and emulsion gel formulation C and D were showed in

Table 2-6. Each result was average of two measurements. CI had a narrow melting point range, which ranged from 219.2 to 230.3℃. PIE had wider melting point range of 128.4~163.5℃. Emulsion gel formulation C and D had almost same melting point range. Comparing CI with PIE, melting point of CI was higher and narrower range than PIE. It indicated CI had a higher molecular weight (DP) (Blecker et al., 2003) and purity than PIE, which coincided with the results of HPLC analysis. In PIE, besides inulin with different DPs, it might also contain other impurities that did not show peak tested by specific column and detector in HPLC.

Table 2-6 Melting point of CI, PIE and emulsion gel formulation C and D, each result was average of two measurements.

Samples	Melting point range (°C)
Commercial inulin (CI) from chicory root	219.2~230.3
Purified inulin extract (PIE) from JA	128.4~163.5
Emulsion without PIE (formulation C)	57.4~60.8
Emulsion with 1% PIE (formulation D)	59.3~62.6

Incorporation of 1% PIE had slight effect on melting point of emulsion gel, since formulation C and D had similar melting point range. This was likely due to a low percentage of PIE. Nevertheless, both of their melting points were much higher than room temperature, making fat-like emulsion gel different from liquid oil.

When CI approached its melting point, it was pulling away from wall of tube and formed a cone of solid (sintering). Whereas PIE powder shrank and began to darken, which indicated decomposition had occurred. There are mainly two factors that affect inaccuracy in this experiment. Due to different texture between CI and PIE, melting behaviors were different, which caused inaccuracy when recording melting temperature. Regarding melting apparatus, although thermometer can read to greater precision, imperfect heat transfer between metal block and sample may also cause error.

2.4 Conclusion

This chapter examined chromatographic profiles of commercial inulin from chicory (CI), purified inulin extract (PIE) and ethanol precipitated inulin (EPI) as well as effect of PIE on appearance and microstructures of emulsion gel.

PIE had a wider degree of polymerization (DP) distribution and wider melting point range than CI. Ethanol helped to precipitate inulin with higher and narrower DPs. Additionally, thermal-induced gels were formed by CI, PIE and CIE solution with 20% concentration. Monoglycerides could structure olive oil and form oleogel. These gels had similar microstructures observed by polarized light microscope.

Emulsion gel was sensitive to temperature during preparation. In nine different formulations of emulsions, formulation 6 (water-in-oil emulsion, oil: water=60:40) had the best appearance and was chosen for optimization. Emulsion gel incorporating lower content of PIE (1%) had a better appearance than that with higher content (10%). In addition, PIE had a positive effect both on appearance and on microstructure of emulsion gel. Incorporation of 1% PIE decreased syneresis and developed smaller droplet sizes. This kind of emulsion gel has a potential application as a fat replacement in food.

Chapter 3: Effect of JA inulin on lipid oxidation and its potential application on pharmaceuticals

3.1 Introduction

Sugars and sugar-like compounds play an important role in defending oxidative stress in plant cells (Moghaddam, Roy, Xiang, Rolland, & Ende, 2010). Stoyanova et al. (2011) revealed that inulin and stevioside had super capacity of scavenging hydroxyl and superoxide radicals. They were more effective than mannitol that is well-known as an antioxidant (Stoyanova, Geuns, Hideg, & Ende, 2011).

Conjugated dienes are produced during oxidation of lipid that contain dienes or polyenes due to position of double bonds shifted caused by isomerization, molecular rearrangement and conjugation formation. Conjugated dienes have strong absorption at 234 nm, which is a good index for measuring lipid oxidation. Therefore, oxidation of polyunsaturated fatty acids can be detected by increased absorbance at 234 nm caused by products of linoleic acid oxidation (Min & Boff, 2002; Vieira & Regitano-D'arce, 1998).

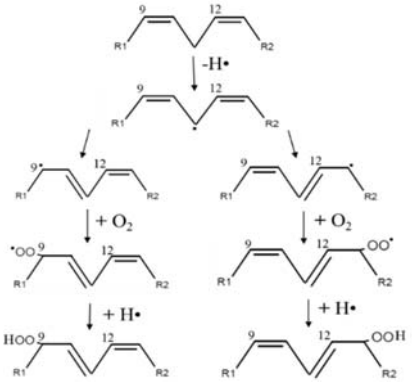


Figure 3-1 Conjugated dienes formation from linoleic acid oxidation, modified from (Min & Boff, 2002).

This chapter was to investigate the effect of JA inulin (PIE and CIE) on linoleic acid oxidation.

3.2 Materials and methods

3.2.1 Chemicals and reagents

Soy lecithin was kindly provided by Grain Process Enterprises limited (Scarborough, ON, CA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), linoleic acid (≥99%), Tween 20 (polyoxyethylene sorbitan monolaurate), inulin from chicory root (commercial inulin) and Folin- Ciocalteu phenol reagent were from Sigma-Aldrich (St Louis, MO, USA). AAPH (2,2'-Azobis (2-amidinopropane) dihydrochloride, 98%) was from Acros Organics (New Jersey, USA). Gallic acid was from Sigma chemical Co. (St. Louis, MO, USA). Sodium carbonate was from VWR International Co. (Mississauga, ON, CA).

3.2.2 Linoleic acid oxidation in an aqueous phase

3.2.2.1 Preparations of ULVs

Liposome dispersion was prepared by film hydration method according to the study of Hosseinian, Muir, Westcott, & Krol (2006) with some modifications. Specifically, 0.5% (v/v) lipid dispersion was prepared by dissolving 20 μL linoleic acid and 30 μL soy lecithin into a vial containing 2 mL chloroform. Thin lipid film was formed at the bottom of vial after chloroform evaporated in fume hood overnight. Phosphate buffer solution (10 mL of 0.05 mol/L, pH 7.4) containing 0.5% (v/v) Tween 20 was added into the vial. This solution was agitated in ultrasonic bath (PS-20, Qingdao, Shandong, China) for 15 min and mixed by a mini-vortexer until it became cloudy.

Homogeneous unilamellar liposome vesicles (ULVs) were produced by extrusion method using Liposofast mini-extruder (Avestin, Inc., Ottawa, ON, CA). 500 μL liposome dispersion passed through a 200 nm porous polycarbonate membrane (Avanti Polar Lipids,

Inc. Alabaster, Alabama) with two support membranes by gently pushing one plunger of filled syringe until solution was transferred to an alternate syringe completely. Extrusion was done back and forth 21 times (as explained previously by Hosseinian et al, 2006 and established in this lab) between the membrane. The 0.5% ULVs dispersion was collected in alternate syringe at the end.

3.2.2.2 Antioxidant assay

Antioxidant activities of commercial inulin (CI) from chicory root, crude inulin extract (CIE), purified inulin extract (PIE) were determined by the method of Liegeois at al. (2000) with modifications. Briefly, 50 µL of 0.5% ULVs dispersion and 50µL of 5 mg/mL samples (final concentration in test in 0.08mg/mL) were added into a quartz cuvette which containing 2.75mL of pH 7.4, 0.05 mol/L phosphate buffer solution. Oxidation reaction was initiated at ambient temperature by adding 150 µL of 40 mmol/L AAPH which acted as a free radical generator. Samples (CIE, PIE, CI) were tested kinetically for 120 min using a Cary 50 Bio UV-visible spectrophotometer (Varian Inc., Australia). Trolox was used as a positive control, which is a well-known water-soluble antioxidant. In the assay without antioxidant, lipid oxidation was carried out in the presence of same amount of phosphate buffer solution (blank control). Absorbances of samples themselves and AAPH in buffer solution at 234 nm were all subtracted (Liégeois, Lermusieau, & Collin, 2000). Each test was performed in triplicates (individually).

3.2.2.3 Statistics analysis

One-way ANCOVA (analysis of covariance) with post-hoc Tukey test was used to find significances between results (α=0.05). Each result was the average of three measurements. Results were significantly different when $P<0.05$.

3.2.3 Interaction of inulin with artificial cell membrane

3.2.3.1 Artificial cell membrane preparation

0.5% liposome dispersion was prepared as described in "section **3.2.2.1**", the only difference was that 50 μL soy lecithin was used instead of 20 μL linoleic acid and 30 μL lecithin. In this case, more lecithin would incorporate in porous polycarbonate membrane, which made it easier to observe difference between incorporated and control one.

Liposome dispersion with and without 1% PIE passed through 200 nm membrane back and forth for 21 times as described in "section **3.2.2.1**" to get membranes for SEM analysis.

3.2.3.2 SEM analysis

Blank polycarbonate membrane, polycarbonate membrane coated by lecithin, membranes treated with and without 1% purified inulin extract (PIE) were observed by conventional scanning electron microscope (SEM) at Nano Imaging Facility Laboratory of Carleton University (Ottawa, ON, Canada). Sample membranes were coated with 7 nm layer Au-Pd and observed under vacuum and room temperature.

3.3 Results and discussion

3.3.1 Antioxidant activity

3.3.1.1 Effect of different samples

Antioxidant properties of CIE, PIE and CI were evaluated by measuring their capacity to inhibit or delay linoleic acid oxidation, which was detected kinetically by increased absorbance of products (conjugated dienes) at 234 nm (Vieira & Regitano-D'arce, 1998). Results were shown in **Figure 3-2**.

CIE had the highest antioxidant activity amongst the treatments and was similar with trolox as there was no significant difference ($P<0.05$) between these two. Whereas PIE had a similar but weaker antioxidant activity compared to CIE as statistical analysis showed no significant differences, but PIE had significantly lower activity than Trolox. CI was similar to blank control, indicating it did not have antioxidant activity (**Figure 3-2**).

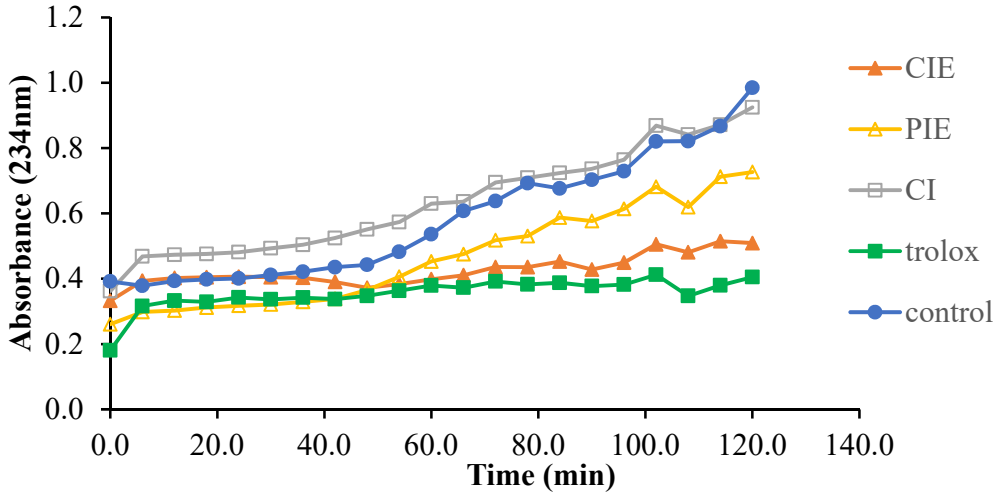


Figure 3-2 Effect of various samples on AAPH-induced linoleic acid oxidation, as tested by absorbance of produced conjugated diene during 120 min, each test was performed in triplicates (individually), data in each curve were the average of triplicate results.

Note: CIE=crude inulin extract from JA; PIE=purified inulin extract from JA; CI=commercial inulin from chicory root; Control=phosphate buffer solution

Trolox curve was almost stable and increased slightly during 120 min run. For blank control (phosphate buffer solution without any antioxidant), it increased dramatically after 50 min, indicating lots of conjugated dienes were produced. Whereas before 50 min, the blank curve remained stable. This was likely due to substrate (ULVs dispersion). As it was mentioned previously (section "**3.2.2.1**"), ULVs dispersion was used as a substrate which contained linoleic acid and soy lecithin (mainly comprised of phospholipid (Scholfield,

1981)). In ULVs, phospholipid encapsulated linoleic acid and protected linoleic acid from oxidation at the beginning of reaction.

In summary, CIE and PIE from Jerusalem artichoke tuber had antioxidant ability against linoleic acid oxidation. CIE had stronger antioxidant ability than PIE. CI did not have antioxidant ability.

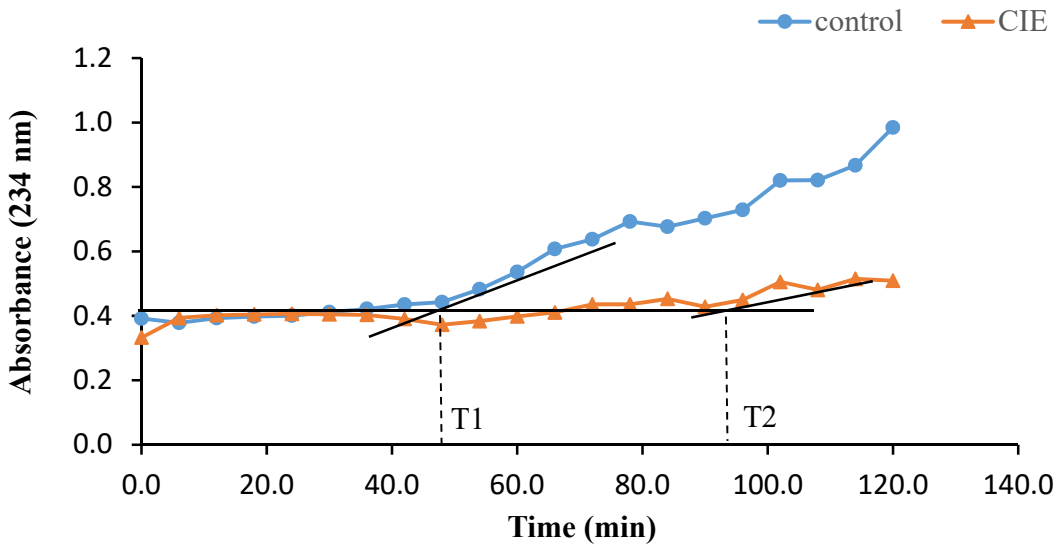


Figure 3-3 Delayed time of lipid oxidation by CIE compared to control, each test was performed in triplicates (individually), data in each curve were the average of triplicate results.

Note: CIE=crude inulin extract from JA; Control=phosphate buffer solution without any antioxidant

Delayed time of linoleic acid oxidation by CIE could be calculated by T2-T1 (**Figure 3-3**). T1 and T2 is the time that linoleic acid began to be oxidized in absence and presence of CIE, respectively. Delayed time by CIE (5 mg/ml) was 42 min.

One possible mechanism in present experiment was that inulin interacted with other compounds remained in CIE and acted as physical barriers (wall material), which encapsulated linoleic acid and formed a shell (Fernandes et al., 2016). This shell protected linoleic acid from oxidation to some extent. If this assumption is true. This is also the

common point that JA inulin played similar roles (wall material) both in “emulsion gel” part and “antioxidants” part. Hincha et al. (2000) revealed that fructan was regarded as protective agents against drought and freezing in plant. In that study, inulin combined with glucose had a capacity of stabilizing liposome, which was probably due to its interaction with liposome, whereas hydroxyethyl starch had no protective effect on liposome (Hincha, Hellwege, Heyer, & Crowe, 2000).

Additionally, CIE antioxidant activity might come from other compounds remained in CIE (Bhagia et al., 2018). The mechanism needs to be further investigated.

3.3.1.2 Effect of different concentrations of CIE

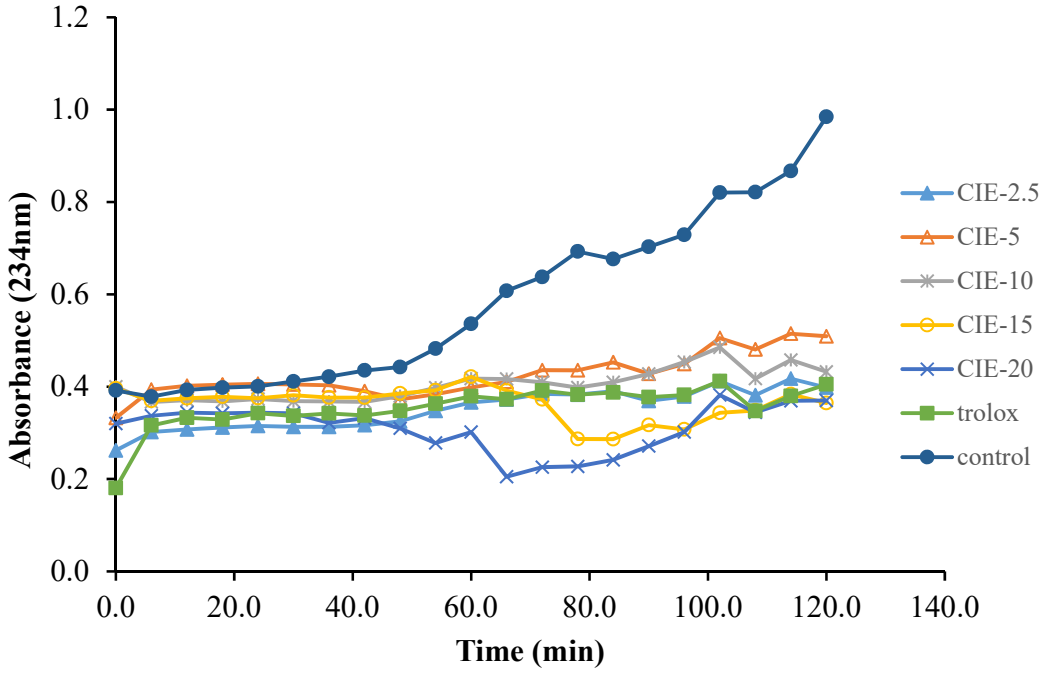


Figure 3-4 Effect of different concentration of CIE on AAPH-induced linoleic acid oxidation, as tested by absorbance of produced conjugated diene during 120 min, each test was performed in triplicates (individually), data in each curve were the average of triplicate results.

Note: CIE-2.5=crude inulin extract from JA with original concentration of 2.5 g/ml; the similar thing for other concentrations.

It is known that active compound is generally dose-dependent (Karadag et al., 2009). To investigate the effect of concentration, CIE samples with five different concentrations were measured, and results were shown in **Figure 3-4**. All tested concentrations of CIE were significantly different than blank control but similar to Trolox (**Figure 3-4**). Therefore, in the range of 2.5-20 mg/ml (final concentration: 0.04-0.33 mg/ml), CIE had antioxidant activity.

3.3.1.3 Effect of different concentrations of PIE

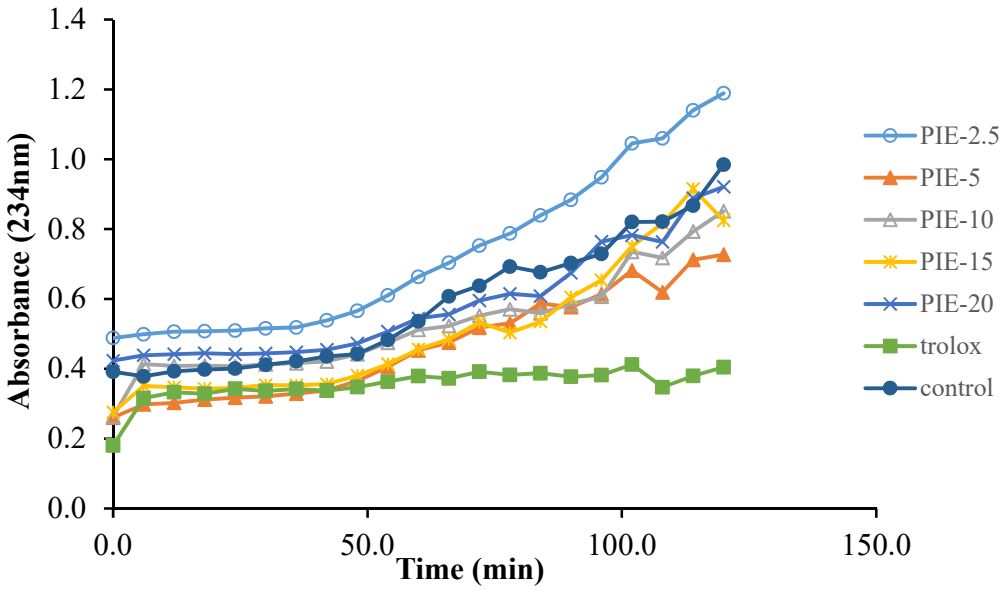


Figure 3-5 Effect of different concentration of PIE on AAPH-induced linoleic acid oxidation, as tested by absorbance of produced conjugated diene during 120 min, each test was performed in triplicates (individually), data in each curve were the average of triplicate results.

Note: PIE-2.5=purified inulin extract from JA with original concentration of 2.5 g/ml; the similar thing for other concentrations.

Lastly, the same way as CIE examination, it was aimed to investigate effect of different concentrations of PIE on prevention of linoleic acid oxidation. Five different concentrations of PIE were run in the same assay. **Figure 3-5** showed the results of this

run. PIE with 2.5 mg/ml (final concentration: 0.04 mg/ml) had the lowest or even no activity compared to all other concentrations and the control. Trolox had significantly higher activity than all samples and control. PIE with 5 mg/ml (final concentration: 0.08 mg/ml) had the highest activity as it was the only treatment that was significantly ($P<0.05$) different than the control (**Figure 3-5**). When concentration was 20 mg/ml (final concentration 0.33 mg/ml), the absorbance of PIE was slightly lower than the control. Therefore, it was assumed that when concentration increased, PIE would have adverse effect on its antioxidant ability. On the other hand, when concentration was too low (2.5 mg/ml), it did not show antioxidant ability.

In conclusion, antioxidant ability of CIE and PIE was concentration dependent and effective concentration range was 5-20 mg/ml (final concentration: 0.08-0.33 mg/ml).

3.3.2 Membrane incorporation and inulin interaction

Figure 3-6 showed schematic diagram of polycarbonate membrane coated with lecithin and interacted with inulin. **Figure 3-7A** displayed SEM micrograph images of 200 nm porous polycarbonate membrane without any treatment. Each pore was seen clearly with around 200 nm diameter and no substance blocking the pores. **Figure 3-7B** showed polycarbonate membrane treated by lecithin. Most of pores were partially blocked and the pore sizes decreased.

Figure 3-7C and **D** displayed membrane treated by liposome dispersion (lecithin) without and with 1% purified inulin extract (PIE), respectively. **Figure 3-7C** could be regarded as artificial cell membrane which consisted of porous polycarbonate membrane coated with lecithin. Simulated cell membrane had lumpy-looking surface with large ridges and grooves (**Figure 3-7C**). Whereas the surface of membrane treated with lecithin

containing 1% PIE was different (**Figure 3-7D**). Most of ridges and grooves were connected, making its surface more even and flat. It is assumed that PIE can interact with lecithin and make it connected on surface. This schematic model diagram was shown in **Figure 3-6**. Alternatively, PIE might facilitate lecithin to pass through the pores of membrane. Simulated cell membrane can be used potentially as a basic in-vitro model. This model can be further modified, either tethered nanoparticles or combined with cells to do cell biology related research. Because of interaction of PIE with lecithin, PIE has potential application as a drug delivery carrier.

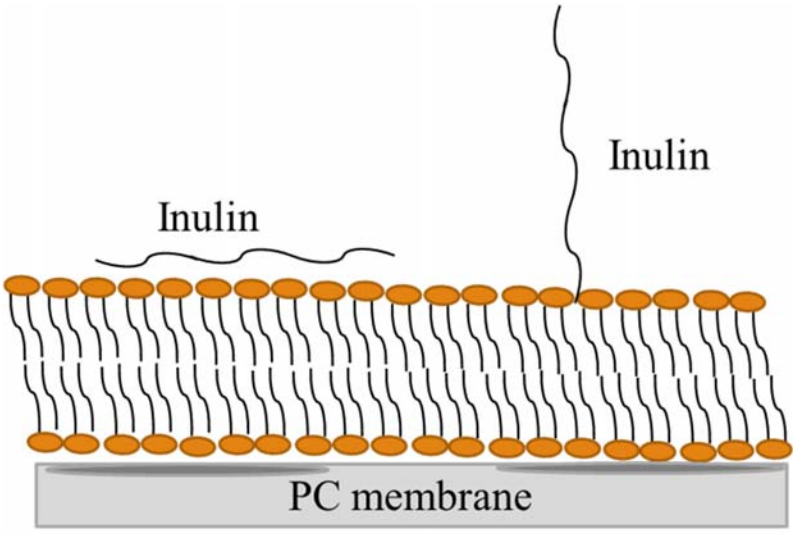


Figure 3-6 Schematic model of porous polycarbonate membrane coated with lecithin and interacted with inulin.

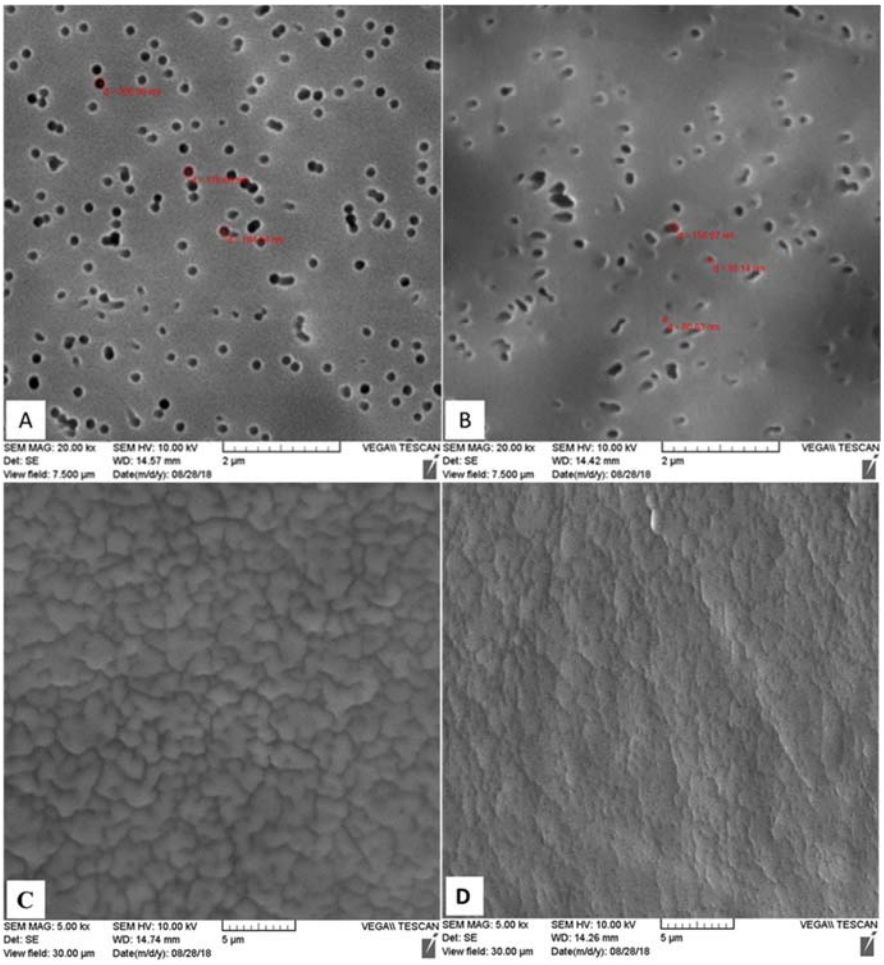


Figure 3-7 SEM micrograph image of 200 nm porous (A) polycarbonate membrane without any treatment; (B) treated by lecithin; (C) polycarbonate membrane treated by lecithin without 1% PIE; (D) with 1% PIE.

Note: SEM=scanning electron microscope

3.4 Conclusion

JA inulin (including crude inulin extract and purified inulin extract) had potential antioxidant ability against AAPH-induced linoleic acid oxidation, as detected by delaying or inhibiting production of conjugated dienes with the range of 5-20 mg/ml (final concentration 0.08-0.33 mg/ml). Crude inulin extract had higher antioxidant ability than

purified inulin extract. Commercial inulin from chicory root did not show antioxidant ability. The mechanism needs to be further investigated.

Porous polycarbonate membrane coated with liposome (lecithin) by the extrusion method can be used potentially as a basic in-vitro cell membrane model. Purified inulin extract could interact with the surface (lecithin) of this membrane model and have a potential application on pharmaceuticals, such as drug delivery carriers.

Chapter 4: General conclusion and future study

In present study, purified inulin extract had a wider range of DPs and melting point than commercial inulin from chicory root. Thermal-induced gels were formed by heating-cooling process after commercial inulin from chicory root, purified inulin extract and crude inulin extract from JA dissolved in distilled water (w/v, 20%). Monoglycerides could structure olive oil and form oleogel. Emulsion gel was sensitive to temperature during preparation. In nine different formulations of emulsions, formulation 6 (oil: water=60:40) had the best appearance and was chosen for further optimization. Emulsion gel incorporating lower content of purified inulin extract (1%) had a better texture than that with higher content (10%). Incorporation of 1% purified inulin extract decreased syneresis and droplet sizes of emulsion gel. This emulsion gel has a potential application as a fat replacement in food.

In addition, JA inulin (purified inulin extract and crude inulin extract) had potential ability to delay AAPH-induced linoleic acid oxidation as tested by absorbance of produced conjugated dienes during 120 min kinetically. Active concentration of purified inulin extract and crude inulin extract ranged from 5 to 20 mg/ml (final concentration 0.08-0.33 mg/ml). Crude inulin extract had a stronger antioxidant ability than purified inulin extract, whereas commercial inulin from chicory root did not have antioxidant ability. A simulated cell membrane model was developed by using porous polycarbonate membrane coated by lecithin through extrusion method, which was a potential supported in-vitro cell membrane model for research related to surface of cell membrane. Scanning electron microscope images showed purified inulin extract made the surface of membrane more even and fat. It

might interact with lecithin that coated on polycarbonate membrane. As a result, purified inulin extract has potential application on pharmaceuticals such as drug delivery vehicles.

In future study, emulsion gel can be further optimized by adopting different techniques and incorporated bioactive compounds to increase stability and health benefits. Techniques such as ultrasound, homogenizer, or spray drying can be used to find which one is the best technique for emulsion gel preparation in terms of stability and quality. Additionally, application of emulsion gel in food products (such as margarine, mayonnaise, sausage, cake products) acted as fat replacement is a promising direction.

The mechanism for antioxidant capacity of purified inulin extract and crude inulin extract can be investigated. Purified inulin extract has a potential application on pharmaceuticals, such as drug delivery carrier. In addition, artificial cell membrane can be combined with nanoparticles or other small molecules as a potential in-vitro model to conduct research that is related to surface of cell membrane.

Appendices

Appendix A

A.1 Statistics analysis of different samples in Figure 3-2

Least Squares Means adjustment for multiple comparisons: Post-hoc using Tukey

SAMPLE	VALUES LSMEAN	LSMEAN Number
CI	0.63363608	1
CIE	0.42440935	2
Control	0.58238263	3
PIE	0.46398897	4
Trolox	0.35452582	5

CI: commercial inulin from chicory root; CIE: crude inulin extract from JA
PIE: purified inulin extract from JA; Control: phosphate buffer solution without samples

Least Squares Means for effect SAMPLE Pr>|t| for H0: LSMean(i)=LSMean(j) Dependent Variable: VALUE					
i/j	1	2	3	4	5
1		<.0001	0.0280	<.0001	<.0001
2	<.0001		<.0001	0.1548	0.0007
3	0.0280	<.0001		<0.0001	<.0001
4	<.0001	0.1548	<.0001		<.0001
5	<.0001	0.0007	<.0001	<.0001	

A.2 Statistics analysis of different concentrations of CIE in Figure 3-4

Least Squares Means adjustment for multiple comparisons: Post-hoc using Tukey

SAMPLE	VALUE LSMEAN	LSMEAN Number
CIE-10	0.40404749	1
CIE-15	0.36327048	2
CIE-2.5	0.35075944	3
CIE-20	0.31003926	4
CIE-5	0.42440935	5
Control	0.58238263	6
Trolox	0.35452582	7

CIE-value: crude inulin extract from JA with different concentrations
Control: phosphate buffer solution without samples

Least Squares Means for effect SAMPLE Pr>|t| for H0: LSMean(i)=LSMean(j) Dependent Variable: VALUE							
i/j	1	2	3	4	5	6	7
1		0.0443	0.0019	<.0001	0.7446	<.0001	0.0055
2	0.0443		0.9690	0.0020	0.0002	<.0001	0.9953
3	0.0019	0.9690		0.0449	<.0001	<.0001	1.0000
4	<.0001	0.0020	0.0449		<.0001	<.0001	0.0193
5	0.7446	0.0002	<.0001	<.0001		<.0001	<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
7	0.0055	0.9953	1.0000	0.0193	<.0001	<.0001	

A.3 Statistics analysis of different concentrations of PIE in Figure 3-5

Least Squares Means adjustment for multiple comparisons: Post-hoc using Tukey

SAMPLE	VALUES LSMEAN	LSMEAN Number
Control	0.58238263	1
PIE-15	0.50466156	2
PIE-20	0.58238787	3
PIE-10	0.52729840	4
PIE-2.5	0.72723281	5
PIE-5	0.46398897	6
Trolox	0.35452582	7

PIE-value: purified inulin extract from JA with different concentrations; Control: phosphate buffer solution without samples

Least Squares Means for effect SAMPLE Pr>|t| for H0: LSMean(i)=LSMean(j) Dependent Variable: VALUE							
i/j	1	2	3	4	5	6	7
1		0.0069	1.0000	0.1476	<.0001	<.0001	<.0001
2	0.0069		0.0069	0.9434	<.0001	0.4975	<.0001
3	1.0000	0.0069		0.1475	<.0001	<.0001	<.0001
4	0.1476	0.9434	0.1475		<.0001	0.0563	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
6	<.0001	0.4975	<.0001	0.0563	<.0001		<.0001
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

References

Aleong, J. M., Frochot, S., & Goff, H. D. (2008). Ice recrystallization inhibition in ice cream by propylene glycol monostearate. *Journal of Food Science*, *73*(9), E463–E468.

Barclay, T., Ginic-markovic, M., Cooper, P., & Petrovsky, N. (2010). Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses. *Journal of Excipients and Food Chemicals*, *1*(3), 27–50.

Barnham, K. J., Masters, C. L., & Bush, A. I. (2004). Neurodegenerative diseases and oxidative stress. *Nature Reviews Drug Discovery*, *3*(3), 205–214.

Beirao-da-costa, Maria Luisa Januario, Maria Isabel Nunes Simao, Filipa Maria Sequeira Leitao, A. E. B. (2005). Characterisation of inulin from chicory and salsify cultivated in Portugal. *Alimentos E Nutrição Araraquara*, *16*(3), 221–225.

Bhagia, S., Ferreira, J. F. S., Kothari, N., Nunez, A., Liu, X., da Silva Dias, N., … Wyman, C. E. (2018). Sugar yield and composition of tubers from Jerusalem artichoke (*Helianthus tuberosus. L.*) irrigated with saline waters. *Biotechnology and Bioengineering*, *115*(6), 1475–1484.

Blecker, C., Chevalier, J. P., Fougnies, C., Herck, J. C. V., Deroanne, C., & Paquot, M. (2003). Characterisation of different inulin samples by DSC:influence of polymerisation degree on melting temperature. *Journal of Thermal Analysis and Calorimetry*, *71*, 215–224.

Böhm, A., Kaiser, I., Trebstein, A., & Henle, T. (2005). Heat-induced degradation of inulin. *European Food Research and Technology*.

Bolouri-Moghaddam, M. R., Roy, K. Le, Xiang, L., Rolland, F., & Ende, W. Van Den.

(2010). Sugar signalling and antioxidant network connections in plant cells. *FEBS Journal*, *277*(9), 2022–2037.

Causey, J. L., Feirtag, J. M., Gallaher, D. D., Tungland, B. C., & Slavin, J. L. (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutrition Research*, *20*(2), 191–201.

Clark, A. H. (1996). Biopolymer gels. *Current Opinion in Colloid & Interface Science*, *1*(6), 712–717.

Dassanayake, L., Kodali, D. R., & Ueno, S. (2011). Formation of oleogels based on edible lipid materials. *Current Opinion in Colloid & Interface Science*, *16*(5), 432–439.

Dave, P. (2012). *Rheological properties of low-fat processed cheese spread made with inulin as a fat replacer*. University of Wisconsin--Stout.

De Wiele, T. Van, Boon, N., Possemiers, S., Jacobs, H., & Verstraete, W. (2004). Prebiotic effects of chicory inulin in the simulator of the human intestinal microbial ecosystem. *FEMS Microbiology Ecology*, *51*(1), 143–153.

Donsì, F. (2018). Applications of nanoemulsions in foods. In *Nanoemulsions* (pp. 349–377).

DrugBank. (n.d.). Inulin. Retrieved from http://www.drugbank.ca/drugs/DB00638

Fadaei, V., Poursharif, K., Daneshi, M., & Honarvar, M. (2012). Chemical characteristics of low-fat wheyless cream cheese containing inulin as fat replacer. *European Journal of Experimental Biology*, *2*(3), 690–694.

Fernandes, R. V. de B., Botrel, D. A., Silva, E. K., Borges, S. V., Oliveira, C. R. de, Yoshida, M. I., … de Paula, R. C. M. (2016). Cashew gum and inulin: new

alternative for ginger essential oil microencapsulation. *Carbohydrate Polymers*, *153*, 133–142.

Funami, T., Funami, M., Yada, H., & Nakao, Y. (1999). Rheological and thermal studies on gelling characteristics of curdlan. *Food Hydrocolloids*, *13*(4), 317–324.

GardenMaine. (2012). Not from Jerusalem, not an artichoke. Retrieved November 4, 2018, from http://gardenmaine.com/2012/09/22/not-from-jerusalem-not-an-artichoke/

Glibowski, P., & Wasko, A. (2008). Effect of thermochemical treatment on the structure of inulin and its gelling properties. *International Journal of Food Science and Technology*, *43*(11), 2075–2082.

Griffin, W. C. (1949). Classification of surface active agents by HLB. *Journal of the Society of Cosmetic Chemists*, *1*, 311–326.

Griffin, W. C. (1954). Calculation of HLB values of non-ionic surfactants. *J.Soc.Cosmet.Chem.*, *5*, 249–256.

Ha, H.-K., Jeon, N.-E., Kim, J. W., Han, K.-S., Yun, S. S., Lee, M.-R., & Lee*, W.-J. (2016). Physicochemical characterization and potential prebiotic effect of whey protein isolate/inulin nano complex. *Korean J Food Sci Anim Resour*, *36*(2), 267–274.

Hamilton, J., Knox, B., Hill, D., & Parr, H. (2000). Reduced fat products-consumer perceptions and preferences. *British Food Journal*, *102*(7), 494–506.

Hartman, K. L., Kim, S., Kim, K., & Nam, J. M. (2015). Supported lipid bilayers as dynamic platforms for tethered particles. *Nanoscale*, *7*(1), 66–76.

Hasenhuettl, G. L., & Hartel, R. W. (2008). *Food emulsifiers and their applications*

(Second Edi). New York: Springer Science +Business Media,LLC.

He, R. R., Li, Y., Li, X. Di, Yi, R. N., Wang, X. Y., Tsoi, B., … Kurihara, H. (2013). A new oxidative stress model, 2,2-azobis(2-amidinopropane) dihydrochloride induces cardiovascular damages in chicken embryo. *PLOS ONE*, *8*(3), 1–11.

Health Canada. (2018). Use of the term "prebiotic(s)." Retrieved January 30, 2019, from http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/labelling/for-industry/health claims/eng/1392834838383/1392834887794?chap=11

Hincha, D. K., Hellwege, E. M., Heyer, A. G., & Crowe, J. H. (2000). Plant fructans stabilize phosphatidylcholine liposomes during freeze- drying. *European Journal of Biochemistry*, *267*(2), 535–540.

Hofberger, R. W. H. H. von E. (2018). Fats, oils and emulsifiers. In *Confectionery Science and Technology* (pp. 85–124). Springer, Cham.

Hongxin, W. (2008). Study on the decoloration of chicory inulin by active carbon Method. *Journal of Anhui Agricultural Sciences*, *36*(7), 2626.

Hosseinian, F. S., Muir, A. D., Westcott, N. D., & Krol, E. S. (2006). Antioxidant capacity of flaxseed lignans in two model systems. *JAOCS*, *83*(10), 835–840.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841–1856.

Huang, G., Chen, Y., & Wang, X. (2011). Extraction and deproteinization of pumpkin polysaccharide. *International Journal of Food Sciences and Nutrition*, *62*(6), 568–571.

Jadhav, K. R., Shaikh, I. M., Ambade, K. W., & Kadam, V. J. (2006). Applications of

microemulsion based drug delivery system. In *Current Drug Delivery* (p. 267–273(7)). Bentham Science Publishers.

Kansy, M., Senner, F., & Gubernator, K. (1998). Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes. *Journal of Medicinal Chemistry*, *41*(7), 1007–1010.

Karadag, A., Ozcelik, B., & Saner, S. (2009). Review of methods to determine antioxidant capacities. *Food Analytical Methods*, *2*(1), 41–60.

Kim, Y., Faqih, M. N., & Wang, S. S. (2001). Factors affecting gel formation of inulin. *Carbohydrate Polymers*, *46*(2), 135–145.

Ku, Y., Jansen, O., Oles, C. J., Lazar, E. Z., & Rader, J. I. (2003). Precipitation of inulins and oligofructose by ethanol and other solvents. *Food Chem*, *81*, 125–132.

Li, L., Li, L., Wang, Y., Du, Y., & Qin, S. (2013). Biorefinery products from the inulin-containing crop Jerusalem artichoke. *Biotechnology Letters*, *35*(4), 471–477.

Li, W., Zhang, J., Yu, C., Li, Q., Dong, F., Wang, G., … Guo, Z. (2015). Extraction, degree of polymerization determination and prebiotic effect evaluation of inulin from Jerusalem artichoke. *Carbohydrate Polymers*, *121*, 315–319.

Liégeois, C., Lermusieau, G., & Collin, S. (2000). Measuring antioxidant efficiency of wort, malt, and hops against the 2,2’-azobis(2-amidinopropane) dihydrochloride-induced oxidation of an aqueous dispersion of linoleic acid. *Journal of Agricultural and Food Chemistry*, *48*(4), 1129–1134.

Lin, W., Yu, C., Triffo, S., & Groves, J. T. (2010). Supported membrane formation, characterization, functionalization, and patterning for application in biological

science and technology. *Current Protocols in Chemical Biology*, *2*, 235–269.

Liu, R., Wang, L., Liu, Y., Wu, T., & Zhang, M. (2018). Fabricating soy protein hydrolysate/xanthan gum as fat replacer in ice cream by combined enzymatic and heat-shearing treatment. *Food Hydrocolloids*, *81*, 39–47.

Majzoobi, M., Mohammadi, M., Mesbahi, G., & Farahnaky, A. (2018). Feasibility study of sucrose and fat replacement using inulin and rebaudioside A in cake formulations. *Journal of Texture Studies*, *49*, 468–475.

Mandracchia, D., Trapani, A., Perteghella, S., Di Franco, C., Torre, M. L., Calleri, E., & Tripodo, G. (2018). A micellar-hydrogel nanogrid from a UV crosslinked inulin derivative for the simultaneous delivery of hydrophobic and hydrophilic drugs. *Pharmaceutics*, *10*(3).

Marangoni, A. G., Idziak, S. H. J., Vega, C., Batte, H., Ollivon, M., Jantzi, P. S., & Rush, J. W. E. (2007). Encapsulation-stucturing of edible oil attenuates acute elevation of blood lipids and insulin in humans. *Soft Matter*, *3*(2), 183–187.

Martins, C. A., Fernández, P. S., & Camara, G. A. (2018). Alternative uses for biodiesel byproduct: glycerol as source of energy and high valuable chemicals. In *Increased Biodiesel Efficiency* (pp. 159–186). Springer, Cham.

McClements, D. J. (2015). Emulsion ingredients. In *Food Emulsions: Principles, Practices, and Techniques* (Third, pp. 99–183). Boca Raton, Florida: CRC Press.

Mert, B., & Demirkesen, I. (2016). Reducing saturated fat with oleogel/shortening blends in a baked product. *Food Chemistry*, *199*, 809–816.

Meyer, D., Bayarri, S., Tárrega, A., & Costell, E. (2011). Inulin as texture modifier in dairy products. *Food Hydrocolloids*, *25*(8), 1881–1890.

Min, D. B., & Boff, J. M. (2002). Lipid oxidation of edible oil. In C. C. Akoh & D. B. Min (Eds.), *Food lipids: Chemistry, nutrition and biotechnology* (Second edition). New York: Marcel Dekker Inc.

Mozaffarian, D., Katan, M. B., Ascherio, A., Stampfer, M. J., & Willett., W. C. (2006). Trans fatty acids and cardiovascular disease. *The New England Journal of Medicine*, *354*(15), 1601–1613.

Ng, S. F., Rouse, J. J., Sanderson, F. D., & Eccleston, G. M. (2012). The relevance of polymeric synthetic membranes in topical formulation assessment and drug diffusion study. *Archives of Pharmacal Research*, *35*(4), 579–593.

Norudin, N. S., Mohamed, H. N., & Yahya, N. A. M. (2018). Controlled released alginate-inulin hydrogel: development and in-vitro characterization. In *AIP Conference Proceedings* (Vol. 2016, pp. 201131–6). American Institute of Physics.

Oh, I. K., & Lee, S. (2018). Utilization of foam structured hydroxypropyl methylcellulose for oleogels and their application as a solid fat replacer in muffins. *Food Hydrocolloids*, *77*, 796–802.

Ojijo, N. K. O., Neeman, I., Eger, S., & Shimoni, E. (2004). Effects of monoglyceride content, cooling rate and shear on the rheological properties of olive oil/monoglyceride gel networks. *Journal of the Science of Food and Agriculture*, *84*(12), 1585–1593.

Pasquali, R. C., Taurozzi, M. P., & Bregni, C. (2008). Some considerations about the hydrophilic–lipophilic balance system. *International Journal of Pharmaceutics*, *356*(1–2), 44–51.

Peyrat-Maillard, M. N., Cuvelier, M. E., & Berset, C. (2003). Antioxidant activity of

phenolic compounds in 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidation: synergistic and antagonistic effects. *Journal of the American Oil Chemists' Society*, *80*(10), 1007–1012.

Pintado, T., Herrero, A. M., Colmenero, F. J., Cavalheiro, C. P., & Capillas, C. R. (2018). Chia and oat emulsion gels as new animal fat replacers and healthy bioactive sources in fresh sausage formulation. *Meat Science*, *135*, 6–13.

Rios, R. V., Garzón, R., Lannes, S. C. S., & Rosell, C. M. (2018). Use of succinyl chitosan as fat replacer on cake formulations. *LWT-Food Science and Technology*, *96*, 260–265.

Roberfroid, M. B., Van Loo, J. A. E., & Gibson, G. R. (1998). The bifidogenic nature of chicory inulin and its hydrolysis products. *The Journal of Nutrition*, *128*(1), 11–19.

Rubel, I. A., Iraporda, C., Novosad, R., Cabrera, F. A., Genovese, D. B., & Manrique, G. D. (2018). Inulin rich carbohydrates extraction from Jerusalem artichoke (*Helianthus tuberosus L.*) tubers and application of different drying methods. *Food Research International*, *103*, 226–233.

Saengthongpinit, W., & Sajjaanantakul, T. (2005). Influence of harvest time and storage temperature on characteristics of inulin from Jerusalem artichoke (*Helianthus tuberosus L.*) tubers. *Postharvest Biology and Technology*, *37*(1), 93–100.

SaffireBlue. (2018). Required HLB values of oils / lipids. Retrieved from http://www.saffireblue.ca/blog/required-hlb-of-oils-and-lipids.htm

Scholfield, C. R. (1981). Composition of soybean lecithin. *Journal of the American Oil Chemists' Society*, *58*(10), 889–892.

Singh, R. ., Singh, T., & Larroche, C. (2019). Biotechnological applications of inulin-rich

feedstocks. *Bioresource Technology*, *273*, 641–653.

Sivandzade, F., & Cucullo, L. (2018). In-vitro blood–brain barrier modeling: A review of modern and fast-advancing technologies. *Journal of Cerebral Blood Flow and Metabolism*, *38*(10), 1667–1681.

Sivapratha, S., & Sarkar, P. (2018). Multiple layers and conjugate materials for food emulsion stabilization. *Critical Reviews in Food Science and Nutrition*, *58*(6), 877--892.

Sołowiej, B., Glibowski, P., Muszyn´ski, S., Wydrych, J., Gawron, A., & Jelin´ski, T. (2014). The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. *Food Hydrocolloids*, *44*, 1–11.

Srinameb, B. orn, Nuchadomrong, S., Jogloy, S., Patanothai, A., & Srijaranai, S. (2015). Preparation of inulin powder from Jerusalem artichoke (*Helianthus tuberosus L.*) Tuber. *Plant Foods for Human Nutrition*, *70*(2), 221–226.

Stoyanova, S., Geuns, J., Hideg, É., & Van Den Ende, W. (2011). The food additives inulin and stevioside counteract oxidative stress. *International Journal of Food Sciences and Nutrition*, *62*(3), 207–214.

Tchoné, M., Bärwald, G., & Meier, C. (2005). Polyphenoloxidases in Jerusalem artichoke (*Helianthus tuberosus L.*). *British Food Journal*, *107*(9), 693–701.

Temkov, M., Petkova, N., Denev, P., & Krastanov, A. (2015). Characterization of inulin from Helianthus tuberosus L. obtained by different extraction methods-comparative study. *Scientific Works of University of Food Technologies*, 461–464.

Van der Beek, C. M., Canfora, E. E., Kip, A. M., Gorissen, S. H. M., Olde Damink, S. W.

M., van Eijk, H. M., … Lenaerts, K. (2018). The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. *Metabolism*, *87*, 25–35.

Verstringe, S., Moens, K., Clercq, D. N., & Dewettinck, K. (2015). Crystallization behavior of monoacylglycerols in a hydrophobic and a hydrophilic solvent. *Food Research International*, *67*, 25–34.

Vieira, T. M. F. S., & Regitano-D'arce, M. A. B. (1998). Stability of oils heated by microwave: UV-spectrophotometric evaluation. *Ciênc. Tecnol. Aliment.*, *18*(4), 433–437.

Walz, M., Hagemann, D., Trentzsch, M., Weber, A., & Henle, T. (2018). Degradation studies of modified inulin as potential encapsulation material for colon targeting and release of mesalamine. *Carbohydrate Polymers*, *199*, 102–108.

Wiele, T. va. de, Boon, N., Possemiers, S., Jacobs, H., & Verstraete, W. (2007). Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. *Journal of Applied Microbiology*, *102*(2), 452–460.

Zahn, S., Pepke, F., & Rohm, H. (2010). Effect of inulin as a fat replacer on texture and sensory properties of muffins. *International Journal of Food Science and Technology*, *45*(12), 2531–2537.

Ziyan, E., & Pekyardimci, Ş. (2003). Characterization of polyphenol oxidase from Jerusalem artichoke (*Helianthus tuberosus.L.*). *Turkish Journal of Chemistry*, *27*(2), 217–225.