Storage and Thermal Effects on the Oxidative Stability and Emulsion Characteristics of
Hemp (*Cannabis sativa* L.) Oil-in-Water Emulsions

by

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Abstract

It has been well established that hemp oil has potential for functional food products and nutraceutical formulations due to its fatty acid profile. However, is its commercial application is limited as a result of its corresponding oxidation susceptibility. To assess the suitability of micro-encapsulation to improve oxidative stability, oil-in-water (1% v/v) emulsions treated with surfactant were exposed to temporal and thermal conditions and examined for resistance to rancidity. Hemp and avocado emulsions were prepared by an ultra-sonication procedure (40% amplitude, processing time 5 min), and a 0.5% (w/v) soy protein and 0.5% (w/v) lecithin suspension was incorporated into treated emulsions as the surfactant. Samples were subjected to conditions of 25°C and 4°C for the 42-day storage trial, as well as short exposure to a 72°C condition for the thermal trial. The diphenyl-1-pyrenylphosphine assay was performed and sulfhydryl group levels were quantified as oxidative parameters. Average particle size, polydispersity, and zeta potential were analyzed as the emulsion characteristics. All emulsions were relatively resistant to further oxidation during the thermal trial. Over the course of the storage trial, emulsions with surfactant exhibited smaller particle sizes and lower polydispersity, compared to non-treated emulsions. Although this encapsulation technology appears promising for physical emulsion traits, there was a tendency for emulsions with surfactant to have higher oxidation levels. Soy lecithin should be re-examined as a food grade emulsifier for highly unsaturated oils, as it may become oxidized itself or catalyze oxidation of the oils, contributing to lipid oxidation levels. Hemp oil's instability during storage and processing will continue to limit its food applications until suitable encapsulation techniques and surfactant materials are identified.

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List of Abbreviations Used

ALA	α-linolenic acid
CBD	Cannabidiol
DHA	docosahexaenoic acid
DPPP	Diphenyl-1-pyrenylphosphine
DTNB	5,5′-dithio-bis(2-nitrobenzoic acid)
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
GLA	Gamma-linolenic acid
LA	Linoleic acid
LEC	Soy lecithin
LEC	Soy lecithin
O/W	Oil-in-water
PI	Polydispersity index
PS	Particle size
PUFA	Polyunsaturated fatty acid
Sabs	Surfactant absent
SEM	Standard error mean
SH	Sulfhydryl groups
SPI	Soy protein isolate
SPI	Soy protein isolate
Spr	Surfactant present
THC	Tetrahydrocannabinol
ZP	Zeta potential

Acknowledgements

Chapter 1: Introduction

1.1 Thesis Overview

Hemp (*Cannabis sativa* L., Cannabaceae) is a versatile herbaceous crop originating from Central Asia, which has been used traditionally for its food, fibre, and medicinal purposes (Vonapartis et al., 2015; Andre et al., 2016). As an ancient fibre crop, hemp is said to have lined the spine of the first copy of the Bible with pages and set Columbus's sails with canvas and rope (Struik et al., 2000; Callaway, 2004; Ranalli & Venturi, 2004). The hemp seed, which can be consumed raw, cooked, or pressed into oil, has been well documented as a primitive source of fibre, protein, and lipids (Callaway, 2004; Matthäus & Brühl, 2008). As well, hemp products have been used to aid in treating and preventing ailments for thousands of years in traditional oriental medicine (Callaway, 2004; Ranalli & Venturi, 2004).

Undoubtedly, the recently concluded 60-year prohibition on hemp (late 1930s to late 1990s) has hindered the scientific investigation of its health and medicinal purposes (Dimić et al., 2008). However, in recent years, the viable crop has experienced a renaissance, with more serious exploration of the crop's potential commencing, accompanied by stringent regulations.

As modern science catches up with ancient knowledge, many studies have confirmed the potential of hemp seed's constituents. Hemp seed oil is a rich source of polyunsaturated fatty acids (PUFAs). Most notably, hemp oil contains omega-6 and -3 fatty acids (FAs), linoleic acid (LA) and α-linolenic acid (ALA), at a ratio of ~3:1, which is regarded as

ideally balanced for human nutrition (Tang et al., 2006; Ditrói et al., 2013; Vonapartis et al., 2015). These FAs must be acquired from the diet, as they are required for proper nutrition but cannot be synthesized by humans (Deferne & Pate, 1996; Kriese et al., 2004; Tang et al., 2006; Dimić et al., 2008; Vonapartis et al., 2015). In addition to serving as precursors to biochemicals that regulate a variety of biological functions, these FAs have been reported to improve heart health, and mitigate other disorders (Deferne & Pate, 1996; Parker et al., 2003). Hemp oil is known to contain trace amounts of phytocannabinoids, which are benefical to human health (Callaway, 2004). The predominant phytocannabinoid in hemp is cannabidiol (CBD), which has been studied extensively and is recognized as a strong bioactive with anti-inflammatory and antioxidant effects (Russo, 2007; Ditrói et al., 2013; Andre et al., 2016). These characteristics of hemp oil raise interests for functional food application strategies.

Typically, food products undergo some form of storage and heat processing prior to consumption, which can cause conformational changes and degradation of the lipids and bioactive compounds if the oil is susceptible to rancidity (Calligaris et al., 2016). Oxidative stability is an important safety parameter of oils, as well as a determinant of food quality and biological activity of lipophilic bioactive molecules (Parker et al., 2003; Uluata & Özdemir, 2012; Calligaris et al., 2016). Unfortunately, although hemp oil products are rising in popularity and hold great promise for health applications, the oxidative stability of the oil has proven very poor during storage and processing. Hemp oil has been shown to be particularly susceptible to oxidation when compared to several other edible oils (Parker et al., 2003; Dimić et al., 2008; Uluata & Özdemir, 2012). This sensitivity of hemp oil to

oxidative deterioration has been tied to its high content of unsaturated FAs, since these unsaturated bonds are vulnerable to attack by oxygen (Belhaj et al., 2010; Uluata & Özdemir, 2012; Prescha et al., 2014).

In recent years, encapsulation techniques have been utilized to preserve and enhance the quality and functionality of edible oils (Huang et al., 2008; Sozer and Kokini, 2008; Ezhil-arasi et al., 2013; Khaled et al., 2014; Devi et al., 2016). Encapsulation may serve to protect the beneficial lipids in hemp oil by creating a physical barrier between the oil and environmental conditions that induce oxidation (oxygen, heat, light).

Although some studies have examined the oxidative stability of hemp oil encapsulated by surfactants in emulsions, the characteristics of the emulsions were not evaluated (Sapino, 2005; Abuzaytoun and Shahidi, 2006; Raikos et al., 2015; Kowalska et al., 2015). Overall, little is known about the stability of hemp oil against oxidation when dispersed in an aqueous food matrix and how the emulsion characteristics relate to its stability. This thesis will provide a review on the hemp crop and encapsulation of edible oils, as well as the results and implications of using micro-encapsulation techniques for stabilizing hemp oil against oxidation in an effort to broaden its food applications.

1.2 Thesis Outline and Objectives

This thesis aims to provide a three-part literature review and manuscript detailing the testing methods and results for the micro-encapsulation of hemp oil. The first section of the literature examines the hemp crop with a focus on its cultivation, prohibition, and resurgence in the food industry. The second section reviews the composition of hemp, health implications of its derivatives, and the oxidative instability of hemp seed oil. The last section outlines encapsulation, emulsification as an encapsulation technique, and the encapsulation of edible oils. The thesis manuscript describes the methods, results, and discussion of an ultrasonication technique for micro-encapsulation of hemp oil. By formulating oil-in-water emulsions and subjecting them to temporal and thermal conditions, the objective was to evaluate whether oil which was micro-encapsulated by surfactant would better resist oxidation, compared to emulsions without surfactant.

I hypothesized that in emulsions with surfactant present, the inner oil would be protected from oxidation and thus lipid oxidation would be lower in oxidation inducing conditions (storage over time and exposure to heat), compared to emulsions without surfactant. I expected to see a greater indication of protein oxidation in emulsions with the addition of the surfactant because it contained protein and this protein would likely oxidize over time as the outer barrier to the oil. I also expected the surfactant to improve emulsion characteristics, lowering the average particle size, polydispersity, and creating a more stable surface charge of the oil droplets. The overall purpose of this work was to examine an encapsulation technique with promise to improve the stability and physical emulsion traits of hemp oil, which would enable its use in fortified food products.

Chapter 2: Literature Review

2.1 Hemp – Ancient Remedy, Prevailing Therapy

The cultivation of hemp can be dated back to China around 2700 BC, and its believed to have then expanded across Asia, eventually making its way to Europe approximately 2000–2200 years ago (Callaway, 2004; Ranalli & Venturi, 2004). Hemp is so deeply rooted in our history, the oldest relics of human industry are fragments of hemp fabric, found in tombs dating back to 8000 BC (Shahzad, 2011; Cherney & Small, 2016). This crop was quite literally sewn into the fabric of who we are as a species. Despite the historic functionality of this multi-purpose crop, global hemp production still only comprises about 0.5% of the total production of natural fibers, and has only recently made its way into health products and the food market (Shahzad, 2011). This can be primarily attributed to the pariah or panacea controversy that dates back a century, centered around the prohibition of one of the oldest plants cultivated by man (Russo, 2007).

2.1.1 Prohibition and Perseverance

There has been no other example in history of such fueled government engagement with a crop, as that with the *Cannabis sativa* L. species. Prohibition deemed hemp guilty by its association with marijuana, which contains the psychoactive compound δ-9-tetrahydrocannabinol (THC) (Tang et al., 2006). Although concerns over the insignificant quantity of THC in industrial hemp have been long disputed by farmers and scientific evidence, the plant fell under the umbrella of "marijuana" in the late 1930s and its production was prohibited in Canada within the Narcotics Control Act (Callaway, 2004; Tang et al., 2006, Agriculture and Agri-Food Canada, 2016).

With the onset of World War II, prohibition was lifted temporarily, when imports of other sources of fiber were unavailable (Callaway, 2004). As an interesting historical note, hemp was of such necessity to the war effort, the United States Department of Agriculture produced an educational video "Hemp for Victory" to encourage farmers to grow hemp (Dir Raymond Evans, 1942). Such promotional efforts led many Americans and Canadians to invest in hemp production and divert farming land towards hemp cultivation, only to subsequently lose their means of a livelihood with reinstated prohibition. Renewal of prohibition after the war caused interest in the industry and production to dwindle and become deferred to other crops (Callaway, 2004; Shahzad, 2011; Mass, 2015). Hemp production persisted in China, the current world leader in production, but in most countries competitive fibre crops like cotton surpassed hemp production and processing technologies for rope, apparel, paper, and other products (Cherney & Small, 2016).

Motivated by the insistence of Canadian activists, research licenses to grow industrial hemp on an experimental basis were issued in 1994 (Laate, 2015). Effective March 12, 1998, under a closely monitored licensing and controlled system by Health Canada, commercial production of hemp was legalized and cultivation commenced in Canada (Laate, 2015; Agriculture and Agri-Food Canada, 2016). Rightfully so, since hemp differs greatly from its psychoactive counterpart marijuana.

While both hemp and marijuana belong to the plant species *Cannabis sativa* L., they are diverse in terms of phytochemical constituents and production strategies (Johnson, 2015). Classification of *Cannabis* as either marijuana or industrial hemp in Canada is based on a

threshold concentration of THC for the hemp cultivar (Cherney & Small, 2016). Cultivation of hemp must be done so with certified seed and must result in a plant with less than 0.3% (w/w) THC in the leaves and flowering heads or 10 µg/g (ppm) THC in the hemp grain, whereas the marijuana plant may contain upwards of 10% (w/w) THC (Russo, 2007; Vonapartis et al., 2015; Johnson, 2015; Agriculture and Agri-Food Canada, 2016). Currently, anyone who wants to participate in the production of hemp (growth, processing, sale, exports, etc.) must be a Canadian resident, submit an application, pass a criminal background check, as well as have no record of drug-related offences in the last 10 years (Agriculture and Agri-Food Canada, 2016). This strict system for monitoring production has effectively prevented any reports linking hemp farms to the drug market for almost 20 years (Cherney & Small, 2016).

Apart from the differences in biochemical composition, the cultivars are also grown to present vastly different morphological features. Hemp grown for fiber or oil are planted with narrower spacing between rows than the marijuana cultivar, and the resulting plants are tall and stalky with few branches, or short with dense branching and flowering, respectively. In addition, industrial hemp is either harvested for the stalk or seeds, whereas the flowering buds are collected for the *Cannabis* drug cultivar (West, 1998; Datwyler and Weiblen, 2006; Agriculture and Agri-Food Canada, 2016). These differences between hemp and marijuana can be in part attributed to the genetics of the plant. Datwyler and Weiblen (2006) identified a 27.2% genetic variation between hemp and marijuana cultivars through amplified fragment length polymorphisms. Hakki et al. (2008) also assessed genome regions between 30 Cannabis plants, hemp and marijuana varieties, and found high

levels of genetic variance. Since recognition of these differences between hemp and mari-juana, hemp cultivation has flourished in Canada. In 2011, 340 cultivation licenses were issued to farmers in Canada who grew more than 39 000 acres of industrial hemp, and this acreage increased to 108 000 in 2014 (Health Canada, 2011; Agriculture and Agri-Food Canada, 2016).

2.1.2 Efficiency and Viability

Cultivation of the crop appeals to farmers because of its hardiness and the lack of inputs required for a successful growing season. The cultivation of hemp is much more efficient and considered less environmentally degrading than many other fibre and oil crops, and it can be grown under a variety of agro-ecological conditions (Struik et al., 2000; Ranalli & Venturi, 2004). As a fibre crop, hemp provides a high yield and has a capacity to grow quickly; it produces 250 percent more fiber than cotton and 600 percent more fiber than flax, with the same amount of land (Struik et al., 2000; Mass, 2015). Hemp is second only to bamboo for efficient land use and biomass yield (Alberta Agriculture and Forestry, 2015).

The crop also requires little inputs and inflicts limited harm to the land. Due to the fast growing, dense canopy, hemp is a natural weed suppressor and can grow without fungi-cides, herbicides and pesticides (Struik et al., 2000; Mass, 2015; Agriculture and Agri-Food Canada, 2016). The anchored root system maintains soil quality by preventing soil erosion and nutrient leaching. As well, the continual shedding of leaves through growing season adds moist organic matter to the soil, replenishing nutrients (Ranalli & Venturi,

2004; Mass, 2015). Hemp is often described as stress tolerant; farmers have expressed complete satisfaction with the growth of hemp crops that have been cultivated steadily for many years on the same land (Mass, 2015; Cherney & Small, 2016).

2.1.3 Revival of Ancient Crop

In recent years, this viable crop has experienced a renaissance. The resurgence of interest in the hemp crop can be attributed to the demand for sustainable agricultural practices, along with the recognition of hemp's superior fibre content and nutritional profile. It is estimated that the hemp market now entails more than 25 000 products, ranging from textiles and clothing, rope, home furnishings, industrial oils, cosmetics, to food and pharmaceuticals (Ranalli & Venturi, 2004; Johnson, 2015; Mass, 2015). The durability and high strength properties of the cellulose-rich fibre from the stalk make it a valuable product for rope, paper, along with reinforcement and construction materials (Callaway, 2004; Ranalli and Venturi, 2004; Tang et al., 2006; Andre et al., 2016). The hemp seed byproduct of fibre processing has often been overlooked, but the seeds have proven a source of great nutritional value and pharmacological properties (Tang et al., 2006; Vonapartis et al., 2015). Within the last decade, hemp seed products have expanded to the food market to include raw oil products like salad dressings and plant protein sources like protein powder (Johnson, 2015; Vonapartis et al., 2015).

In today's world, food products are not just considered a source of nutrients. Consumers have become increasing interested in the way their diet can contribute to their overall health, beyond basic nutrition (Klaypradit & Huang, 2008; Sozer & Kokini, 2008). Even

over a decade ago, two-thirds of grocery shoppers reported that their purchases were highly influenced by a pursuit of preventing, managing, or treating a specific health condition (Parker et al., 2003). Since this time, food scientists have targeted such consumer demands by investigating additional health benefits and bioactive properties of food ingredients to formulate functional foods. In recent years, some animal- and plant-derived oils, like hemp oil, have earned a reputation for not only cooking and alimentary services, but also possessing medicinal and pharmaceutical potential (Uluata & Özdemir, 2012).

In particular, cold pressed hemp oil has become commercially popular, since its viewed as a natural and safe oil product with high nutritional value (Parker et al., 2003; Prescha et al., 2014). The cold-pressing procedure passes the raw seed material through a conventional screw press, without the addition of harsh chemical solvents and high heat treatments (Parker et al., 2003; Dimić et al., 2008). It has been demonstrated that this process retains more of the naturally beneficial components of the seeds, including valuable unsaturated FAs and bioactive substances, by minimizing degradative changes in the oil (Parker et al., 2003; Dimić et al., 2008; Uluata & Özdemir, 2012; Prescha et al., 2014). The resulting hemp meal byproduct after cold pressing the oil can be processed into a high fibre supplement or a high protein product to utilize the entire seed (Vonapartis et al., 2015).

In Canada, the steadily increasing production of hemp is now mostly driven by demands for its oilseed (Cherney & Small, 2016). In fact, Canadian businesses have led the hemp seed industry worldwide for the last two decades, while introducing a variety of innovative food and health products to the marketplace (Cherney & Small, 2016). This success

with hemp oilseed does not come as a complete surprise, since traditionally Canada has been profitable with the production of many other oilseeds, like Canola, flaxseed, and soybean, and has established applicable production and processing technologies (Cherney & Small, 2016). There are currently numerous Canadian companies working to fulfill the demand for hemp seed products – including Hemp Oil Canada Inc., Hempola Valley Farms, Manitoba Harvest, Ruths Hemp Foods, Cool Hemp, Natures Path, etc. As the global leader in hemp-based foods, Manitoba Harvest works together with over 125 Canadian farms to supply products to approximately 7000 retail stores across the U.S. and Canada (Manitoba Harvest Hemp Foods, 2017). In addition, this company saw a 500 percent growth in sales from 2010 to 2015 (Bikis, 2015). From 2010 to 2015, Canada experienced annual growth of 25 percent in its national acreage of industrial hemp (Alberta Agriculture and Forestry, 2015). Experts predict hemp oil production will continue to thrive, with health food products and pharmaceutical applications ensuing (Alberta Agriculture and Forestry, 2015; Cherney & Small, 2016).

2.2 Composition and Quality of Hemp

2.2.1 Composition

The major constituents of hemp seed include crude protein (20-25%), PUFA rich lipids (25-35%), carbohydrates (20-30%), insoluble fibre (10-15%), and approximately 5% ash (Table 1; Deferne & Pate, 1996; Calloway 2004; Silversides & Lefrancois, 2005; House et al., 2010; Vonapartis et al., 2015). The protein composition of hemp seed is comparable to soy beans and egg whites in terms of essential amino acid content (Callaway, 2004). The hemp seed protein meal, as a byproduct of oil extraction, is well-suited for human and

animal consumption. Hemp protein consists mainly of the high-quality, easily digested proteins edestin and albumin (Callaway, 2004; Tang et al., 2006; Matthäus & Brühl, 2008; Vonapartis et al., 2015). A composition and digestibility analysis, based on hemp varieties in Western Canada, revealed that hemp seed contains all essential amino acids and that the protein from hemp seed is highly digestible in both its whole form or as a hemp seed meal, though removal of the outer hull improves protein digestibility (House et al., 2010). Just three tablespoons of whole hemp seeds provides 11 grams of protein (Alberta Agriculture and Forestry, 2015).

The primary product, hemp oil, provides an appreciable amount of the unsaturated linoleic (omega-6), alpha-linolenic (omega-3), and gamma-linolenic FAs. As mentioned, the essential fatty acids (EFAs) omega-6 and -3 are in approximately a 3:1 ratio, favourable for human nutrition. Hemp seed oil also has several minor constituents contributing to its potential as a health food product. The cited quantities of macro constituents and phytochemicals may vary due to such a broad variety of existing hemp cultivars, which are grown, processed, and stored under diverse conditions.

Table 1. Content of important major and minor constituents of hemp seed and hemp oil.

Hemp	Constituent	Content	Author(s)
Hemp seed	Carbohydrate	20-30[a]; 27.6[a]	Deferne & Pate, 1996; Calloway 2004
	Crude fat	25-35[a]; 33.2[a]; 30.4[a]; 29.2[a]	Deferne & Pate, 1996; Silversides & Lefrancois, 2005; House et al., 2010; Vonapartis et al., 2015
	Crude protein	20-25[a]; 24.8[a]; 24.9[a]; 24[a]; 25.6[a]	Deferne & Pate, 1996; Calloway 2004; Silversides & Lefrancois, 2005; House et al., 2010; Vonapartis et al., 2015
	NDF	37.2[a]; 32.1[a]; 35.7[a]	Silversides & Lefrancois, 2005; House et al., 2010; Vonapartis et al., 2015
	ADF	23.5[a]; 27.8[a]	House et al., 2010; Vonapartis et al., 2015
	Ash	5.6[a]; 5.8[a]; 4.8[a]; 5.5[a]	Calloway 2004; Silversides & Lefrancois, 2005; House et al., 2010; Vonapartis et al., 2015
Hemp oil	Cannabidiol (CBD)	10[d]; 4.18-243.68[d]	Leizer et al., 2000; Petrovic et al., 2015
	Linoleic acid (LA, 18:2n-6)	51.9–55.7[c]; 52-62[c]; 54.3[b]; 54.47[b]; 56.2[c]; 56.07[c]	Dimić et al., 2008; Leizer et al., 2000; Oomah et al., 2002; Kriese et al., 2004; Petrovic et al., 2015; Vonapartis et al., 2015; Smeriglio et al., 2016
	alpha-linolenic acid (ALA, 18:3n-3)	12.3–15.3[c]; 12-23[c]; 19.1[b]; 21.36[b]; 17.2[c]; 15.98[c]	Dimić et al., 2008; Leizer et al., 2000; Oomah et al., 2002; Kriese et al., 2004; Petrovic et al., 2015; Vonapartis et al., 2015; Smeriglio et al., 2016
	gamma-linolenic acid (GLA, 18:3n-6)	0.8-2.46[c]; 3-4[c]; 3.6[c]; 1.99[b]	Dimić et al., 2008; Leizer et al., 2000; Oomah et al., 2002; Kriese et al., 2004
	β-tocopherol	0.6[b]; 0.16[b]; 0.64[b]	Oomah et al., 2002; Kriese et al., 2004; Smeriglio et al., 2016
	γ-tocopherol	73.3[b]; 21.68[b]; 91.57[b]	Oomah et al., 2002; Kriese et al., 2004; Smeriglio et al., 2016
	α-tocopherol	3.4[b]; 1.82[b]; 19.74[b]	Oomah et al., 2002; Kriese et al., 2004; Smeriglio et al., 2016
	δ-tocopherol	2.5[b]; 1.2[b]; 2.09[b]	Oomah et al., 2002; Kriese et al., 2004; Smeriglio et al., 2016

a- % Hemp seed fresh weight
b- % Hemp seed oil
c- % Total fatty acids
d- mg/kg Hemp oil

As EFAs, omega-6 and -3 must be incorporated into the diet (Deferne & Pate, 1996; Tang et al., 2006; Vonapartis et al., 2015). These FAs are needed to build healthy cells and maintain brain and nerve function (Alberta Agriculture and Forestry, 2015). Historically, the ratio of these FAs obtained in the diet was approximately 1:1, but dietary habits have

changed dramatically in the last 100 years and this ratio is now estimated to be closer to 10-20:1, indicating a deficiency in omega-3 FAs (Ruiz Ruiz et al., 2017). Many have linked this omega-6 dominant diet to the growth of the soybean market and its incorporation into cheap and processed foods. For instance, one study found that the consumption of soybean oil increased more than 1000-fold from 1909 to 1999 (Blasbalg et al., 2011). Omega-6 and -3 FAs are not interconvertible; they do not have the same effects physiologically. Omega-6 FAs are considered to be pro-inflammatory, while omega-3s have an anti-inflammatory effect. A degree of inflammation is essential for protection against infection and recovery for injury, but excessive inflammation may lead to inflammatory diseases (Ruiz Ruiz et al., 2017).

Due to deficiencies in the diet, nutritionists suggest the consumption of oils rich in omega-3, and the consumption of oils with a 2:1 to 4:1 omega 6:3 ratio is preferable (Alberta Agriculture and Forestry, 2015; Simopoulos et al., 2016). The denotation of omega-3 lipids is due to the double carbon-carbon bond at the third carbon from the methyl terminus of the FA carbon chain, and omega-6 lipids are distinguishable by the position of the first double bond. Of the FAs that fit this chemical composition criteria, the most commonly discussed for health purposes are ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Ghorbanzade et al., 2017; Ruiz Ruiz et al., 2017). ALA is present at high levels in certain plant oils like hemp, whereas the richest source of EPA and DHA is fish oil (Ghorbanzade et al., 2017; Ruiz Ruiz et al., 2017). Edible oils with ALA content can also serve as a precursor from which EPA and DHA can be derived through chemical conversions. In humans, DHA makes up a large portion of the FAs in the cortex, retina, and

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testis. This omega-3 FA is also a critical structural lipid in the human brain (Simopoulos et al., 2016). However, adequate provision of longer chain FAs may require direct dietary sources, as the conversion of ALA to EPA and DHA is limited.

As shown in Table 1, linoleic acid concentrations in hemp oil ranges from 64-72% of the total FA composition. Notably, hemp oil contains a significantly higher level of EFAs compared to common edible oils like canola and olive; 80% of FA composition composed of EFAs compared to 37% and 9%, respectively (Dimić et al., 2008). The same FA analysis reveals a higher content of LA and ALA in hemp seed oil compared to canola and olive oils, in addition to a more favourable ratio between these FAs (Dimić et al., 2008). While some other edible oils, like flax seed oil, have a high omega-3 content, they require more inputs and land to produce. In addition, the stalk or byproduct material from flax oil seed harvest is often considered waste, whereas the whole plant can be utilized in a hemp seed production (Struik et al., 2000; Mass, 2015). Nutritional recommendations indicate that of the daily caloric intake, 15-20% should be from the consumption of fats, and approximately one third of these fats should be the EFAs in the 3:1 ratio. As such, it is estimated this dietary goal can be met with just 3 tablespoons of hemp seed oil (Leizer et al., 2000; Alberta Agriculture and Forestry, 2015).

Apart from an excellent nutritional content, hemp seed also contains unique and beneficial phytochemicals. Phytocannabinoids are naturally occurring cannabinoids that are unique to the cannabis plant (Russo, 2007). Cannabinoids are recognized as pharmacologically

powerful hemp bioactives, which function as a result of their interaction with the endocannabinoid system in humans (Andre et al., 2016). This system has receptors that respond exclusively to cannabinoids, either from endogenous or cannabis plant sources. The diversified physiological effects of cannabinoids take place when they bind to and activate specific G protein-coupled receptors. One of the cannabinoid receptors that has been identified is the cannabinoid receptor type 2, CB2 receptor (Sanchez et al., 2001; Massi et al., 2004). The activation of the CB2 receptor leads to a number of immunological and neural responses, but is devoid of psychological effects (Sanchez et al., 2001). The CBD cannabinoid has the ability to regulate cellular signaling pathways and initiate therapeutic strategies by triggering this receptor. Although there are structural similarities between CBD and THC, their conformations differ; THC carbon rings are planar while CBD rings are at right angles to each other (Fig.1). This prevents CBD from binding to the CB1 receptor and inducing psychoactive effects, but allows it to bind to the CB2 receptor (Burstein, 2015).
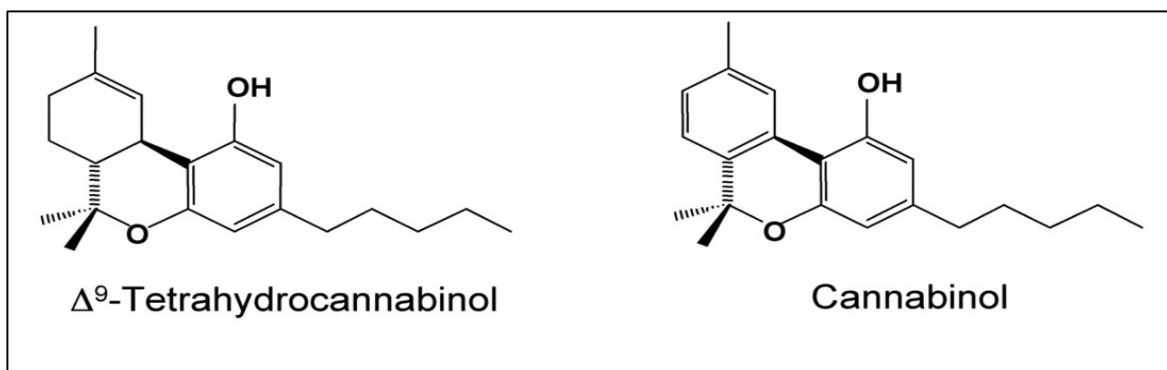
Figure 1. Chemical structures of tetrahydrocannabinol and cannabidiol, modified from Rao and Kaminski (2006).

Cultivated hemp varieties have a particularly high content of CBD and low content of THC (Leizer et al., 2000). Although CBD content is higher than THC, it is still detected at relatively low levels in hemp seed oil; 4.18-243.68 mg/kg (Petrovic et al., 2015). This is because both the production and storage of CBD is in the glandular structures (secretion tissue on plant epidermis) of the plant, so its presence in hemp oil is due to the resin transferring onto the seed during harvesting (West, 1998; Leizer et al., 2000; Andre et al., 2016). The wide range of CBD content detected is primarily due to the amount of resin retained by the seed coat during processing, as well as the varying hemp varieties and their associated cultivation conditions (Petrovic et al., 2015). However, the presence of CBD, even in trace amounts, can provide significant health benefits, as outlined in section 2.5.2 (Leizer et al., 2000; Callaway, 2004; Petrovic et al., 2015; Andre et al., 2016).

Hemp seed oil also contains the tocopherol isomers β-tocopherol, γ- tocopherol, α- tocopherol, and δ-tocopherol, with the γ- tocopherol derivative present in the highest quantity (Oomah et al., 2002; Kriese et al., 2004; Vonapartis et al., 2015; Smeriglio et al., 2016). Tocopherols are natural antioxidants that have been proposed to reduce the risk of cardiovascular diseases, cancer, and other oxidative degeneration related disorders (Kriese et al., 2004; Vonapartis et al., 2015). In addition, terpenes and phenolic compounds have been detected, which contribute to the odour/flavour and intrinsic antioxidant activity (Vonapartis et al., 2015; Andre et al., 2016). The flowers, roots, and leaves of hemp contain mono- and sesquiterpenes, with monoterpenes contributing to the volatile terpene profile

(Andre et al., 2016). The colour of hemp oil and some odour characteristics can be attributed to its chlorophyll content, which is a lipophilic yellowish-green pigment (Sapino et al., 2005).

These characteristics of hemp seed raise interests for food application strategies. In addition to its nutritive value, hemp oil has recognized potential as a source of bioactives and may act as a carrier of additional lipid based bioactives in a food system.

2.2.2 Hemp for Health

Although hemp oil was integrated into ancient therapy regimes, only recently has its health care role been reintroduced into the modern marketplace. Hemp oil is currently advertised primarily as a natural health product for body care purposes, as a vegetable oil for salad dressings, or taken directly as a supplement. Prevailing health products focus on the superior lipid profile of the hemp seed and the phytocannabinoid content.

The essential omega-6 and -3 FAs serve vital biological functions and may improve cardiovascular health, protect against inflammatory diseases, amongst other health benefits (Deferne & Pate, 1996; Parker et al., 2003; Gökmen et al., 2011; Ghorbanzade et al., 2017). CBD also has pharmacological properties that make it potential therapeutic agent. Since it was first isolated in the late 1930s, CBD has been found to have therapeutic potential for disorders involving inflammation and anxiety, as well neuroprotective effects, and anti-cancerous attributes (Massi et al., 2004; Vaccani et al., 2005; Castillo et al., 2010; Jones et al., 2010).

Since hemp prohibition was lifted a mere ~20 years ago, only in recent years has the hemp seed been investigated for its biological actions beyond basic nutrition. Much room remains for hemp oil promotion as a functional food product. The hemp food sector is growing, and with increased consumer awareness and product availability the health applications of the hemp oil are expected to expand (Agriculture and Agri-Food Canada, 2016; Alberta Agriculture and Forestry, 2015).

2.2.3 Polyunsaturated Fatty Acids

The dietary inclusion of hemp seed for cardiovascular health has been examined in many studies. Consumption of PUFAs is linked to decreasing serum concentrations of triglycerides and low-density lipoprotein cholesterol, subsequently improving heart contractile ability and blood flow (Schwab et al., 2006; Richard et al., 2007). In a randomized, double-blind crossover design, Schwab et al. (2006) conducted a study involving supplementation of the human daily diet with 30 ml of hemp oil for 4 weeks and detected positive changes in the serum lipid profile. Particularly, the serum cholesteryl esters and triglycerides had improved FA content with greater LA and GLA present, compared to diets supplemented with flaxseed oil (Schwab et al., 2006). Hemp oil supplementation also improved participants' cholesterol profile (more high density lipoprotein cholesterol), compared to flaxseed oil (Schwab et al., 2006). Although both oils contain high amounts of EFA LA and ALA, hemp oil's ratio of 3:1 is considered nutritionally balanced, while flaxseed has an almost inverse ratio. Since these FAs compete for enzymes during their metabolism to longer, more unsaturated FAs, too much omega-3 can has been associated with making longer omega-6 FAs less available.

Dietary hemp seed has also provided cardio-protective effects against ischemia in 12-week study conducted with rats. Rats fed a 5% or 10% hemp seed-supplemented diet for the duration of the study experienced significant elevation in plasma ALA and GLA levels, as well as ALA levels in the heart tissue. After the diet, post-ischemic heart performance was assessed and the ischemic condition was reduced in rats that had consumed hemp seed compared to diets supplemented with 1% palm oil or partially delipidated hemp seed oil. Since ALA was the only fatty acid with elevated levels in the heart tissues, the authors attributed the changes in the hearts' ability to recover from ischemia-reperfusion insult and recovery of maximal contractile to the ALA content in hemp seed (Al-Khalifa et al., 2007).

Richard et al. (2007) also incorporated hemp seed into rat diets at 5% and 10%, but assessed its ability to reduce platelet aggregation, compared to 1% palm oil. They found that the integration of hemp seed into the rat diet increased plasma PUFAs, particularly, increasing the ALA and LA levels significantly. They credit this adjusted plasma lipid profile for reducing the onset of platelet aggregation after diet supplementation, this was not achieved by palm oil. The diminished likelihood of clot formation has implications for reducing the incidences of myocardial infarctions and strokes (Richard et al., 2007).

As another indication of hemp-mediated improved heart health, Prociuk et al. (2008) examined the effect of dietary hemp seed for 8 weeks in rabbits. Elevated plasma levels of PUFAs, primarily GLA, were accompanied by an improved defense against hypercholesterolemia, and indirectly, lessened risk for platelet aggregation and myocardial infarction

(Prociuk et al., 2008). Current understanding shows a close relationship between the FA content of hemp oil and indicators of cardiovascular health.

2.2.4 Cannabidiol

The cultivation and processing of hemp plants to contain significant quantities of the CBD phytocannabinoid has not commercialized in Canada. Currently, hemp leaves and flowers cannot undergo CBD extraction without a particularly rare license available through Health Canada. Until hemp-derived CBD is removed from the list of controlled substances, farmers can only harvest its seed and stalk, which contains only trace amounts of the compound (Health Canada, 2011; Succi, 2017). Even so, CBD and its potential as a bioactive should not be overlooked. Especially considering the new cannabis legislation arising, which may lift the narcotic label on hemp products for its lack of psychoactive effects (Succi, 2017). Hemp-dervived CBD allows consumers to take advantage of the plant's additional health benefits without any unwanted psychoactive ramifications (Leizer et al., 2000; Petrovic et al., 2015).

Since the first study exhibiting the anti-cancer effects of cannabis phytochemicals in 1975 by Munson et al., there have been major advances in the investigation of cannabinoids therapeutically. Evidence suggests that phyto-, endo-, and synthetic cannabinoids contain properties that could aid in the treatment of various cancer types, including brain (Massi et al., 2004; Vaccani et al., 2005), prostate (Sarfaraz et al., 2005), breast (Ligresti et al., 2006), skin (Blazquez et al., 2006), pancreas (Carracedo et al., 2006), and colon cancer (Cianchi et al., 2008). Both *in vitro* and *in vivo* models support the hypothesis that the anti-cancer

effect is due to the role cannabinoids play in the regulation of pathways which control cell survival and death. As such, cannabinoids can lead to different cellular mechanisms causing anti-proliferative, anti-metastatic, anti-angiogenic, and pro-apoptotic responses (Sarfaraz et al., 2005; Alexander et al., 2009).

Massi et al. (2004) tested the effect of introducing CBD to human glioma cell lines U87 and U373 in both *in vitro* and *in vivo* models and concluded CBD produced antitumor activity. Upon the addition of CBD to either culture medium of cell lines, a reduction in mitochondrial oxidative metabolism was noted, as well as a reduction in glioma cell viability. Doses of CBD (0.5mg/mouse/5 days per week injected near the tumor for the duration of the 23-day study) significantly inhibited the growth U87 human glioma cells that were subcutaneously implanted in the mice (Massi et al., 2004).

Ligresti et al. (2006) examined the effect of adding cannabinoid extracts to the MDA-MB-231 breast cancer cell line. Growth of subcutaneous MDA-MB-231 tumors in mice were inhibited by CBD injections, and the authors deduced that CBD was able to induce apoptosis of the cancer cells via the activation of the CB2 receptor. Of several natural cannabinoids tested, the CBD extract proved to provide the most potent cytotoxic effects against breast cancer cells, with significantly lower abuse to healthy cells (Ligresti et al., 2006). Such findings, in addition to studies of CBD effects on skin, pancreas, prostate, and other cancers, have encouraging implications in oncology. Since most cancers originate from uncontrolled or improperly managed cellular growth, these pro-apoptotic and anti-proliferative mechanisms of CBD are promising (Sarfaraz et al., 2005). Although further work

is needed to confirm the clinical capacity for CBD-induced apoptosis in cancer cell lines, its potential as a non-psychoactive, antineoplastic agent is intriguing (Ligresti et al., 2006).

Several cannabinoids have exhibited the ability to mediate symptoms of neurodegenerative diseases and reduce compromising damage. The mechanisms by which CBD exerts its neuroprotective effects are not entirely understood, but they may be augmented by antioxidant and anti-inflammatory properties (Castillo et al., 2010; Jones et al., 2010). Since the activation of CB1 receptors is consequently associated with psychoactive ramifications and potentially neurodegenerative symptoms upon long term activation, the investigation of CBD is increasingly important for neurological disorders.

Several studies have indicated that CBD enhanced neuroprotection and recovery from hypoxic-ischemic brain injury resulting from oxygen deprivation (Castillo et al., 2010; Pazos et al., 2012; Pazos et al., 2013). CBD may hold promise as a therapeutic agent to prevent reduced motor and cognitive function resulting from hypoxic-ischemic injury, but data from well-controlled, double-blind, clinical trials are lacking. CBD remedies have also been shown to lessen the severity of epileptic symptoms by exerting anticonvulsant properties and reducing seizure incidences (Jones et al., 2010; Jones et al., 2012). Treatment of many multiple sclerosis (MS) symptoms (spasticity, pain, and hindered bladder control) is often achieved with a CBD/THC combination supplement, as outlined by several clinical trials (Vaney et al., 2004; Wade et al., 2004; Langford et al., 2013).

At present, CBD used therapeutically appears to aid with the treatment and symptomatic relief of several neurodegenerative issues. A recent review of preclinical studies on anti-cancer properties of CBD indicate that positive results were typically achieved with relevant concentrations and models, however, more research is necessary to make this potential a clinical reality (Fowler, 2015). This also applies to the research thus far with CBD as a therapeutic agent for neuroprotection and recovery from brain injuries (Devinsky et al., 2014). CBD as a remedy for epileptic symptoms is supported by clinical trials, and reviews of human data indicate the next steps are dosing and efficacy trials for epilepsy (Devinsky et al., 2014). More research is needed in the area of hemp-derived CBD for these and other ailments, but post-prohibition restrictions on hemp products may pose further challenges for investigation and development of therapies.

2.2.5 Oxidative Instability

Before reaching the consumer, hemp oil must maintain its functional food quality during processing, storage, shipment, and final preparation (Mohammadi et al., 2016). The stability of this oil during typical food storage and preparation conditions determines its potential food applications and nutritive value, as well as customer satisfaction (Parker et al., 2003; Calligaris et al., 2016). Although the cold-press process of extracting hemp oil retains much of the hemp seed's composite value, the resulting oil product is highly susceptible to going rancid (Dimić et al., 2008). Once rancid, the bioactive potential of hemp oil to promote cardiovascular health, provide neuroprotection, and influence the remedy of other ailments is reduced.

The rancidity of hemp oil can be primarily attributed to lipid peroxidation (Calligaris et al., 2016). Lipid peroxidation is the process in which reactive oxygen species cause degradation and damage to lipids (Calligaris et al., 2016). When an excess of oxidative species arises in a system, beyond the capacity for endogenous antioxidants to neutralize their actions, an imbalance occurs, leading to the production of reactive peroxides and free radicals (Fig. 2).
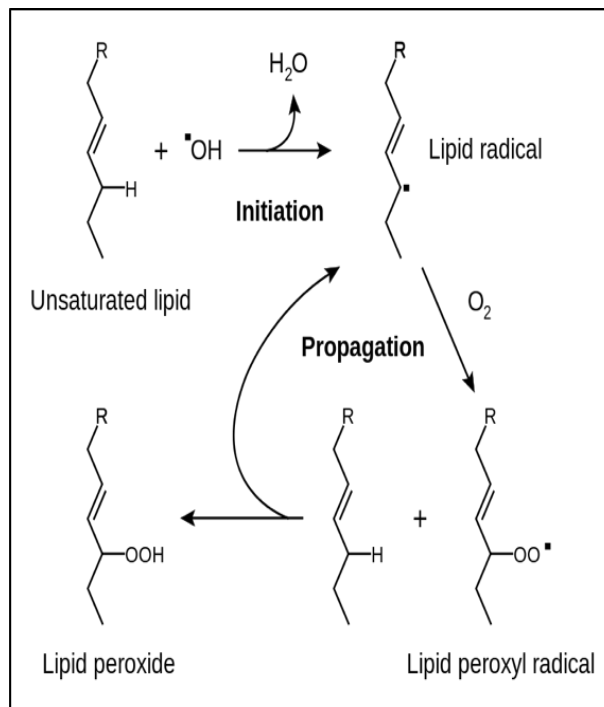
Figure 2. Mechanism of lipid peroxidation, modified from Young and McEneny (2001)

The most easily sensed qualities that are deteriorated are flavour, smell, and colour, which reduce consumer acceptability (Uluata & Özdemir, 2012; Calligaris et al., 2016; Mohammadi et al., 2016). Apart from repelling consumption, this rancidity changes the oil properties, which depletes the nutritional value and results in the reduction of lipophilic compound biological activity (Dimić et al., 2008; Calligaris et al., 2016; Mohammadi et al., 2016). In addition, excessive oxidation can cause the formation of harmful, or even toxic, by-products, which are potentially cytotoxic and genotoxic (Belhaj et al., 2010; Calligaris et al., 2016; Mohammadi et al., 2016).

The degree to which lipid peroxidation will occur is affected by several intrinsic factors, including the type of packaging, the temperature, exposure to light, and other environmental conditions (Parker et al., 2003; Abuzaytoun and Shahidi, 2006; Belhaj et al., 2010; Calligaris et al., 2016). However, it is the content of unsaturated FAs, especially ALA, that most directly contributes to the degree of lipid peroxidation (Abuzaytoun and Shahidi, 2006; Prescha et al., 2014). Dimić et al. (2008) examined cold pressed hemp seed oil from seven hemp varieties and found a correlation (R^2=0.8586) between an increase in the content of total unsaturated FAs and a decrease in oxidative stability; the more unsaturated FAs the hemp oil contained, the quicker the onset of oxidation at a high rate, as indicated by the induction period using the Rancimat method. Unsaturated FAs are sensitive to oxidative deterioration because they contain weakly bound hydrogen, compared to saturated FAs, making them vulnerable to attack by reactive oxygen species and subsequent degradation (Belhaj et al., 2010; Sun-Waterhouse et al., 2012; Uluata & Özdemir, 2012; Prescha et al., 2014).

Lipid oxidation can be measured and monitored using several different methods. A fluorescence spectroscopy method with the fluorescent probe diphenyl-1-pyrenylphosphine (DPPP) was used to monitor lipid oxidation of emulsions for the purpose of this work. Accelerated oxidation methods, as with the Rancimat apparatus, are not necessary to quantify levels of lipid oxidation because this reagent is known to be a sensitive and selective reagent, which can react quantitatively with peroxides in numerous solvents (Mosca et al., 2010; Wheatly, 2000). Reproducibility has been compared to chromatography mass spectrometry quantification of peroxides, without the onerous sample preparation (Wheatly,

2000). DPPP itself is not fluorescent; it reacts with lipid hydroperoxides stoichiometrically to yield the fluorescent product DPPP-oxide, thus providing an indication of hydroperoxides present (Fig. 3). The level of fluorescence can be considered to correspond with the level of lipid peroxidation.
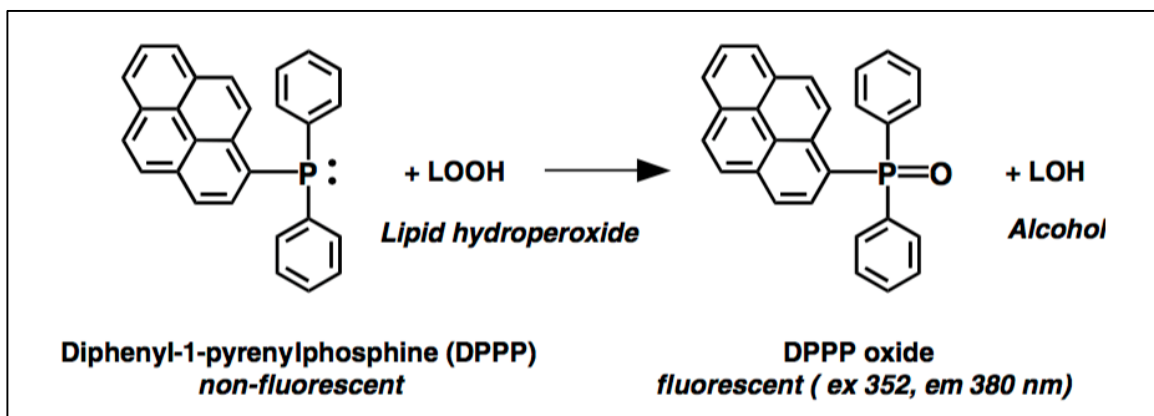
Figure 3. Reaction of diphenyl-1-pyrenylphosphine (DPPP) with lipid hydroperoxides to form fluorescent DPPP-oxide, modified from Kawai et al. (2007).

If stored correctly after production, an unopened bottle of hemp oil may last up to 14 months, though manufacturers' best before dates are typically 9 months after the product has been packaged (Manitoba Harvest Hemp Foods, Winnipeg, Manitoba; Chi Hemp Industries Inc., 2017). In comparison, several manufacturers list the best before date for olive oil as 18-24 months after packaging (Scarafia, 2017).

Hemp oil has proven less stable than many other edible oils due to its high content of unsaturated FAs (Parker et al., 2003; Uluata & Özdemir, 2012). For instance, Dimić et al. (2008) used the accelerated oxidative test (Rancimat method) and observed that the induction period of hemp oil was 6.4 to 7.6 hours at 100°C, whereas induction times for canola and olive oil at even higher temperatures have been reported to range from 12-17 and 6-

11, respectively (Läubli & Bruttel, 1986; Frega et al., 1999; Metrohm, 2017). Only a short time frame of exposure to heat and oxygen produced products indicative of oxidation in hemp oil. Uluata and Özdemir (2012) also noted the susceptibility of hemp oil to oxidation, in comparison to other unconventional oils (radish, terebinth, stinging nettle, laurel). The higher degree of vulnerability to oxidation was indicated by the peroxide values, the Rancimat method, and other quantitative oxidation assays. The oils containing the most unsaturated FAs were noted to have greater oxidative instability, as indicated by lower induction times at 110°C; hemp oil had the lowest induction period (1.32 hours), followed by stinging nettle seed oil (5.57 hours), radish seed oil (8.02 hours), terebinth seed oil (37.55 hours), and laurel oil (43.44 hours; Uluata and Özdemir, 2012). Similarly, hemp oil was found to be the least stable compared to cranberry, carrot, and caraway seed oils by Parker et al. (2003), using the oil stability index, as a measurement of the resistance of lipids to oxidation. This instability reduces hemp oil quality during processing, storage, and cooking, as well limits the potential for incorporating hemp oil in foods (Prescha et al., 2014).

For this reason, hemp oil cannot be used as a frying or baking oil, and may not withstand processing conditions if incorporated into food products like fortified juice or yogurt. Hemp oil can be ingested raw and could substitute other oils in salad dressings, hummus, pesto or other food products which are not exposed to heat or other oxidative-inducing conditions. The very attributes that make the omega rich oil valuable for consumption also limits its application in the food industry. Thus, several studies examining this susceptibility to oxidation have suggested employing protective measures to ensure the high nutritive

value and bioactive components of the oil are preserved (Abuzaytoun and Shahidi, 2006; Dimić et al., 2008; Calligaris et al., 2016).

2.3 Encapsulation

It has been well established that the unsaturated FA content of many edible oils creates potential for functional food products and nutraceutical formulations. Limited is their commercial application due to corresponding oxidative susceptibility. Driven by increasing consumer demand for PUFA edible oils and oil-infused functional foods, food scientists have begun to implement encapsulation techniques to address issues of storage stability, processing, and formulation of food products.

One method researchers have developed is the protection of oils by encapsulating them in a physical matrix that acts as a barrier and reduces detrimental effects of the external environment (Sun-Waterhouse et al., 2012; Ruiz Ruiz et al., 2017). Encapsulation technology for edible oils, both on the micro- and nano-scale, involves a relative magnitude of oil entrapped by a wall material of either protein, carbohydrate, or lipid origin, using various techniques (Klaypradit & Huang, 2008; Ghorbanzade et al., 2017). This method can protect functional ingredients and create better characteristics for incorporation into foods (Huang et al., 2008; Ezhilarasi et al., 2013). Encapsulation can be achieved by many approaches, such as emulsification, homogenization, spray-drying, and ultrasonication (Sozer and Kokini, 2008; Ezhilarasi et al., 2013).

2.3.1 Emulsification as Encapsulation Method

Each emerging encapsulation method has its own merits, but food grade micro-emulsions are particularly popular for promoting better oxidative stability of edible oils rich in PUFAs (Huang et al., 2008; Kentish et al., 2008; Calligaris et al., 2016). Oil-in-water (O/W) emulsions are especially useful for the delivery of hydrophobic bioactive compounds in the oils and incorporation into food products (Kentish et al., 2008). These emulsions are characterized by small droplets of oil (range 0.1 to 100 μm), which are dispersed within water by different mixing methods and typically involve the incorporation of a surfactant to diffuse the interfacial tension between the oil and water (Huang et al., 2008; Leong et al., 2009; Khaled et al., 2014). Such emulsions protect the inner core of oil and lipophilic compounds, while allowing the oil to be incorporated into aqueous food systems.

2.3.2 Mixing Methods

To form an O/W emulsion, sufficient agitation must be applied to cause the otherwise immiscible liquids to disperse and mix. High-energy methods are typically required to form micro-sized droplets of oil within the water (Chen and Tao, 2005; Leong et al., 2009; Ezhilarasi et al., 2013). Small droplet size is necessary to achieve solution characteristics important for consumer acceptance (optically appealing and appropriate consistency), as well as provide suitable food production qualities like homogeneity (Kentish et al., 2008; Leong et al., 2009; Khaled et al., 2014). Edible oils have been successfully encapsulated in emulsions with a variety of techniques, including: emulsification, homogenization, spray-drying, and ultrasonication (Sozer and Kokini, 2008; Ezhilarasi et al., 2013). Ultrasonic emulsification uses high intensity ultrasound power to separate and scatter the oil in the small

droplets in the water phase.

2.3.3 Surfactant Material

Apart from the mixing strategy to form emulsions, a surfactant material is normally used to stabilize the newly formed oil droplets and to create a consistent mixture throughout (Chen and Tao, 2005; Kentish et al., 2008; Leong et al., 2009). Most studies emphasize that the key parameter for the stability of an emulsion is selecting the appropriate surfactant(s) to form the wall material around the oil core (Tadros, 2013). The surfactant material has properties which allow it to adsorb to the surface of the oil droplets and form a protective membrane (McClements and Decker, 2000). In the case of O/W emulsions, the hydrophobic portion of the surfactant interacts with the oil, while the hydrophilic portion interacts within the water (Fig. 4). The surfactant plays a critical role in controlling the interfacial tension between emulsion phases, retaining the oil, and preventing droplet deformation and aggregation (Leong et al., 2009; Khaled et al., 2014).
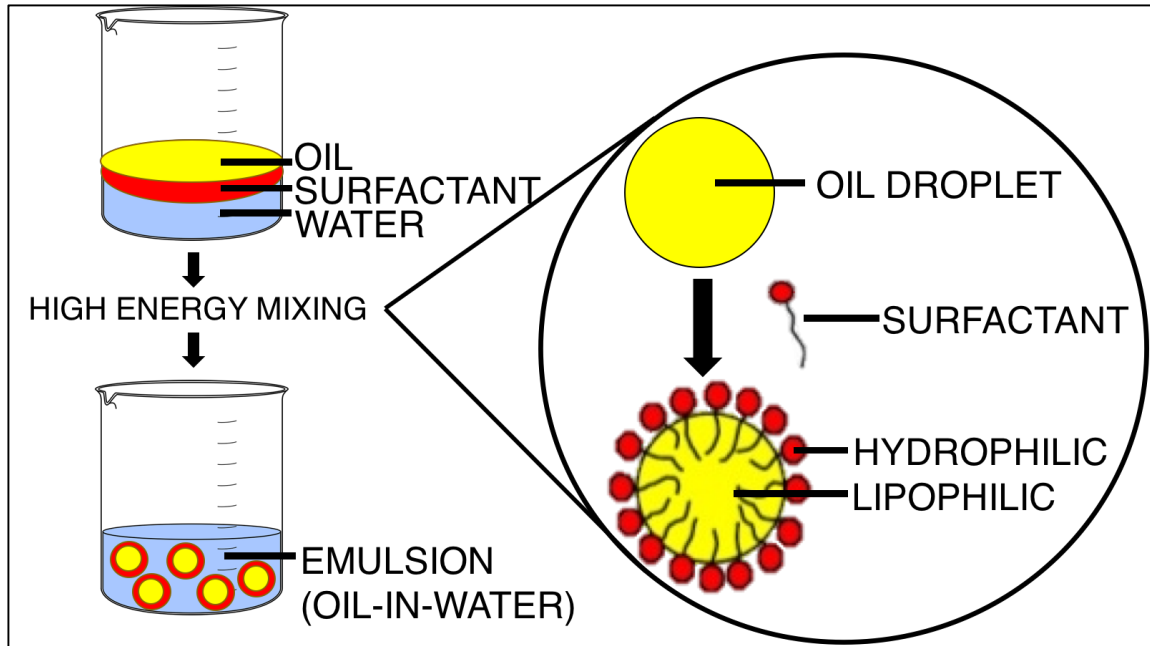
Figure 4. Schematic of how oil-in-water emulsions with surfactant are formed.

How successfully the formation of an emulsion is can be measured by several parameters. The droplet size, homogeneity, and stability of emulsions are all important characteristics to assess emulsion suitability for food applications. Firstly, smaller emulsion droplets are more known to be more physically stable than larger emulsion droplets, and when droplets are smaller, creaming or separation of the oil and water phases is less likely (Klaypradit & Huang, 2008). Polydispersity index (PI) provides the degree of homogeneity of particle sizes, higher values indicate a broader distribution of particle sizes (primary particle sizes differing more extensively as this value increases). This parameter reflects the consistency and homogeneity of an emulsion. Zeta potential (ZP) values are an indicator of dispersion stability, with values ±40 to ±60 indicating the dispersion is less likely to coagulate. Droplet charge within an emulsion is an important factor in determining stability, since charged

particles repel each other and prevent aggregation (McClements and Decker, 2000; Tadros, 2013).

Several different materials can serve as a surfactant during emulsion formation. Wall materials may include: polysaccharides of plants (such as pectin or starch), food proteins (such as soy or whey proteins), or emulsifiers (such as lecithin or Tweens; Huang et al., 2008). The food industry is limited to using agents for encapsulation that are edible, biodegradable, and stable in the food matrix. Carbohydrate sources have desirable drying properties and often provide good oxidative stability for edible oils, but their use is limited by interfacial properties that cause poor oil encapsulation efficiency (Wang et al., 2011; Shamaei et al., 2017). Most commonly used for food-grade emulsions are sources of amphiphilic proteins and phospholipids (McClements and Decker, 2000).

Protein sources are often employed for encapsulation due to their strong amphiphilic surface properties, which are well suited to encapsulate hydrophobic core materials (Wang et al., 2011; Ma et al., 2014; Shamaei et al., 2017). Milk proteins and soy protein are most commonly used because they are safe for consumption, available in abundance and inexpensive raw materials (Wang et al., 2011; Ma et al., 2014). In particular, a preference for soy protein as a surfactant is due to its added nutritional value, digestion ease, emulsification ability, and solubility properties (Molina Ortiz et al., 2009; Leong et al., 2009; Ma et al., 2014).

Soybean seeds have a protein content of approximately 40%, with the 7S (β-conglycinin)

and 11S (glycinin) protein fractions as the major components (Xu et al., 2011). Once de-hulled and defatted, soybean meal is further refined by aqueous or mild alkali extraction to produce soy protein isolates (SPI) which are ~90% protein. The SPI is then typically neutralized for optimum solubility. Soy protein isolates orientate their hydrophobic portions towards the immersed oil droplets and are adsorbed into the interface, while their hydrophilic portions interact with the surrounding aqueous phase (Xu et al., 2011).

For the purpose of this work, absorbance spectroscopy with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) reagent was used to evaluate the level the soy protein surfactant oxidation. The detection of reactive sulfhydryl groups, as an indication of protein oxidation, was completed using the DTNB reagent by absorbance measurements. DTNB, commonly known as Ellman's reagent, has been the most popular reagent for spectrophotometric measurement of

Figure 5. Reduction of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) to form detectable 2-nitro-5-thiobenzoic acid (TNB), modified from Thermo Scientific (2011)

protein sulfhydryls since its introduction in 1959 (Riener et al., 2002). Thiol groups present in protein can protect lipid compounds against oxidative damage due to their ability to react with free radicals and reductive capacity. This antioxidant action forms the target of DTNB (the conjugate base of a free sulfhydryl group) (Thermo Scientific, 2011). This provides a thiolate anion to react with DTNB, forming a measurable yellow-colored product, 2-nitro-5-thiobenzoic acid (TNB), which exhibits intense light absorption at 412 nm (Fig. 5; Riener

et al., 2002; Thermo Scientific, 2011). At a neutral pH, the DTNB assay reagent has high specificity for sulfhydryl groups and requires a relatively short reaction time (Thermo Scientific, 2011). This assay was completed to determine the level of protein surfactant oxidation. Higher levels of sulfhydryl groups can be considered as increased levels of protein oxidation.

Soy lecithin, a phospholipid emulsifier, is also useful for dispersing lipophilic substances within an aqueous solution (Belhaj et al., 2010). Lecithin (LEC) that is used in the food industry is predominately manufactured from soybean, since it is in abundance and cost is low. Crude soybean oil contains approximately 1-3% phospholipids, and after extraction and purification soy LEC contains about 65-75% phospholipids (Xu et al., 2011). Incorporation of LEC into food grade emulsions is usually at 0.1-2% (Xu et al., 2011). LEC has a relatively simple configuration; with a glycerol backbone, a hydrophobic tail group, and a hydrophilic head group. The hydrophobic tail group consists of unsaturated FAs and extends into the oil droplet in an emulsion droplet, while the polar head group containing phosphoric acid with choline faces the water phase (Horn et al., 2011; Xu et al., 2011).

In some cases, protein alone cannot effectively encapsulate oil and LEC is added to achieve the desired functionality (Xu et al., 2011). Surface active protein like SPI and phospholipids like LEC behave differently at the interfacial layer due to their different configuration, but their hydrophobic and hydrophilic portions orientate themselves the same within an emulsion and are both commonly used surfactants (Decker et al., 2005; Horn et al., 2011). Studies have shown that the presence of phospholipids in combination with soy protein

have enhanced the homogenous dispersity of oil droplets within aqueous solutions and improved emulsion stability (Scuriatti et al., 2003; Xu et al., 2011). The encapsulation of edible oils in these matrices offers better delivery of stable, unsaturated functional ingredients (Ruiz Ruiz et al., 2017).

2.3.4 Encapsulation of Edible Oils

Methods of encapsulation form a barrier that is less permeable to oxygen diffusion and environmental conditions that may enhance oxidative deterioration, while also masking unfavourable attributes (Wang et al., 2011). This process has been used extensively over the past two decades for preserving and enhancing the quality and functionality of edible oils (Huang et al., 2008; Sozer and Kokini, 2008; Ezhilarasi et al., 2013; Khaled et al., 2014; Devi et al., 2016). In terms of consumer acceptability, encapsulation can mask the unpleasant taste or smell of edible oils and incorporate them into desirable functional foods (Wang et al., 2011). By reducing particle size and creating a hydrophilic outer layer, encapsulated edible oils are better positioned to be solubilized and bioavailable (Sozer & Kokini, 2008). Encapsulation has particularly been shown to promote better oxidative stability of edible oils entrapped in the protective wall materials (Wang et al., 2011; Gökmen et al., 2011; Gallardo et al., 2013; Ghorbanzade et al., 2017).

Encapsulation techniques have been implemented for a variety of edible oils, including fish oil, avocado oil, flaxseed oil, and others (Table 2). Encapsulation of fish oil is particularly popular since it provides a direct dietary source of EPA and DHA (Burdge & Calder, 2005). Ghorbanzade et al. (2017) used a homogenizer and probe sonicator to encapsulate fish oil

with a liposomal formulation from soy LEC and sunflower oil. This method was developed to avoid the high temperatures utilized by the more common spray-drying process, which has the potential to accelerate oxidation. They found that the fish oil was efficiently encapsulated ($92.22 \pm 0.19\%$) and the retention of DHA and EPA was improved in encapsulated form compared to free fish oil (57% compared to 27%, and 12% compared to 6%, respectively). Along with approximately 50% better omega-3 preservation, the overall acceptability (color, taste, texture) of yogurt fortified with the encapsulated fish oil was higher compared to the free fish oil. Wang et al. (2011) found that fish oil was successfully encapsulated by barley protein using a homogenizer, a microfluidizer system, followed by spray drying. Oxidative stability of the encapsulated oil versus free oil was tested in different states (dry and aqueous solutions) over a storage trial of 8 weeks at 40 °C. The barley protein capsules possessed a strong ability to protect fish oil against oxidation. Hydroperoxide levels, as a measure of oxidation during the storage trial, indicated that the crude fish oil deteriorated more greatly than the protein encapsulated fish oil; maximum level of 350 meq peroxide/kg oil versus 45–76 meq peroxide/kg oil. Klaypradit & Huang (2008) used a novel ultrasonic atomizer technique to test the feasibility of chitosan based encapsulation of fish oil. In this case, they found that a combination of chitosan and maltodextrin as wall materials stabilized formulations and maintained high EPA and DHA content in the oil.

Table 2. Summary of methods and materials used to achieve various edible oil-encapsulation objectives.

Oil type	Method	Wall materials	Objective	Author(s)
Fish oil	homogenizer, probe sonicator	liposomal formulation (soy lecithin, sunflower oil)	Prevent strong odors and rapid deterioration	Ghorbanzade et al., 2017
	homogenizer followed a microfluidizer system, then spray dry	barley protein	Improve oxidative stability	Wang et al., 2011
	emulsification, ultrasonic atomization, and freeze drying	chitosan, maltodextrin, and whey protein isolate	Assess feasibility of chitosan-based encapsulation and new ultrasonic atomizer encapsulation technique	Klaypradit and Huang, 2008
Flaxseed oil	spray drying, homogenizer	high amylose corn starch	Lower lipid oxidation and formation of harmful compounds in breads during baking	Gökmen et al., 2011
	spray drying	gum arabic, maltodextrin, methyl cellulose, and whey protein isolate	Improve resistance to oxidation	Gallardo et al., 2013
Annatto seed oil	freeze-drying, spray-drying, and high intensity ultrasound	gum arabic	Stabilizing the bioactive compounds	Silva et al., 2015
Avocado oil	co-extrusion	alginate and hydroxypropyl methylcellulose	Improve the storage stability using phenolic antioxidant fortification (phloridzin or BHT) and encapsulation	Sun-Waterhouse et al., 2012
Walnut oil	spray drying	skim milk powder, Tween 80, and maltodextrin	Preserve essential fatty acids, tocopherols, and phytosterols.	Shamaei et al., 2017
Pomegranate seed oil	spray drying	Skim milk powder	Prevent deterioration of the seed oil	Goula and Adamopoulosb, 2012

Dietary trends have begun to favour plant-based oils, particularly those which are cold-pressed without chemical extraction methods. A review on dietary consumption of fats from 1990 to 2010 found that global plant-based omega-3 fat intake has increased (Micha et al., 2014). For this reason, encapsulation of plant sources of highly unsaturated fatty

acids have also been explored (Ruiz Ruiz et al., 2017). Gökmen et al. (2011) worked on developing a functional bread enriched with flaxseed. High amylose corn starch proved effective in encapsulating flaxseed oil, while maintaining bread quality and reducing formation of oxidation products and harmful compounds, compared to incorporating free flaxseed oil. Kentish et al. (2008) and Leong et al. (2009) used an ultrasonication method to form flax and sunflower oil in water nano-emulsions, respectively.

These encapsulation strategies are promising to protect the dietary fats of edible oils, as well as their lipophilic endogenous bioactive constituents, and potentially deliver lipophilic nutraceuticals or supplements (Ezhilarasi et al., 2013). For food applications, emulsions can protect the bioactive compounds from interactions with the food ingredients and enhance their physical stability in food formulations (Khaled et al., 2014). As an efficiently grown crop with highly nutritional constituents, hemp seed oil warrants investigation of encapsulation processing strategies to improve its stability for food and health applications.

Chapter 3: Methods

3.1 Emulsion Formation

3.1.1 Source of Oils

The hemp oil was obtained from raw cold pressed extraction (Manitoba Harvest Hemp Foods, Winnipeg, Manitoba)**.** The avocado oil was also obtained from raw cold pressed extraction (President's Choice, New Zealand).

3.1.2 Surfactant Preparation

The SPI was produced from defatted soy meal (Bulk Barn Foods Ltd., Truro, NS, Canada) by alkali extraction (pH 4.6) with NaOH 1M in reference to the Molina Ortiz et al. (2009) method. The SPI was brought to a neutral pH (~7). The LEC was made from soy LEC granules (Bulk Barn Foods Ltd., Sydney, NS, Canada), which were ground to a fine powder.

3.1.3 Ultrasonication Process

A 500 Watt ultrasonic processor (Vibra Cell Ultrasonic Processor, Model VC 505) equipped with an ultrasonic probe with a 13mm diameter was used for emulsion formation in this study (Sonics and Materials Inc., Connecticut, US). The surfactant-treated O/W emulsions had a 0.5% (w/v) SPI and 0.5% (w/v) LEC suspension added to the 1% (v/v) of oil in water, whereas the surfactant suspension was not added to non-treated emulsions. Emulsions stabilized solely by either SPI or LEC were found to separate after emulsion formation. Sonication was performed for 2 min with a 5-sec pulse (on, off), followed by a 2 min break, and another 2 min with a 5-sec pulse. The amplitude of probe oscillation was

set at 40% in order to effectively mix the solution. The 2 min break and 5-sec pulse method was employed to reduce heat accumulation in emulsions. Three separate batches of each emulsion were formed on the same day, and aliquots were stored in capped amber 2ml vials for testing (Table 3). Resulting samples from the sonification protocol included emulsions with surfactant present (Spr) and absent (Sabs): Hemp Sabs, Hemp Spr, Avocado Sabs, and Avocado Spr.

Table 3. Amount of oil and surfactant components used to formulate hemp and avocado emulsions.

	Composition		
Emulsion	Oil (v/v %)	Isolated Soy Protein (w/v %)	Soy Lecithin (w/v %)
Hemp Spr	1	0.5	0.5
Hemp Sabs	1		
Avocado Spr	1	0.5	0.5
Avocado Sabs	1		

3.2 Preliminary Trial

A preliminary trial was conducted to establish expected parameters for encapsulation protocols and stability measurements. Hemp and avocado emulsions were prepared as indicated above in Table 3. All emulsions were subjected to storage at room (25°C) and refrigeration temperature (4°C) in the absence of light for the duration of 23 days. Each temperature condition (fridge and water bath) contained individual aliquots of all samples to be used on days of analysis. Aliquots were stored in capped amber 2ml vials, with 1 aliquot of each sample removed on testing days for evaluation. During storage of emulsions, aliquots of emulsions were collected for analysis of oxidative parameters on days 3, 5, 7, 9, 12, 18, and 23. Testing on day 0 of emulsion formation as well as other testing days were

interrupted due to inclement weather, resulting in the irregular pattern of testing days noted.

3.3 Primary Trials

After reviewing results of the preliminary trial, storage and thermal trials were conducted with a new set of emulsions. The storage trial was extended to 42 days, with consistent measurements of oxidative parameters taken. A thermal trial was added to examine the effect of a heat treatment on emulsion oxidative stability.

3.3.1 Storage Trial

Emulsions were stored in the same manner and exposed to the same storage temperatures as section 3.2. During storage of emulsions, aliquots of emulsions were collected for analysis of oxidative parameters on days 0, 6, 12, 18, 24, 30, 36, and 42, and on days 0 and 42 for emulsion characteristics.

3.3.2 Thermal Trial

Emulsions were immersed in water at 72°C for 15 sec to simulate the pasteurization process (high-temperature, short-time) aqueous beverage products or yogurt infused with the emulsions could endure as part of food processing, according to the FDA standard of pasteurization time/temperatures (FDA, 2003). Oxidative parameters were evaluated pre- and post-heat treatment.

3.4 Oxidative parameters

3.4.1 Fluorometric Detection of Lipid Peroxides

Relative lipid peroxide content in emulsions was assessed using the diphenyl-1-pyrenylphosphine (DPPP; Molecular ProbesTM, OR, U.S) assay, by the fluorescence of DPPP upon reaction with lipid peroxides, an accepted method to monitor lipid oxidation during oil storage (Khaled et al., 2014; Prescha et al., 2014). In triplicate, samples of 200 µL and 10 µL of 10 µM DPPP were added to a fluorescence micro-plate, in the absence of light. After 15 min, the fluorescence intensity of the assay mixture, due to oxidative reaction product, DPPP oxide, was measured at an excitation and emission wavelength of 351 nm and 380 nm read, respectively (Takahashi et al., 2001). Triplicates were averaged to yield individual emulsion batch measurements.

3.4.2 Sulfhydryl Groups Based on Molar Absorptivity

Measurement of sulfhydryl groups (SH) was performed using Ellman's reagent as reported by Udenigwe et al. (2013), with modifications. Briefly, in triplicate, 1.00 mL of samples were vortexed with 1.00 mL of Tris-Glycine (0.1 M, pH 8, 5% SDS) and 20 µL 5,5'-dithiobis-2-nitro benzoic acid in a microfuge tube, then incubated at 40°C for 15 min. Assay mixtures were centrifuged at 5000 rpm for 10 min, then an aliquot of 200 µL from the supernatant was removed and added to a clear bottom 96-well plate, and the absorbance of 2-nitro-5-thiobenzoic acid (TNB) was measured at 412 nm. The reported molar absorptivity of TNB, E, 14 150 M^{-1} cm^{-1}, was used to calculate the SH content, expressed as µM SH per mg protein, as shown in Fig. 6. (X, Y, and Z are variables, depending on the absorbance value). Triplicates were averaged to yield individual emulsion batch measurements.

$$E = \frac{A}{bc} \quad \text{where } A = \text{absorbance, } b = \text{path length in centimeters, } c = \text{concentration in moles/liter (=M)}$$

Solving for concentration gives the following formula: $c = \dfrac{A}{b\mathrm{E}}$

$A = $ W, $b = $ 1, and E = 14,150M^{-1}cm^{-1}. Therefore, $c = \dfrac{W}{1(14,150)} = $ X   M

Account for dilution factors as follows:

The total volume of the solution being measured is

       1.00mL of   Reaction Buffer
    +1.00mL of   Unknown Sample
    +0.02mL of   Ellman's Reagent Solution
      1.02mL of   solution

If the concentration of the assay solution is   X   M,   then 1.02mL of that solution contains

     1.02mL $\dfrac{1L}{1000mL} \times$ (X   moles/L) =   Y   moles

These   Y   moles of sulfhydryl in the assay solution were contributed by the original   1.00mL sample. Therefore, the concentration of free sulfhydryl in the original unknown sample is

      $\dfrac{\text{Y moles}}{1.00mL} \times \dfrac{1000mL}{L} = $ Z   M

Figure 6. Example calculation of the sulfhydryl concentration, modified from Thermo Scientific, 2011.

3.5 Emulsion characteristics

The physical properties of the emulsions were characterized by the Horiba Particle Analyzer (Nano Partica SZ-100 series). The mean particle diameter, polydispersity index, and zeta potential of the emulsions were determined using this combined dynamic light scattering/laser doppler electrophoresis instrument. For measurements of the droplet size (nm), the dispersion (polydispersity index), and surface charge, emulsions were diluted 1:100 and measured with the refractive indices of water (1.330).

3.6 Statistical Analysis

Oxidation assays were performed in triplicate of each emulsion batch and emulsion charactertistic measurements were also taken in triplicate of each emulsion batch. Data are represented as a mean of three batches for emulsion samples ± standard error of the mean (SEM). The effect of the surfactant treatment on hemp and avocado oil-in-water emulsions was assessed using a repeated measures design, in the mixed model method of Statistical Analysis System (SAS) 9.4 (SAS Institute Inc., 2014). The validity of the model assumptions (normal distribution and constant variance of the error terms) was verified by examining the residuals as described in Montgomery (2013). Transformations were applied for some of the constituents to achieve normality; however, the means shown were back-transformed to the original scale. Statistical difference ($P<0.05$) between samples was analyzed as level of significance. Correlation between the oxidative parameters and emulsion characteristics was analyzed by Spearman's correlation coefficient.

Chapter 4: Results and Discussion

4.1 Storage Trial

4.1.1 Lipid Oxidation

Hemp and avocado O/W emulsions exhibited similar trends concerning their relative levels of lipid peroxidation throughout the storage trial. Initially (day 0), hemp Spr emulsions demonstrated significantly higher levels of lipid peroxidation compared to Sabs emulsions (P<0.05), and this divergence was consistent over the course of the storage trial (Fig. 7). Apart from an increase in lipid oxidation from day 0 to 6, no further oxidation was evident over the course of the trial for hemp Spr emulsions. Hemp Sabs emulsions maintained a significantly lower level of lipid peroxidation level throughout the trial compared to Spr emulsions, with three periods of slight changes in oxidation levels (day 12 to 18, 18 to 24, and 36 to 42). These small but significant fluctuations in oxidation levels for Spr and Sabs emulsions presented no consistent trend over time.

Figure 7. Lipid peroxidation of hemp Spr and Sabs O/W emulsions for duration of primary storage trial (SEM 1.04). Values are a mean of three emulsion batches at 25℃ and 4℃ on each day. Different letters indicate a significant difference ($P<0.05$) between means was detectable.

Likewise, avocado Spr emulsions had higher levels of lipid peroxidation compared to Sabs emulsions, initially and throughout the storage trial (Fig. 8, $P<0.05$). Similarily to hemp Spr emulsions, avocado Spr emulsions exhibited an initial increase in oxidation from day 0 to 6 then maintained a stable level for the remainder of the storage trial. The initial increase in lipid oxidation in both hemp and avocado Spr emulsion samples can be attributed to the fact that, as a chain reaction, lipid oxidation propagates if already formed lipid hydroperoxides are present (Horn et al., 2011). There were also an additional few time periods where a change in oxidation can be observed for the avocado emulsions during the trial, but the Spr and Sabs emulsions remained distinctly different from each other and generally

stable over time. These findings are consistent with the hemp and avocado results from the 23-day preliminary trial; lipid oxidation levels in Spr emulsions were higher throughout the storage trial, compared to Sabs emulsions (Appendix: Figure A & B).
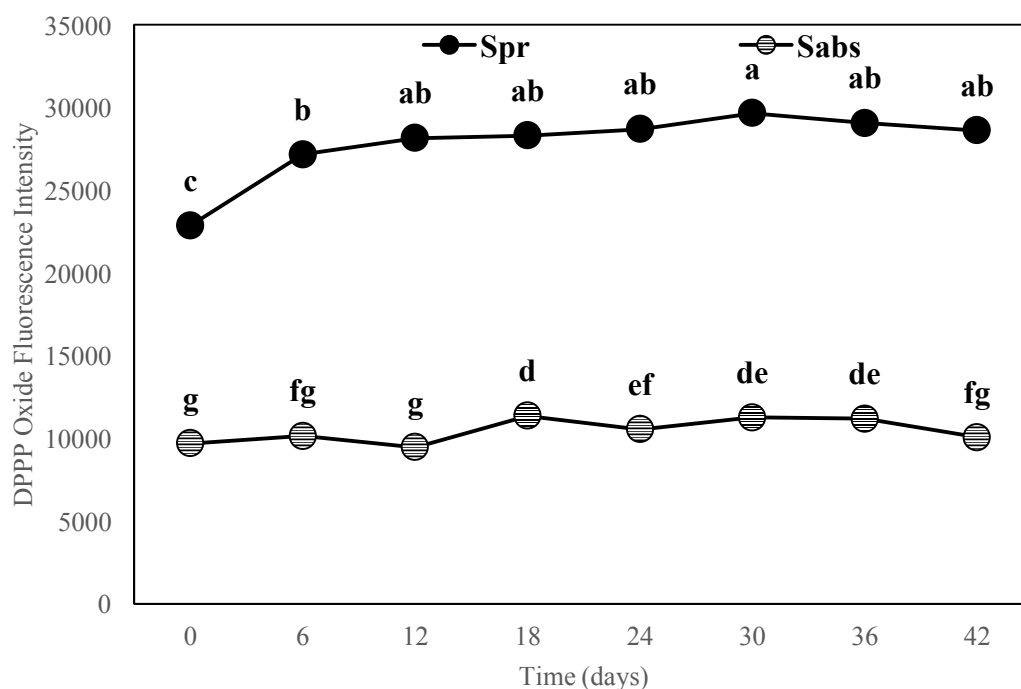

Figure 8. Lipid peroxidation of avocado Spr and Sabs O/W emulsions for duration of primary storage trial (SEM 1.03). Values are a mean of three emulsion batches at 25°C and 4°C on each day. Different letters indicate a significant difference ($P<0.05$) between means was detectable.

Although shelf life of the raw oils extended beyond the duration of the storage trial, it was predicted that oxidative products would began to accumulate before the end of the products' shelf life. Results from the preliminary trial were consistent with Raikos et al. (2015), which concluded that oxidative markers remained relatively unaffected during

storage of hemp oil emulsions (formed with hemp seed powder and LEC as the surfactant) at 4℃ for 10 days. For this reason, the storage time was extended to 42 in the secondary trial, although the emulsions continued to resist significant oxidative changes throughout this longer time period.

The different storage temperatures had relatively no impact on the levels of lipid oxidation of the hemp and avocado emulsions (Fig. 9 & 10). Similarly, no apparent trend concerning the impact of storage temperature on levels of oxidation can be determined from the preliminary trial results; Spr emulsions exhibited similar levels of oxidation whether they were stored at 25℃ or 4℃, as did Sabs emulsions (Appendix: Figure A & B).
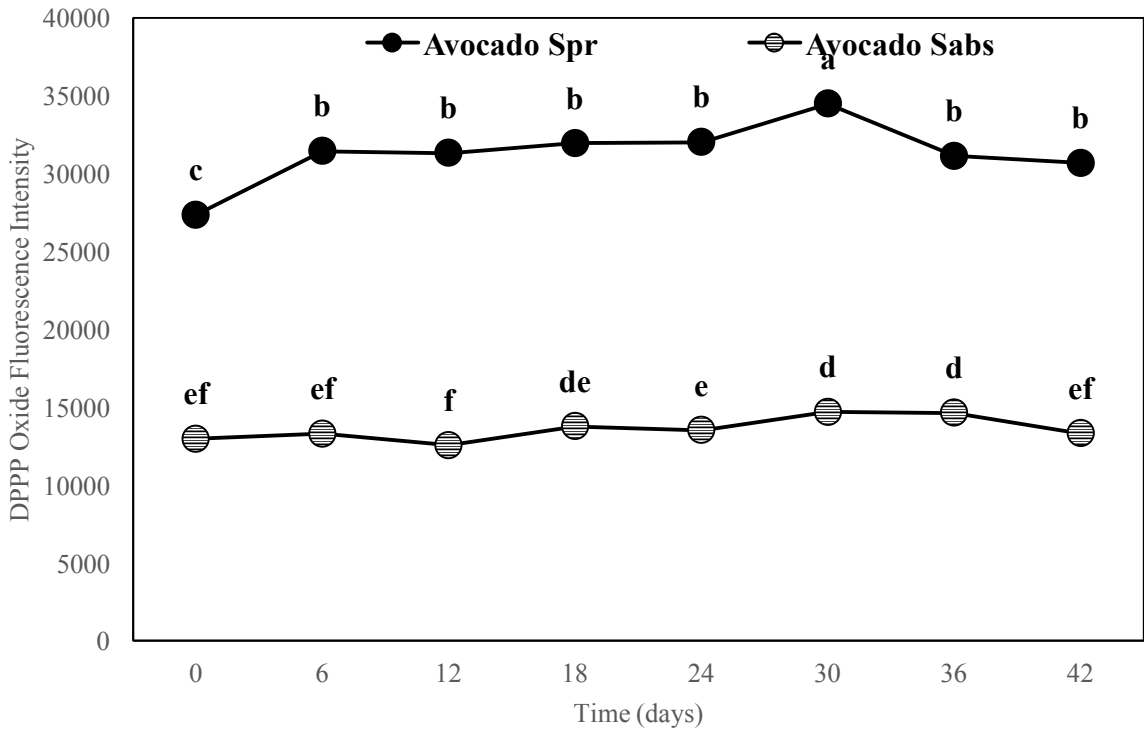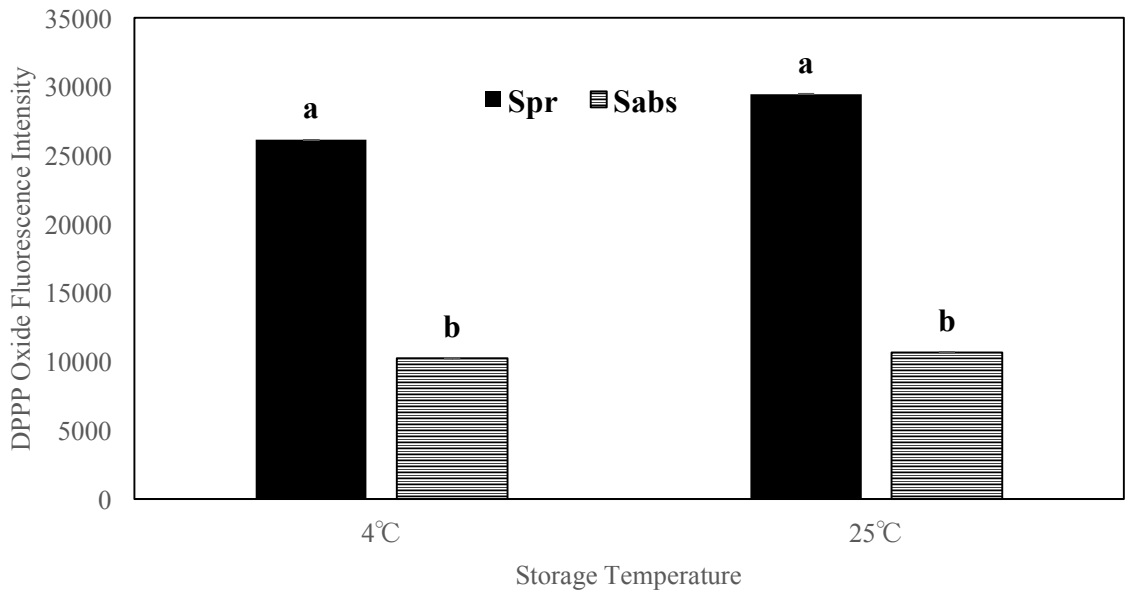

Figure 9. Lipid peroxidation of hemp Spr and Sabs O/W emulsions at 4℃ and 25℃, for duration of primary storage trial (SEM 1.04). Values are a mean of Spr and Sabs emulsion batches at 25℃ and 4℃. Different letters indicate a significant (*P*<0.05) difference between means was detectable.
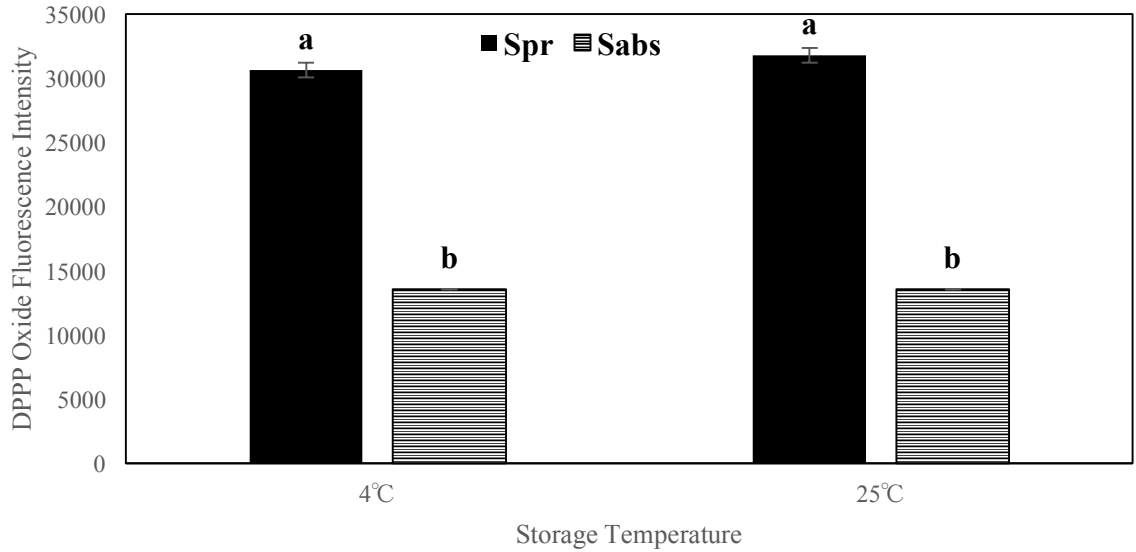
Figure 10. Lipid peroxidation of avocado Spr and Sabs O/W emulsions at 4℃ and 25℃, for duration of primary storage trial (SEM 1.04). Values are a mean of Spr and Sabs emulsion batches at 25℃ and 4℃. Different letters indicate a significant difference ($P<0.05$) between means was detectable.

The storage temperatures were selected based on the manufacturers suggestions for storage of the oils, potential recommendations for storage conditions of oil-infused functional foods, and to reflect possible consumer choices. It is recommended by the respective manufacturers to store hemp oil at "refrigeration temperatures before and after opening" and avocado oil in a "cool dark area after opening", as well as to keep the oils in their original dark opaque containers. The cold storage temperature was chosen to determine the stability of the emulsions if the oils were used as ingredients in food that normally require low temperatures to prolong their self-life (e.g. fortified yogurt or juice). The warmer storage temperature was used as an indication of how the oxidative and physical stability of the emulsions would be altered at approximately room temperature. The oxidative stability of the emulsions at refrigeration and room temperatures for 6 weeks is promising for their potential as food products.

Had the emulsions been exposed to light during their storage, this stability may have been more compromised. The oil producers recommended the oils remain in dark opaque containers. Thus, the emulsions were transferred to and stored in opaque amber microfuge tubes, in conditions without natural or artificial light for the duration of the study. In addition, aliquots of emulsions were kept sealed and only removed and opened on the single testing date they were used for, each testing day using new aliquots. Raikos et al. (2015) found that oxidative markers indicated significantly greater lipid oxidation for hemp emulsions exposed to light, which was attributed to photosensitized oxidation.

It can be noted that the emulsions were not exposed to lipid oxidation-evoking conditions (temporal or thermal), as primary lipid oxidation products were not significantly increased by these variables. For the short-term storage of 42 days, the emulsions appear resistant to further lipid peroxidation at refrigeration (4℃) and room temperature (25℃) when exposure to light and oxygen is minimized. Had the emulsions been tested for lipid oxidation levels after storage at a higher temperature or beyond the manufacturers best before date, a change in levels of oxidation may have become apparent.

Despite LEC being one of the most popular food-grade emulsifiers, whether the emulsions contained the LEC surfactant or not was the primary factor impacting the level of lipid peroxidation (Decker et al., 2005; Xu et al., 2011). The difference between levels of lipid peroxidation in Spr and Sabs emulsions can be observed following emulsion formation on the first day of the storage trial. The LEC and/or oil must be the lipid source(s) for oxidation leading to significantly greater peroxide production in the Spr emulsions, compared to Sabs

emulsions. LEC may have been susceptible to attack by reactive species itself or promoted oxidation of the encapsulated oils at the oil-surfactant interface.

Firstly, the LEC may have catylzed oxidation of the oil due to its iron content. The LEC used contained iron (8% daily value per 100g) which can promote lipid oxidation in O/W emulsions (Horn et al., 2011). As shown by the chain of reactions in Figure 11, transition metals like iron can act as catalysts and can accelerate lipid oxidation through the decomposition of lipid hydroperoxides (Fenton reaction) into highly reactive per-
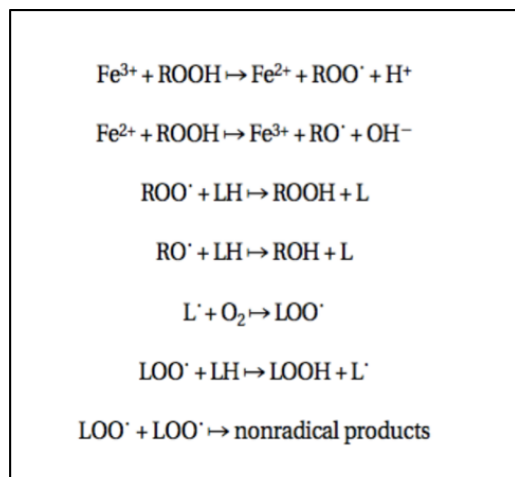
$$Fe^{3+} + ROOH \mapsto Fe^{2+} + ROO^{\cdot} + H^{+}$$

$$Fe^{2+} + ROOH \mapsto Fe^{3+} + RO^{\cdot} + OH^{-}$$

$$ROO^{\cdot} + LH \mapsto ROOH + L$$

$$RO^{\cdot} + LH \mapsto ROH + L$$

$$L^{\cdot} + O_2 \mapsto LOO^{\cdot}$$

$$LOO^{\cdot} + LH \mapsto LOOH + L^{\cdot}$$

$$LOO^{\cdot} + LOO^{\cdot} \mapsto nonradical\ products$$

Figure 11. Mechanism of iron catalyzed lipid peroxidation, modified from McClements and Decker (2000).

oxyl and alkoxyl radicals (McClements and Decker, 2000; Jomova and Valko, 2011). This interaction between hydroperoxides and transition metals occurs at the droplet surface and is noted as one of the most common causes of oxidative instability in emulsions (McClements and Decker, 2000). The sugar content in the LEC used (4g sugar per 100g) may have further promoted this oxidation in Spr emulsions by reducing iron to its most active states (Fe^{3+} and Fe^{2+}), which can catalyze the generation of reactive radicals to further impose oxidative damage (McClements and Decker, 2000). If this is the case, soy LEC could be substituted for LEC from marine sources or eggs, without iron content. Soy LEC was chosen for this study due to its common use in the food industry and studies that expressed success with using soy LEC to encapsulate highly unsaturated oils, including hemp

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oil, for improved oxidative and/or physical stability (Abuzaytoun and Shahidi 2006; Ghor-banzade et al., 2017).

The pH of the emulsion solution may have been another factor leading to more lipid per-oxide production. It has been found that LEC as a surfactant has functioned poorly in emul-sions at moderate to high pH levels. In fact, O/W emulsions with LEC at pH of 7 have exhibited higher levels of lipid oxidation than the bulk oil (Horn et al., 2011). The emul-sions evaluated in the current study are suspected to have been at a pH of approximately 7, as the SPI was brought to a final pH of 7, the oils are typically at a 6-7 pH, and the primary component of the emulsions was water (Alkalife, 2013). In this case, LEC may have inef-fectively encapsulated the oil at pH ~7 and heightened oxidative levels in emulsions.

The LEC may been susceptible to oxidation as the surfactant at the oil interface or as excess surfactant as micelles within the emulsion. Excess phospholipids can compete with the protein at the O/W interface and form free micelles within the outer aqueous solution. These surfactant micelles may have been more vulnerable to oxidation. As well, micelles can influence oxidation levels by interacting with pro-oxidant transition metals and lipid hydroperoxides (McClements and Decker, 2000; Horn et al., 2011). Since soy LEC has a high unsaturated FA content (~80%), the increased levels of oxidation may also have been its phospholipid capsule or free micelles oxidizing, rather than the encapsulated oil (Horn et al., 2011).

Future work could confirm the notion that LEC contributed to lipid oxidation levels by

replicating the experiment with an added lone control of a LEC emulsion, or other emulsi-fying agents could be explored. This would provide insight into whether the oxidation of the oil was provoked due to levels of iron and sugar present in LEC, or if LEC was directly contributing to oxidation levels. Whether LEC is directly or indirectly increasing oxidation, a stable surfactant is required to minimize production of reactive species in emulsion systems so food-grade emulsions maintain their functional properties while resisting rancidity.

4.1.2 Protein Oxidation

Throughout the trial, a significant interaction between the storage temperatures, surfactant, and time was found for SH quantity in hemp emulsions (Fig. 12, $P<0.05$). Both initially and over time, the hemp Spr emulsions stored at 4℃ indicate higher levels of protein oxidation than the hemp Sabs emulsions also stored at 4℃ (Fig. 13). These emulsions appear to follow the same trend over time as the quantities of SH detected fluctuate, with significantly different levels of SH apparent at the beginning and end of the trial. As for emulsions stored at 25℃, only initially a significantly higher level of protein oxidation was detected in the Spr emulsions compared to the Sabs emulsions (Fig. 14). Levels of SH in emulsions at 25℃ then fluctuate with no evident trend between Spr and Sabs emulsions for the remainder of the trial.

Figure 12. Quantification of sulfhydryl group content in hemp O/W emulsions, stored at 4 ℃ and 25℃, for duration of primary storage trial (SEM 5.33). Values are a mean of three emulsion batches on each day.

Figure 13. Quantification of sulfhydryl group content in hemp O/W emulsions, stored at 4℃ for duration of primary storage trial (SEM 5.33). Values are a mean of three emulsion batches on each day. The asterisk (*) denotes significant difference ($P<0.05$) between means was detectable.
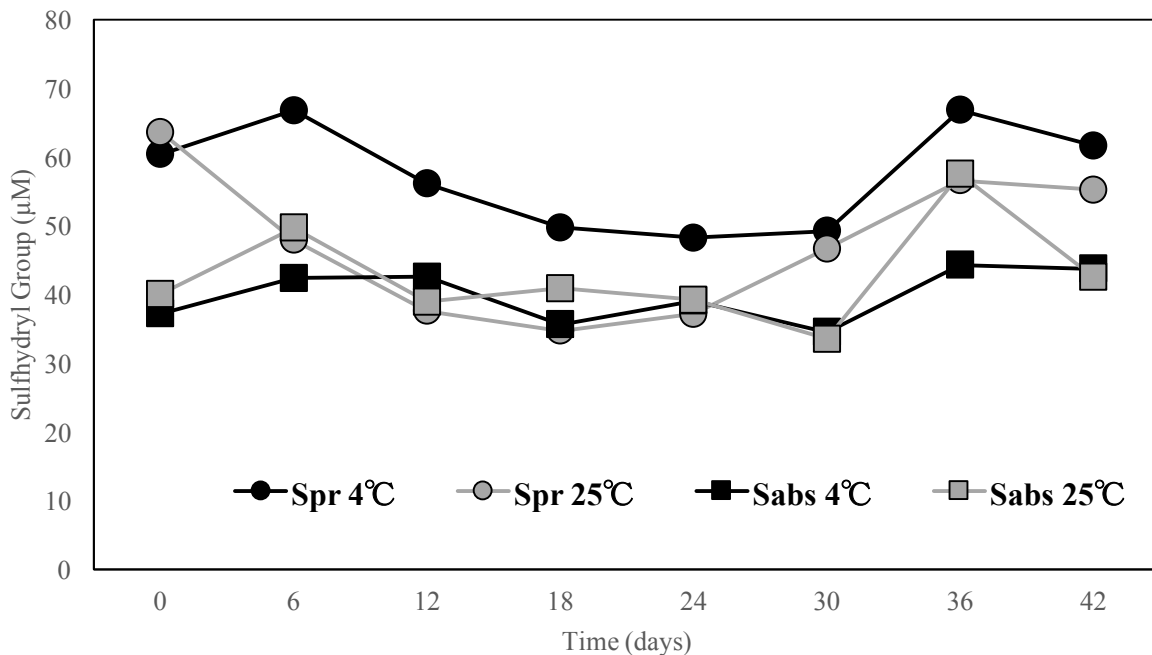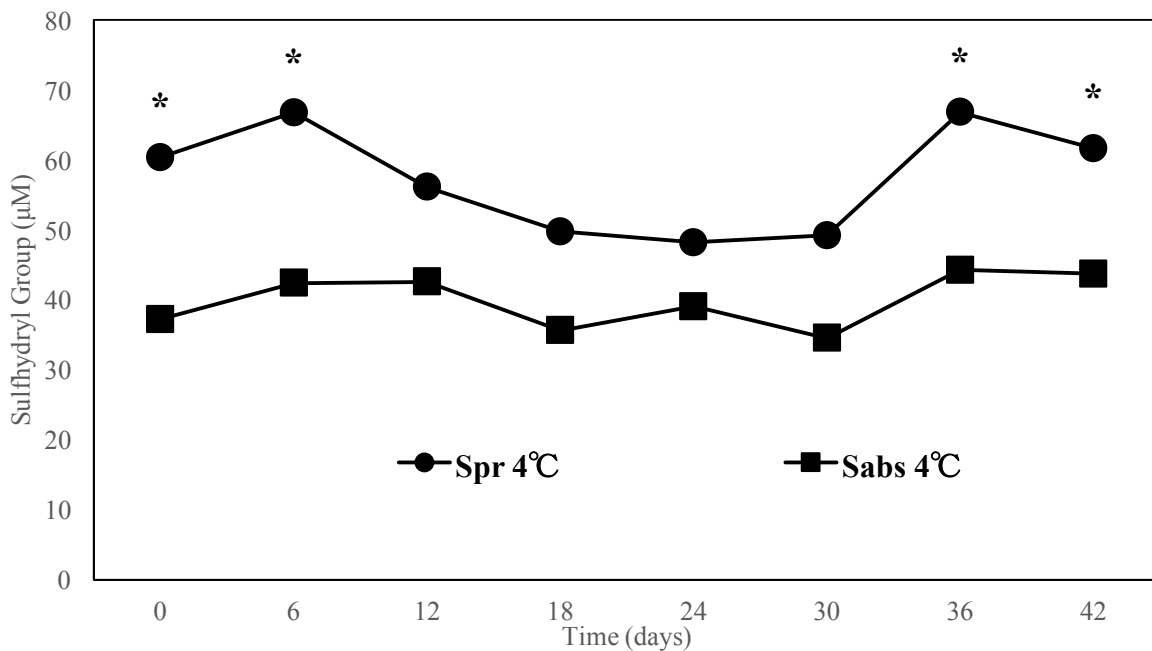
Figure 14. Quantification of sulfhydryl group content in hemp O/W emulsions, stored at 25℃, for duration of primary storage trial (SEM 5.33). Values are a mean of three emulsion batches on each day. The asterisk (*) denotes significant difference ($P<0.05$) between means was detectable.

In a similar manner, avocado Spr emulsions stored at 4℃ indicated higher levels of protein oxidation initially and throughout the storage trial, compared to Sabs emulsions at 4℃ (Fig. 15). Additionally, avocado emulsions stored at 25℃ initially indicated a higher level of protein oxidation in Spr emulsions compared to Sabs emulsions, then the levels of SH in both emulsions vary with time (Fig. 16).

Figure 15. Quantification of sulfhydryl group content in avocado O/W emulsions, stored at 4℃ for duration of primary storage trial. Values are a mean of three emulsion batches on each day.
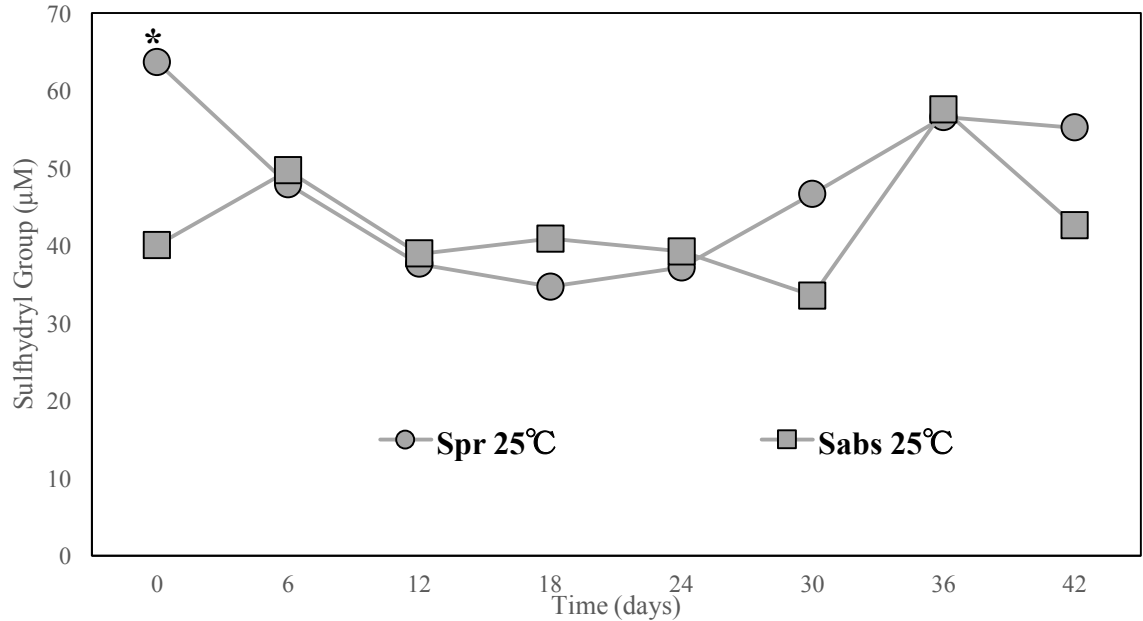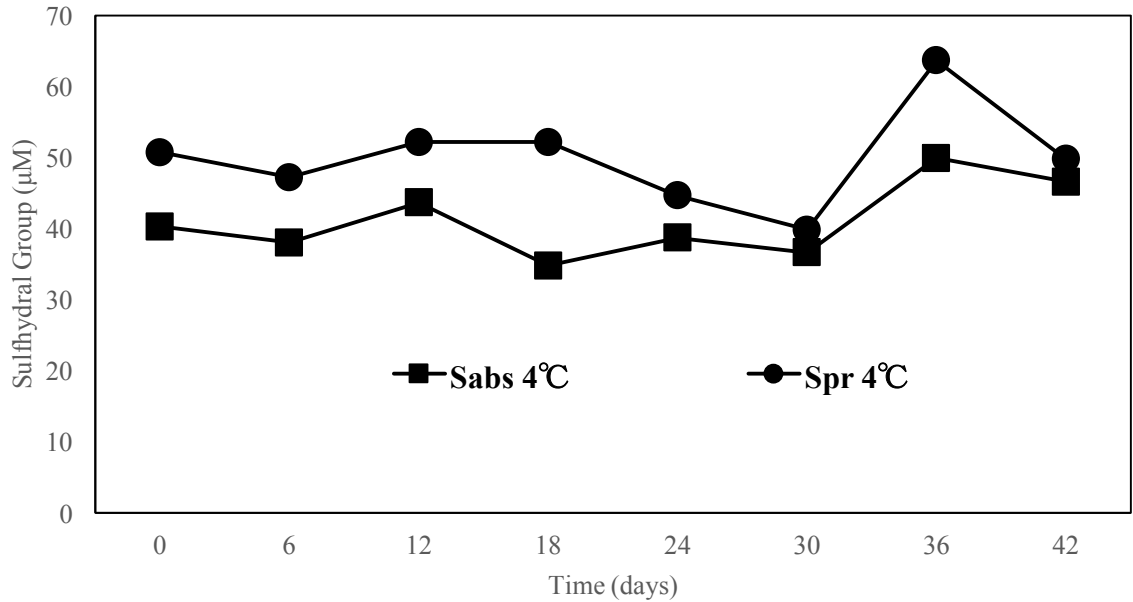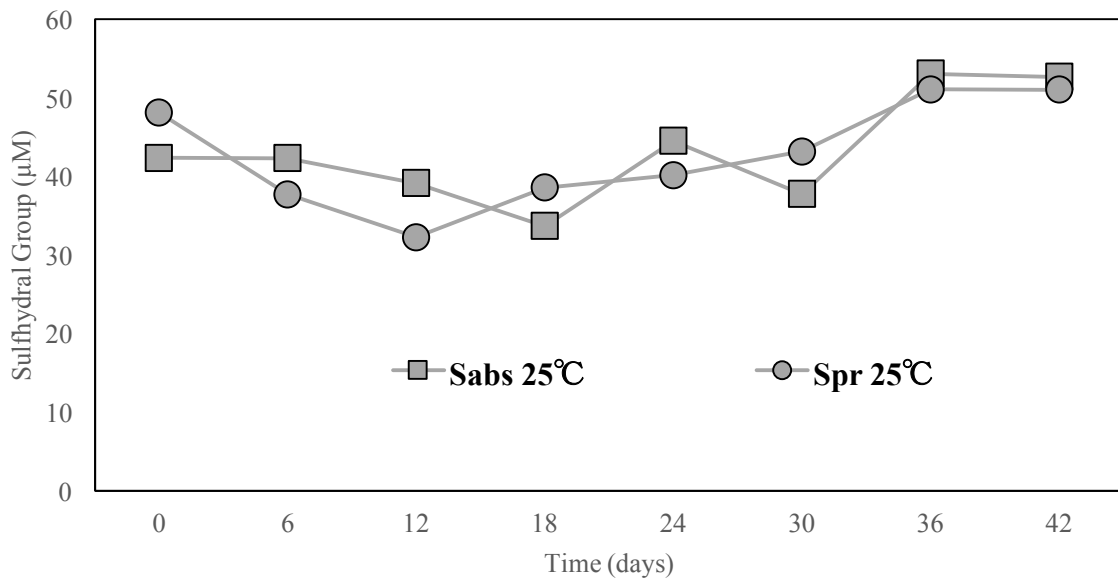

Figure 16. Quantification of sulfhydryl group content in avocado O/W emulsions, stored at 25℃ for duration of primary storage trial. Values are a mean of three emulsion batches on each day.

The SH content of hemp emulsions (Spr 4℃, Spr 25℃, Sabs 4℃, and Sabs 25℃) appears

to generally rise in the beginning of the trial, fall, then rise towards the end of the trial (Fig.

10). In contrast, the avocado emulsions remain more stable with time, apart from a signif-

icant rise in SH at the end of the trial (Fig. 17 & 18). Some studies have reported issues

regarding the kinetic reliability of SH content of over time. The sulfhydryl content has been

noted to fluctuate over time due to the figuration of protein structures changing with ag-

gregation and new SH becoming available for quantification (Riener et al., 2002). The

quantifiable SH depends on the oxidative state of the emulsion at the time of measurement
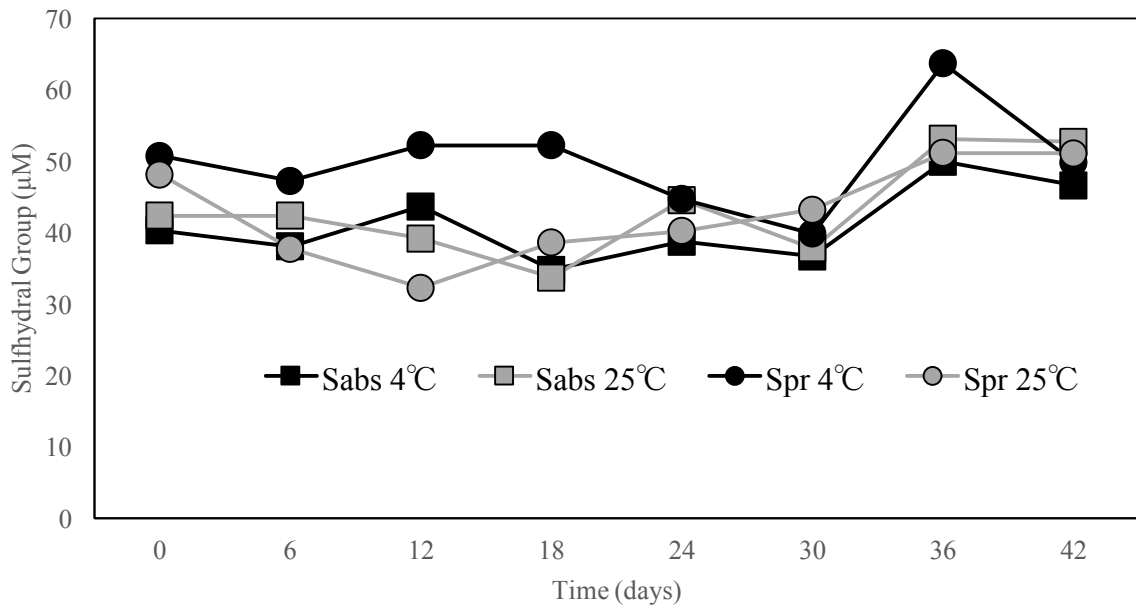
and available protein sulfhydryls.

Figure 17. Quantification of sulfhydryl group content in avocado O/W emulsions, stored at 4 ℃ and 25℃, for duration of primary storage trial. Values are a mean of three emulsion batches on each day.

Figure 18. Quantification of sulfhydryl group content in avocado O/W emulsions for duration of primary storage trial (SEM 2.52). Values are the mean of SH measurements of all avocado emulsions on each day. Different letters indicate a significant difference (P<0.05) between means was detectable.

The storage temperatures of both hemp and avocado emulsions had no consistently significant effect on protein oxidation. With both oils, the SH content quantified over time for Sabs emulsions at 4℃ and 25℃, remains very similar (Fig. 19 & 20). This is also true for both the oils Spr emulsions, although a greater disparity of SH content between the Spr emulsions at 4℃ and those at 25℃ can be noted for both oils (Fig. 21 & 22). Temperature appeared to provoke a more sporadic production and detection of SH. Higher temperatures have been reported to accelerate emulsion degradation, due to changes in interfacial tension in degree of adsorption of emulsifiers (Chen and Tao, 2005; Mosca et al., 2010). Thus, the availability and structure of the surface SPI on emulsion droplets may be more dynamic at higher temperatures. However, there is no significant trend over time, or with regards to surfactant, concerning the impact of storage conditions on SH content.

Figure 19. Quantification of sulfhydryl group content in hemp Sabs O/W emulsions, stored at 25℃ and 4℃ for duration of primary storage trial (SEM 5.33). Values are a mean of three emulsion batches on each day.

Figure 20. Quantification of sulfhydryl group content in avocado Sabs O/W emulsions, stored at 4 ℃ and 25℃, for duration of primary storage trial. Values are a mean of three emulsion batches on each day.
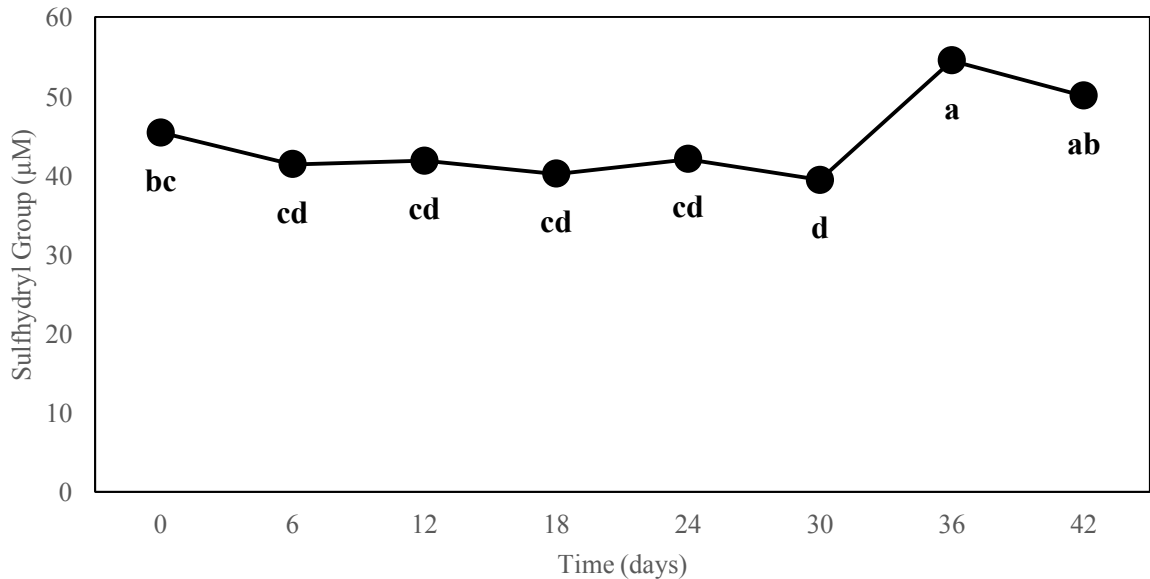
Figure 21. Quantification of sulfhydryl group content in hemp Spr O/W emulsions, stored at 25℃ and 4℃ for duration of primary storage trial (SEM 5.33). Values are a mean of three emulsion batches on each day. The asterisk (*) denotes a significant difference (P<0.05) between means was detectable.
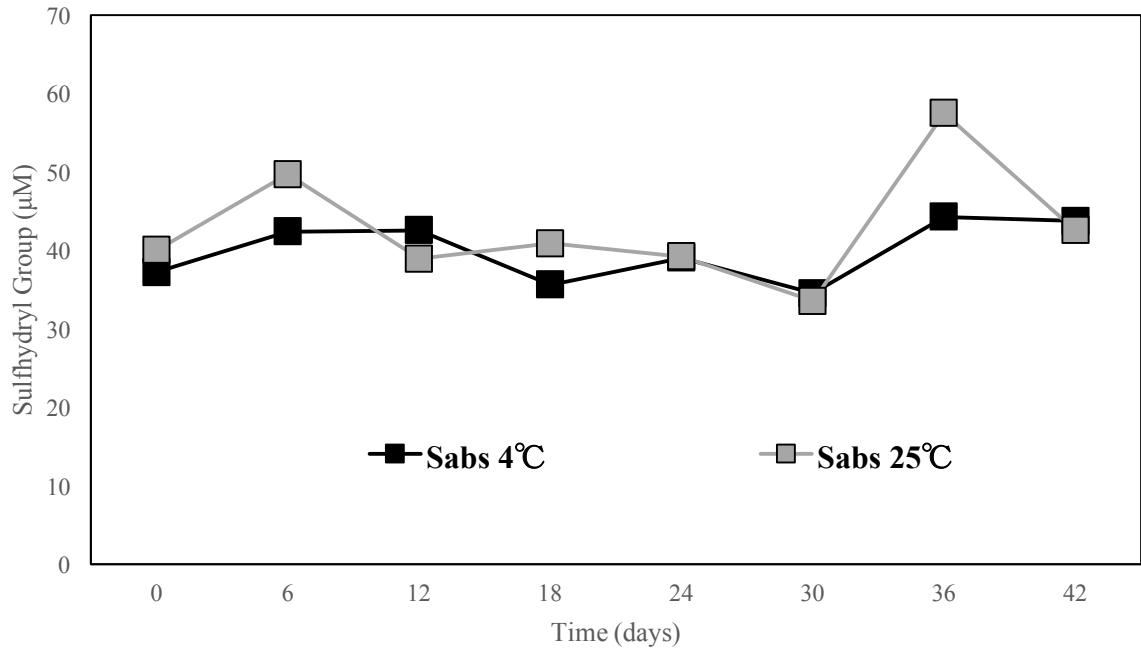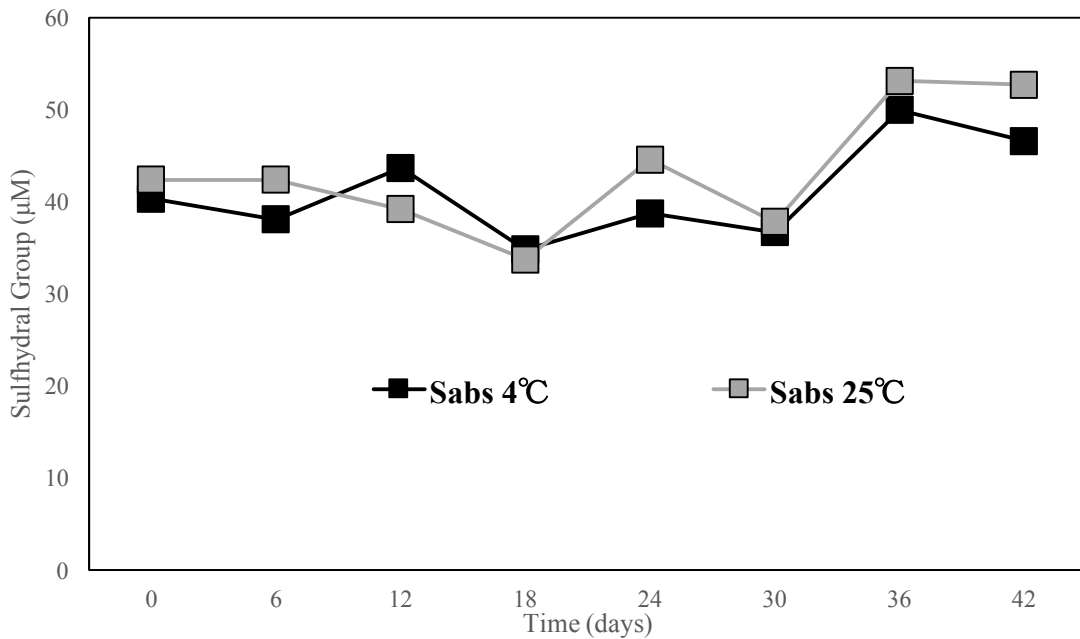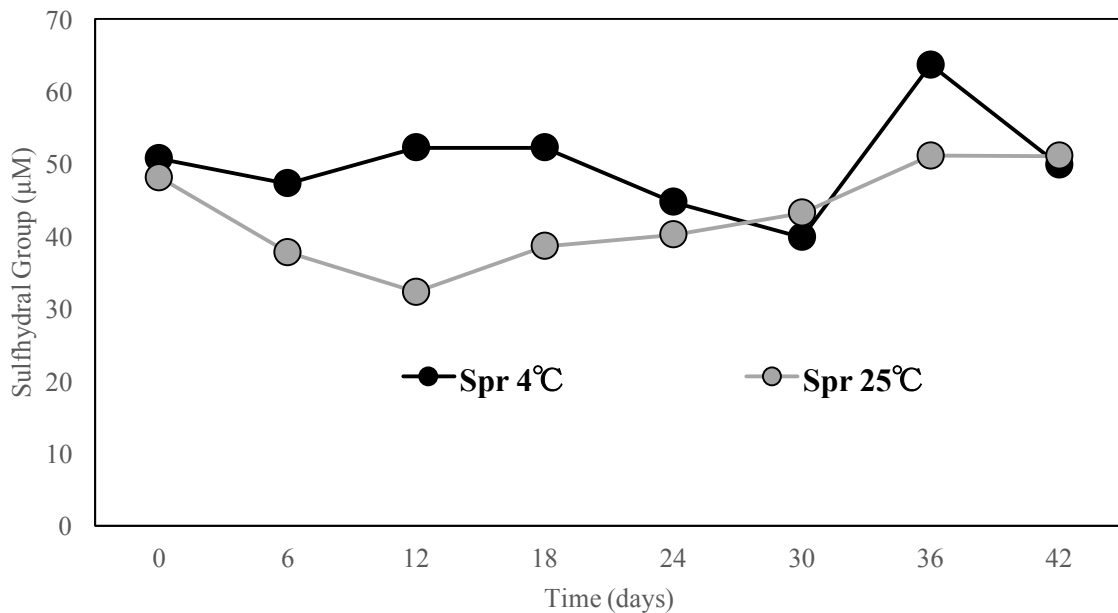

Figure 22. Quantification of sulfhydryl group content in avocado Spr O/W emulsions, stored at 4 ℃ and 25℃, for duration of primary storage trial. Values are a mean of three emulsion batches on each day.
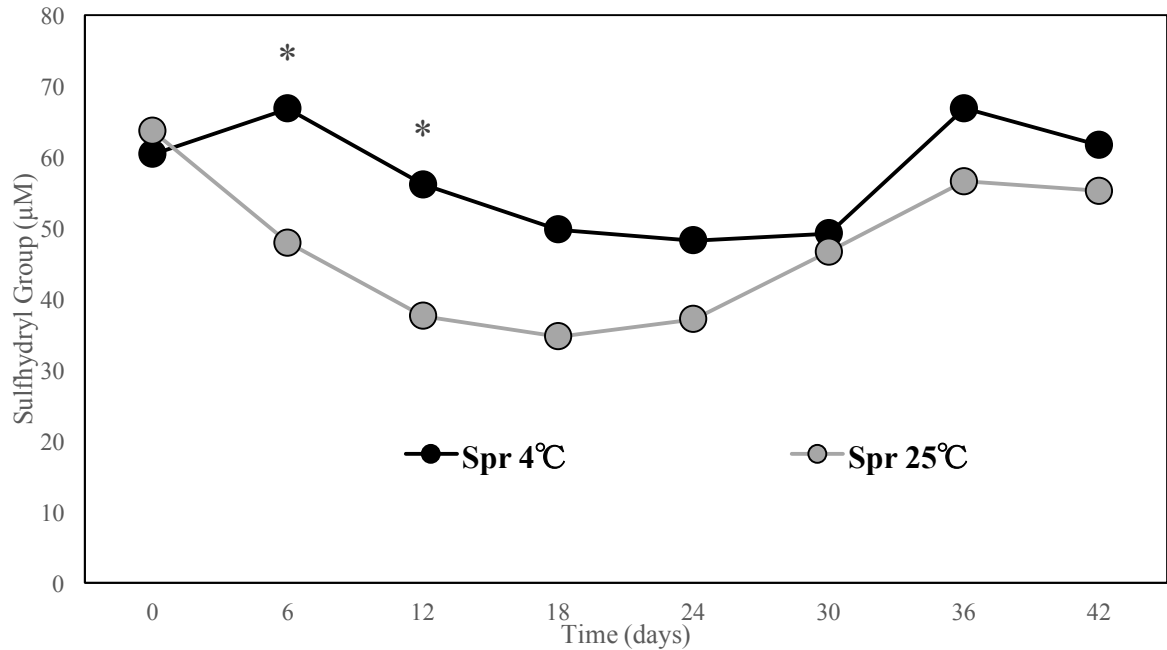
At the beginning of the storage trial, both hemp and avocado Spr emulsions exhibit a higher

level of protein oxidation than the Sabs emulsions (Fig. 23), which remained apparent for

emulsions stored at 4℃ but not at the higher storage temperature of 25℃. It was not ex-

pected the O/W emulsions without surfactant would have any quantifiable SH content.
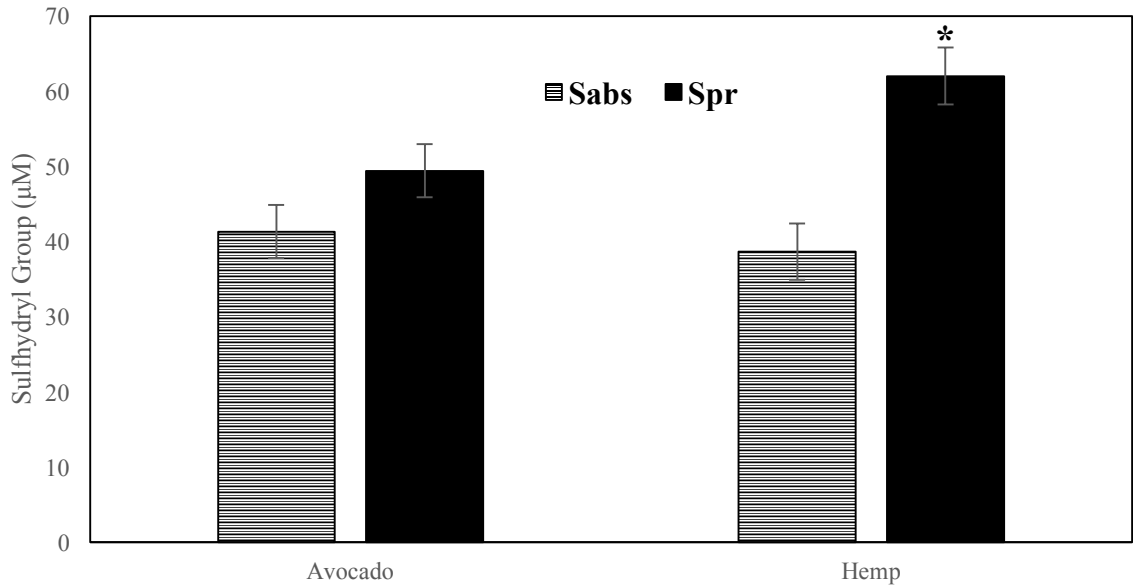
Figure 23. Initial quantification of average sulfhydryl group content in hemp and avocado O/W emulsions on day 0 of primary storage trial. Values are the mean of all SH measurements for Spr and Sabs emulsions for avocado and hemp. The asterisk (*) denotes significant difference ($P<0.05$) between means was detectable.

Firstly, the SPI was introduced to the emulsions at 0.5% (w/v), and only a fraction of the

protein contains the amino acids with detectable sulfhydryl groups. Studies have reported

a wide range for the SH content of SPI (2−8 μmol SH/g protein), and this content can be

poorly accessible depending on the protein structure and its figuration on the surface of the

oil droplet (Brosnan and Brosnan, 2006; Ruan et al., 2013). The interactions of LEC-SPI

at the interface of the oil can cause the formation of protein-LEC complexes, changing the

structure and conformation of the protein over time (Xu et al., 2011).

In addition, considering the amount of protein incorporated into the emulsion, the SH content present may not be high enough for a reliable detection with DTNB. Sensitivity of DTNB has been reported as detecting 20 µM to 1000 µM SH (Molecular Probes, 2001; Riener et al., 2002). Also, underestimation of actual SH can occur to an incomplete reaction with DTNB, even with prolonged time frames (Riener et al., 2002).

It is also possible the bulk oils do have a detectable quantity of SH due to the presence of glutathione, which led to indistinguishable differences in SH content between Spr and Sabs emulsions at certain temperatures and time periods. Glutathione (GSH) is an important antioxidant present in plants, and is believed to contribute to hemps ability to decontaminate heavy metal polluted soils (Ahmed et al., 2016). This may be the source of SH content detected in Sabs emulsions.

Overall, the DTNB assay proved to be an insufficient for detecting the level of SPI oxidation. The use of other reagents for this purpose, like 4,4′-dithiodipyridine (DTDP), have become increasing popular and are believed to react more fully with protein sulfhydryls (Riener et al., 2002).

4.1.3 Emulsion Characteristics

4.1.3.1 Particle Size

Initially, hemp and avocado Spr emulsions had smaller average particle sizes (PS) compared to Sabs emulsions (Fig. 24). After emulsion formation, hemp Spr emulsions had an

average PS of 1803.3 ± 205.52 nm, compared to 2472.4 ± 205.52 nm in Sabs emulsions

(P=0.0609). This difference was not maintained over trial, and particle size of both Spr and

Sabs emulsions increased significantly (Fig. 25, P<0.05) to similar average particle sizes

(3164.1 ± 205.52 nm and 3149.1 ± 247.64 nm, respectively).
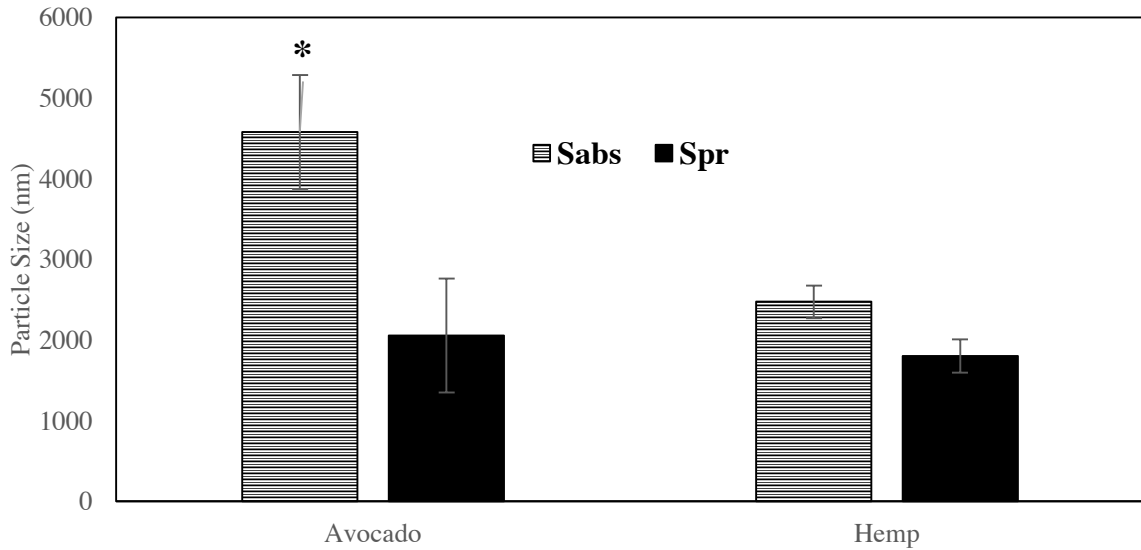
Figure 24. Initial average particle size of avocado Sabs and Spr and hemp Sabs and Spr O/W emulsions. Values are the mean of all PS measurements for Spr and Sabs emulsions for avocado and hemp. The asterisk (*) denotes significant difference (P<0.05) between means was detectable.
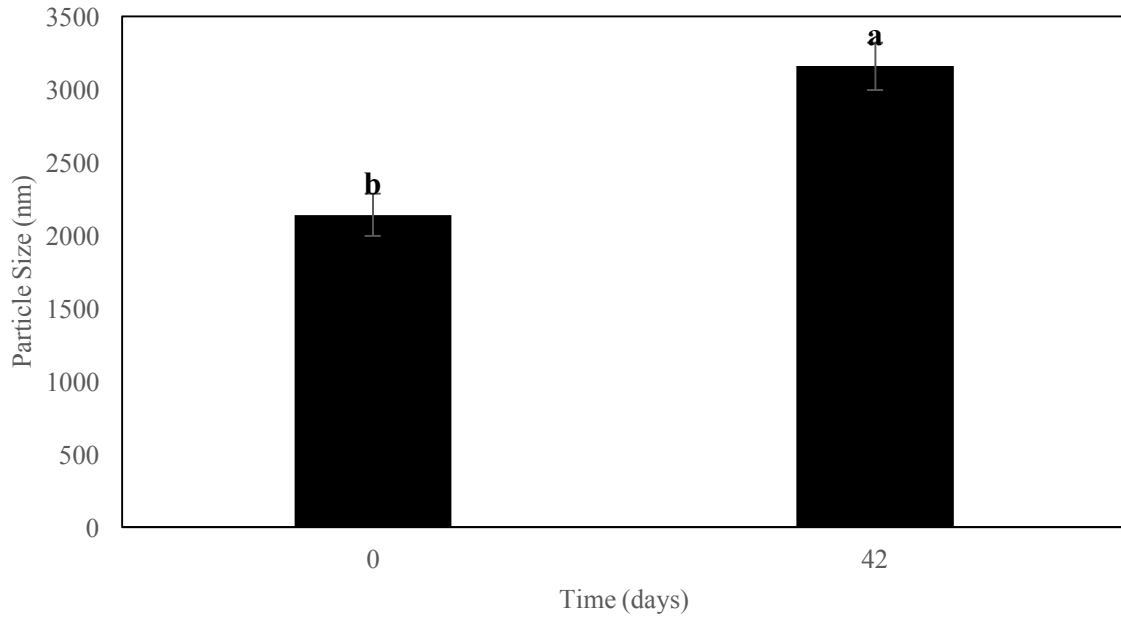
Figure 25. Average particle size of hemp O/W emulsions at the start and end of primary storage trial (SEM 145.33 and 160.91 for day 0 and 42, respectively). Values are the mean of all PS measurements for hemp emulsions. Different letters indicate a significant difference (P<0.05) between means was detectable.

The presence of surfactant also caused avocado emulsions to have a significantly smaller average PS (Fig. 26). Initially, avocado Spr and Sabs emulsions had average particle sizes of 2055.9 ± 706.28 nm and 4581.1 ± 706.28 nm, respectively ($P<0.05$). This difference between Spr and Sabs PS was maintained, with final average particle sizes increasing to 2588.0 ±7 89.25 nm and 6697.5 ± 789.25 nm, respectively ($P<0.05$).
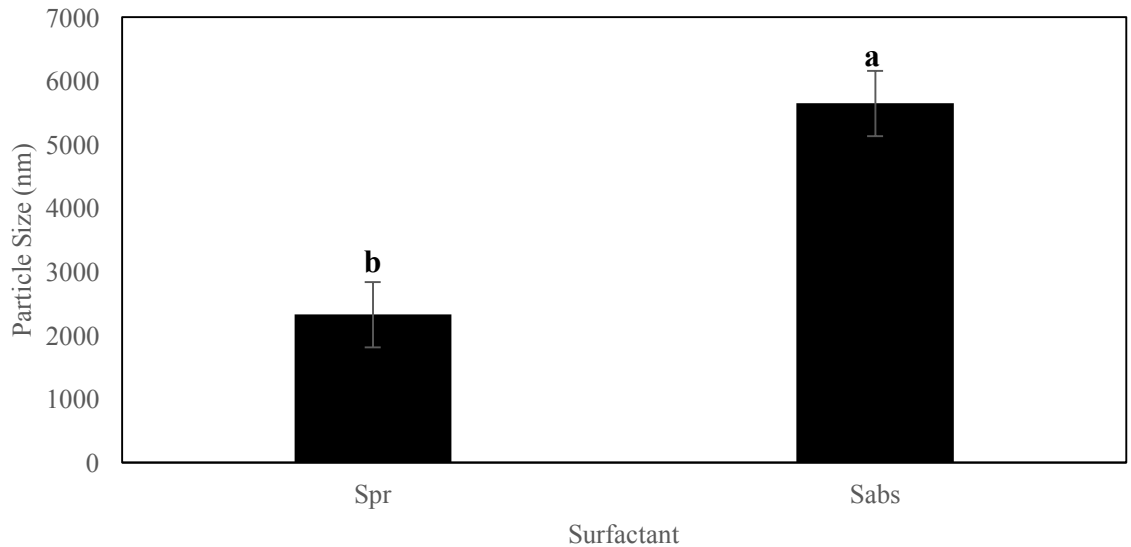
Figure 26. Average particle size of avocado O/W emulsions during the primary storage trial (SEM 512.51). Values are the mean of all PS measurements for Spr and Sabs emulsions for avocado. Different letters indicate a significant difference (P<0.05) between means was detectable.

Abuzaytoun and Shahidi (2006) conducted a study focused on optimizing physical stability of hemp emulsions by varying amounts of oil incorporated and length of time of homogenizing. They looked at emulsion characteristics like particle and dispersion index, as well as centrifuge tests (indicating emulsion resistance to destabilization of O/W distribution). Two emulsions formed by Abuzaytoun and Shahidi (2006) were of comparable composition to this study. These two emulsions were 10% (w/w) hemp oil, 83.75% water, and 5.2% LEC, differing in shear mixing time (1.5 min and 6 min). Particle size values for these emulsions were 3.35 ± 0.02 μM (mean ± SD) for 2 min mixing time and 3.80 ± 0.01 μM (mean ± SD) for 6 min mixing time. In this study, average particle size of hemp Spr emulsions were initially 1.80 ± 0.21 μM (mean ± SEM), increasing to 3.16 ± 0.21 μM (mean ± SEM) on day 42. Smaller particle size may have been achieved in this study due to the ultrasonication process or lower oil to water ratio.

Despite a smaller initial size of particles in hemp and avocado Spr emulsions, the lipid and protein oxidation levels of these emulsions were greater at the start of the trial, compared to Sabs emulsions. There was no significant correlation between PS of emulsions and lipid peroxidation or protein oxidation over the course of the storage trial, though the Spr emulsions with smaller average particle sizes were observed to oxidize more greatly (Table 4 & 5).

Table 4. Spearman correlations for the oxidative parameters and emulsion characteristics of hemp emulsions in the primary storage trial

	Oxidative Parameters	
Emulsion Characteristics	DPPP	SH
PS	-0.179 (p=0.425)	-0.317 (p=0.151)
PI	-0.498 (p=0.018)	-0.532 (p=0.011)
ZP	-0.356 (p=0.088)	-0.163 (p=0.448)

Table 5. Spearman correlations for the oxidative parameters and emulsion characteristics of avocado emulsions in the primary storage trial

	Oxidative Parameters	
Emulsion Characteristics	DPPP	SH
PS	-0.386 (p=0.062)	-0.057 (p=0.793)
PI	-0.768 (p<0.0001)	-0.399 (p=0.073)
ZP	-0.575 (p<0.0001)	-0.322 (p=0.125)

In previous reports, the presence of SPI and LEC was associated with decreased particle size in Spr emulsions (Scuriatti et al., 2003). The decreased size of the particles in the Spr emulsions may be a factor in the increased lipid oxidation. Mosca et al. (2010) observed that a decrease of particle size in water-in-olive oil emulsions caused a greater initial rate of hydroperoxide formation. Consistent with what we observed in the hemp and avocado Spr O/W emulsions, this may be due to greater surface area for attack by reactive species

and increased contact with pro-oxidative metal ions within the emulsion, as proposed by Mosca et al., 2010; Horn et al., 2011. While decreased particle size using SPI and LEC may improve the physical characteristics of oil-infused food products, it may do so at the expense of oxidative stability and shelf life.

Hemp emulsions stored at 4℃ maintained a smaller average PS than those at 25℃ (Fig. 27, *P*<0.05). Particle size of avocado emulsions was relatively unaffected by storage temperature. As mentioned with the SH detection, higher temperatures can impact the surface properties of emulsions by altering the degree of surfactant adsorption (Chen and Tao, 2005; Mosca et al., 2010). In this case, hemp emulsions appear more vulnerable to changes with temperature.
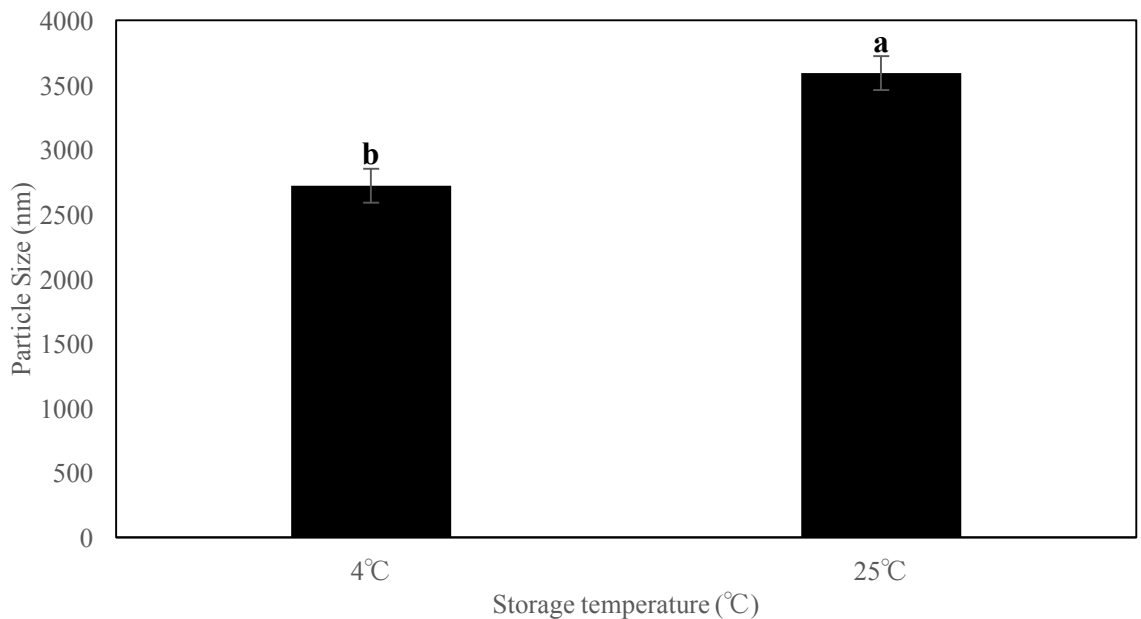
Figure 27. Average particle size of hemp O/W emulsions at storage temperatures 25℃ and 4℃ on day 42 of primary storage trial (SEM 130.2). Values are the mean of all PS measurements for hemp emulsions at 25℃ and 4℃. Different letters indicate a significant difference (P<0.05) between means was detectable.

4.1.3.2 Polydispersity Index

The average PDI of hemp Sabs emulsions remained higher than Spr emulsions throughout the storage trial (Fig. 28; 2.20 ± 0.51 versus 1.65 ± 0.2, respectively), though this difference was more evident in emulsions stored at 4℃ (2.23 ± 0.23 versus 1.11 ± 0.23). From the start to the end of the trial, the PDI of all hemp emulsions increased; Spr from 1.42 to 1.79 and Sabs from 1.92 to 2.63 (SEM 0.25). By the end of the trial, the average PDI of hemp emulsions stored at 25℃ was much higher than those at 4℃ ($P=0.071$).
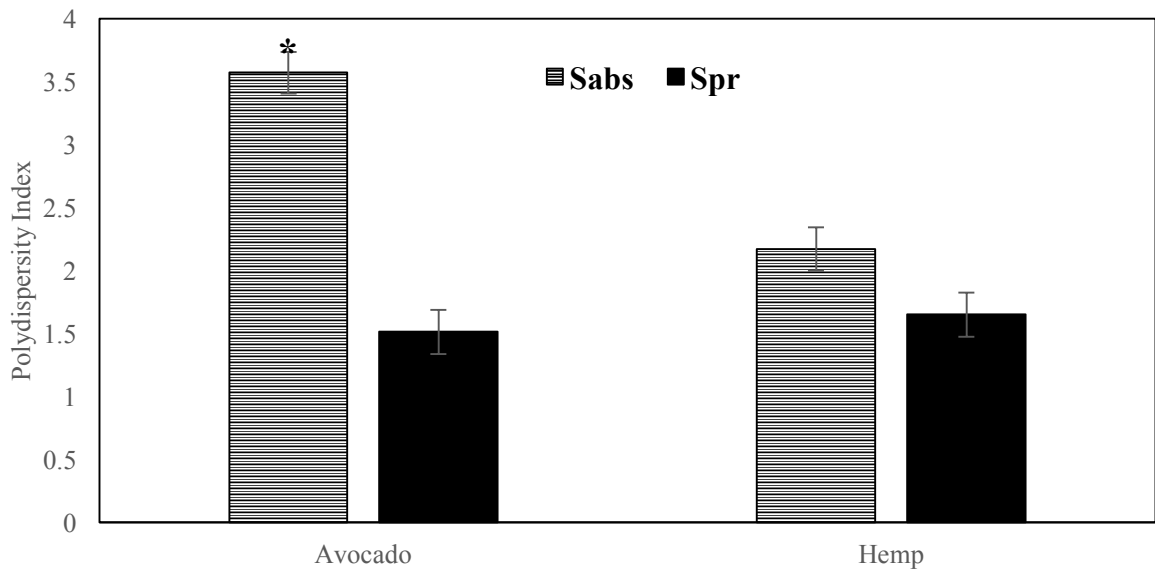
Figure 28. Polydispersity of avocado Sabs and Spr O/W emulsions and hemp Sabs and Spr O/W emulsions. Values are a mean of the PI for all Spr and Sabs emulsions for avocado and hemp. The asterisk (*) denotes significant difference (P<0.05) between means was detectable.

Avocado Sabs emulsions also exhibited higher PI than Spr emulsions (Fig. 29; 3.57 +/ - 0.17 versus 1.51 ± 0.18, $P<0.05$). Similarly, to hemp, avocado Spr emulsions had lower PDI values than sabs initially and at end of trial *(P<0.05)*, although the PDI for Spr emulsions did not change significantly over time, nor did the Sabs emulsions. As noted with PS

of emulsions, PDI of avocado emulsions was relatively unaffected by storage temperature or the duration of the storage trial.

The values obtained for PDI are comparable to that found by Abuzaytoun and Shahidi (2006) for hemp emulsions. The PDI of hemp emulsions for 10% hemp oil emulsions ranged from 1.87 to 1.62 ± 0.01 (mean ± SD), while the PDI for hemp emulsions in this study were initially 1.42, increasing to 1.79 on day 42 (SEM 0.25).
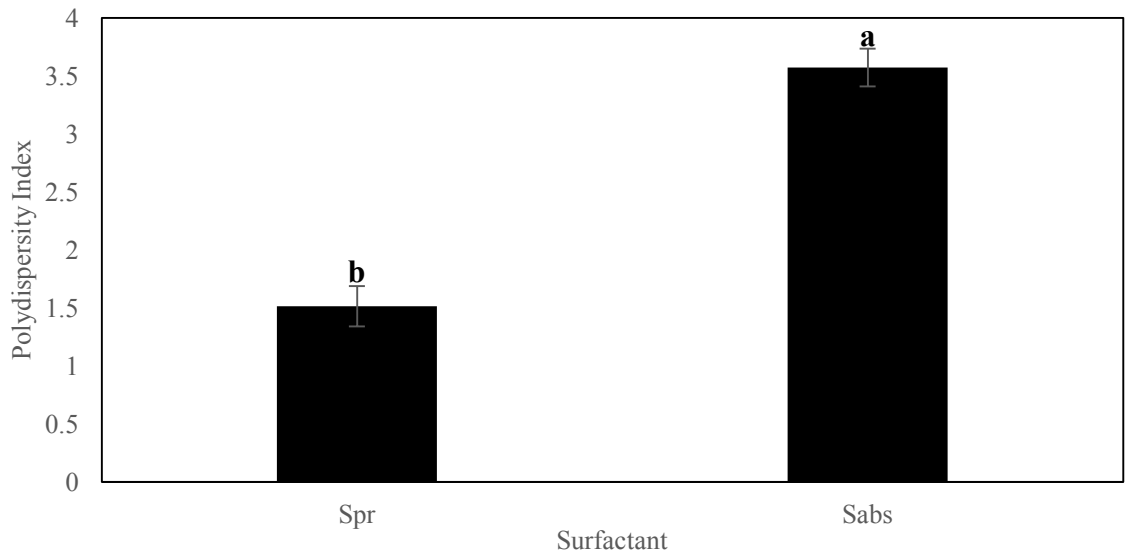
Figure 29. Polydispersity of avocado O/W emulsions (SEM 0.18 and 0.17 for Spr and Sabs, respectively). Values are a mean of the PI for all Spr and Sabs emulsions for avocado. Different letters indicate a significant difference ($P<0.05$) between means was detectable.

Negative correlations were found for the degree of lipid oxidation and PDI values for hemp and avocado (corr = -0.498, $P<0.05$ and corr = -0.768 ($P<0.05$, respectively). As the PDI values of emulsions increase, the level of lipid oxidation decreases, while emulsions with

lower levels of oxidation had higher PDI values (Table 6 & 7). The emulsions that held

low PDI values were the Spr emulsions, while the Sabs emulsions had higher values for

PDI. Despite the Spr emulsions having greater homogeneity than the Sabs emulsions, they

also had greater lipid oxidation levels. Similarly, to emulsions with smaller particle sizes

being more oxidized, the more homogenous and less the particle sizes differed within the

emulsion, the more oxidized the emulsions were.

The PDI and PS values were also found to have a significantly positive correlation for

hemp (Table 6, corr = 0.67, p = 0.001) and avocado emulsions (Table 7, corr = 0.957, p <

0.001). The more the particle size increased, the less consistent the particle sizes within the

emulsions were.

Table 6. Spearman correlations for the emulsion characteristics of hemp emulsions in the
primary storage trial

Emulsion Characteristics	PS	PDI
PDI	0.668 (p=0.001)	
ZP	-0.509 (p=0.016)	-0.205 (p=0.361)

Table 7. Spearman correlations for the emulsion characteristics of avocado emulsions in
the primary storage trial

Emulsion Characteristics	PS	PDI
PDI	0.957 (p<0.0001)	
ZP	0.077 (p=0.721)	0.528 (p=0.014)

Relative levels of oxidation at the same time points cannot be compared directly between

hemp and avocado, as the bulk oils used in the trial were extracted and processed at differ-

ent times, by different manufacturers, and thus likely started at different initial levels of

oxidation at the beginning of the trial. However, it can be noted how the characteristics of

the different O/W emulsions were affected by storage conditions. Overall, hemp emulsions appear less resistant to emulsion property changes than avocado emulsions. The particle size of hemp emulsions changed significantly over time and with storage at a higher temperature, while avocado emulsions did not. Similarly, the PDI of hemp emulsions increased over the storage trial and was greater at the storage temperature of 25℃, while avocado emulsions were not significantly impacted by time or storage conditions.

This relative instability of hemp emulsion characteristics, compared to those of avocado emulsions, could be due to greater unsaturated FA content. The degree of oxidation and the physical nature of emulsions have complex relationship. It is expected that the unsaturated FAs in the emulsions oxidized more quickly and extensively, due to their vulnerability to be attacked by reactive species; hemp has often proved less stable than other edible oils (Belhaj et al., 2010; Sun-Waterhouse et al., 2012; Uluata & Özdemir, 2012; Prescha et al., 2014). A greater extent of lipid oxidation can deteriorate emulsion qualities, as the surfactant capsule reacts with reactive peroxides and subsequently deteriorates, causing increases in particle size and aggregation within the emulsion (Tadros, 2013). Also, if the bulk hemp oil was initially more deteriorated than avocado oil it would have been more likely for the emulsion to undergo conformational changes.

4.1.3.3 Zeta Potential

Initially, hemp Spr emulsions provided slightly more negative ZP values than Sabs emulsions, but over the course of the trial, as all the values became significantly $P<0.05$) more negative, and Spr emulsions became significantly ($P<0.05$) more negative than Sabs emulsions (Fig. 30). Avocado Spr emulsions were also significantly more negative than Sabs,

but for both the beginning and the end of trial (Fig. 31, *P*<0.05). While there was no significant correlation between ZP and lipid or protein oxidation of emulsions, ZP values of Spr emulsions indicate greater emulsion stability than Sabs, despite greater levels of oxidation. As shown in Figure 32, zeta potential of Spr emulsions for both hemp and avocado was significantly greater than for Sabs emulsions.

Figure 30. Zeta potential of hemp O/W emulsions at the start and end of primary storage trial (SEM 1.79). Values are the mean of all Spr and Sabs emulsions. Different letters indicate a significant difference (P<0.05) between means was detectable.

Figure 31. Zeta potential of avocado O/W emulsions at the start and end of primary storage trial (SEM 0.67). Values are the mean of all Spr and Sabs emulsions. Different letters indicate a significant difference (P<0.05) between means was detectable.
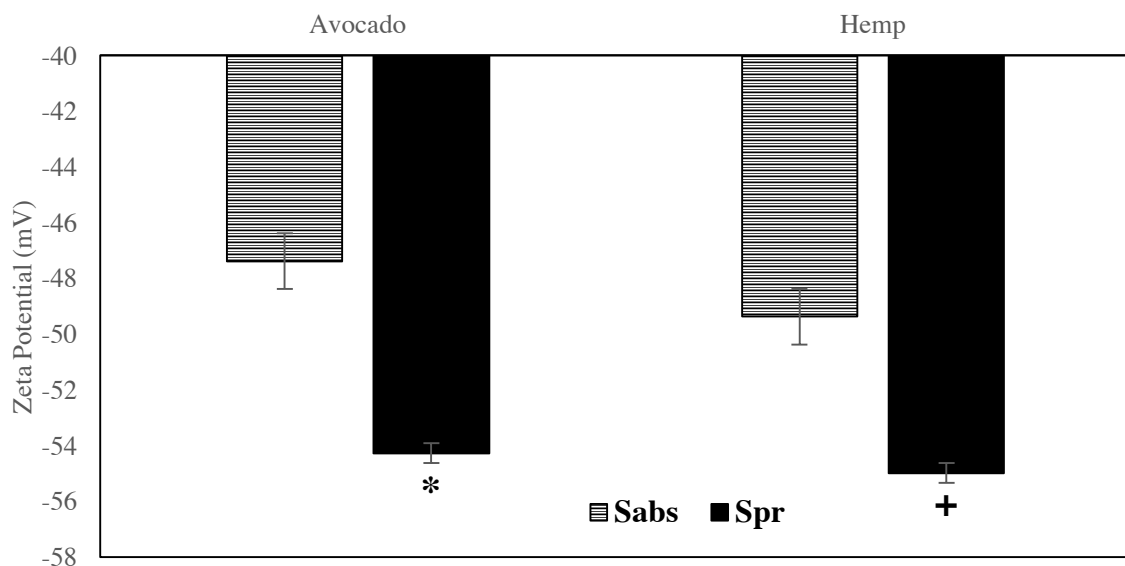
Figure 32. Zeta potential of avocado Sabs and Spr O/W emulsions and hemp Sabs and Spr O/W emulsions. Values are the mean of all ZP measurements for Spr and Sabs emulsions for avocado and hemp. The asterisk (*) denotes a significant difference (P<0.05) between avocado Sabs emulsions means and avocado Spr emulsions means was detectable, the plus symbol (+) denotes a significant difference (P<0.05) between hemp Sabs emulsions and hemp Spr emulsions was detectable.

Zeta potential of hemp emulsions appears unaffected by the different storage conditions, but storage at 25℃ resulted in more negative ZP values for avocado emulsions, compared to 4℃, by the end of the trial (Fig. 33).
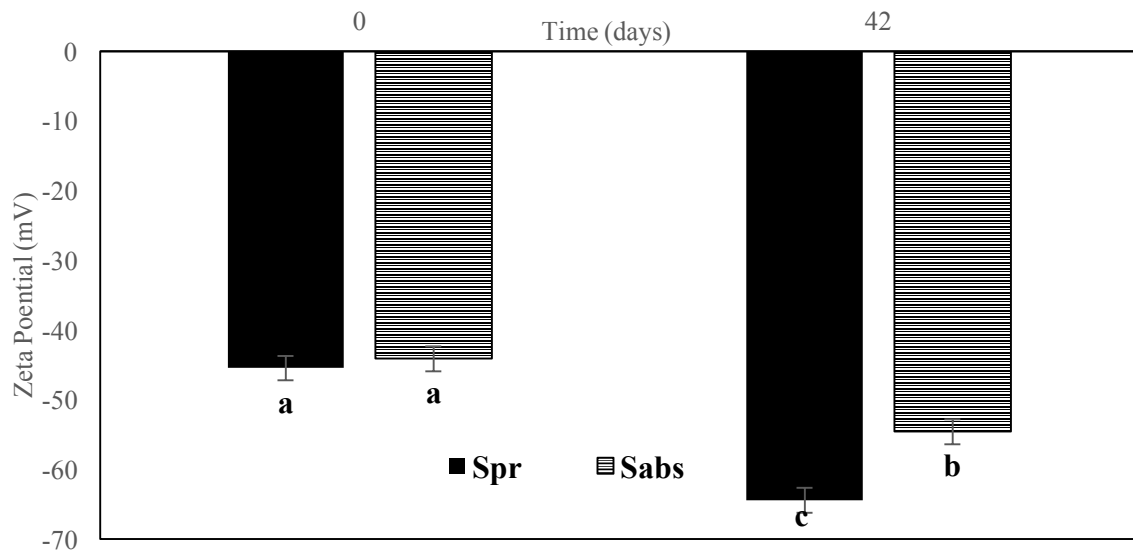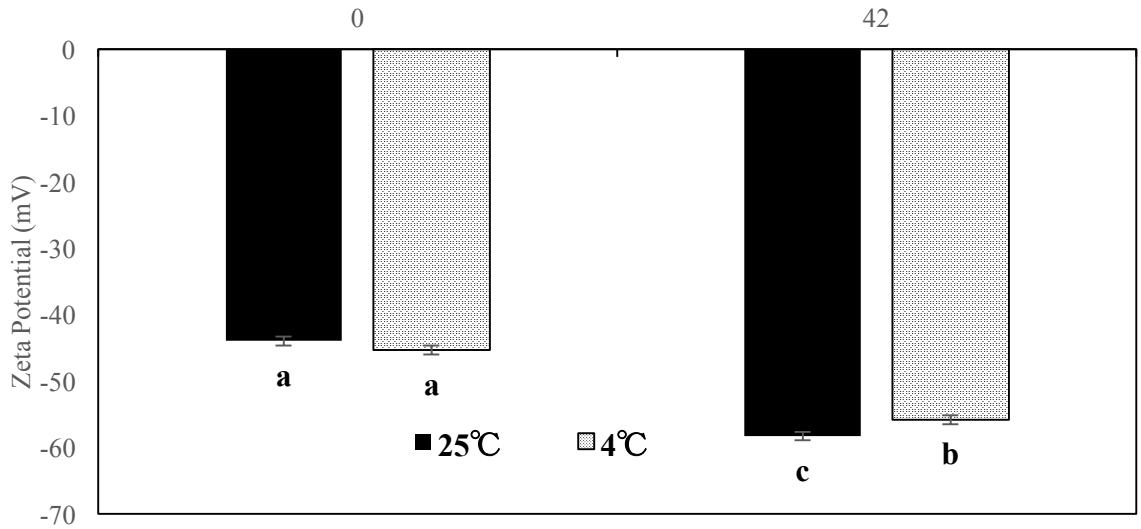

Figure 33. Zeta potential of avocado O/W emulsions at the start and end of primary storage trial (SEM 0.67). Values are a mean of three emulsion batches on each day. Different letters indicate a significant difference (P<0.05) between means was detectable.

It can be noted that the more negatively charged droplets in Spr emulsions would have a stronger affinity to attracting the oppositely charged iron ions present in the emulsions, due to attractive forces between molecules with opposite charges. This may promote the iron-catalyzed reaction of lipid oxidation (Donnelly et al., 1998; McClements and Decker, 2000).

4.2 Thermal Trial

4.2.1 Lipid Oxidation

The levels of lipid and protein oxidation were evaluated pre- and post- the brief exposure to high heat. As observed in the storage trial, the Spr emulsions for both oils indicated significantly higher levels of oxidation compared to the Sabs emulsions both pre- and post- the heat treatment (Fig. 34 & 35, $P<0.05$). The different storage temperatures and exposure to heat caused no significant change in oxidation levels (Fig. 36).

Raikos et al. (2015) exposed hemp emulsions to heat treatments of 50, 60, 70, 80 and 90°C for 10 min and only observed significant decrease in oxidative stability of lipids after emulsion exposure to 70°C for 10 min. An incremental, though not significant, decrease of the emulsions' resistance to lipid oxidation was observed with increasing temperatures (Raikos et al., 2015). In this study, a longer induction time to higher temperatures (compared to 72°C for 15 sec) may have induced a more evident response of lipid oxidation. Heat stability for a short time does not appear to be an issue for hemp and avocado emulsions, although they might be able to withstand food processing conditions with extended exposure to high temperatures.

Figure 34. Lipid peroxidation of hemp Spr and Sabs O/W emulsions pre- and post- thermal trial. Values are the mean of all Spr and Sabs emulsions. Different letters indicate a significant difference (P<0.05) between means was detectable.
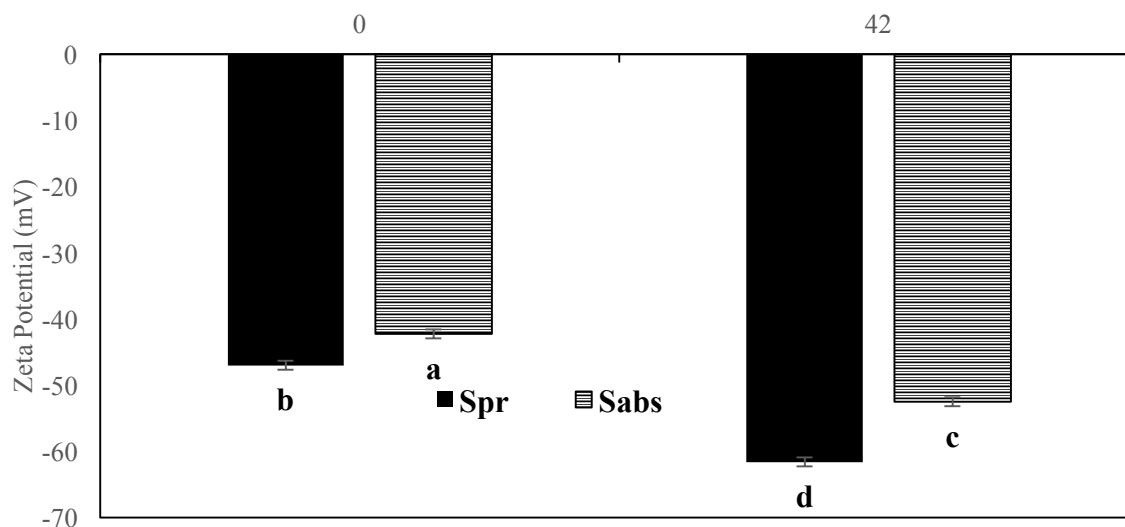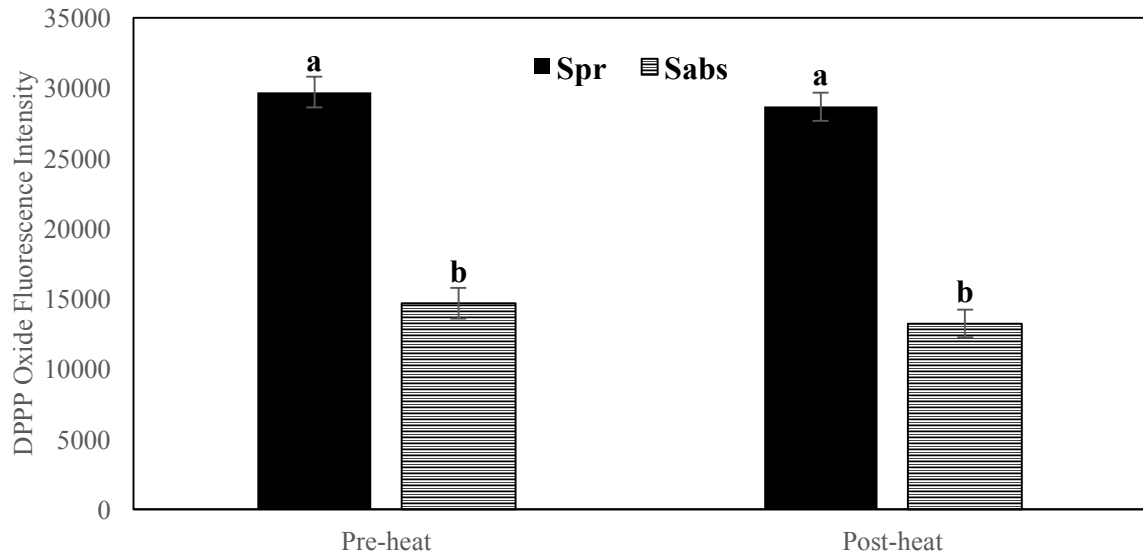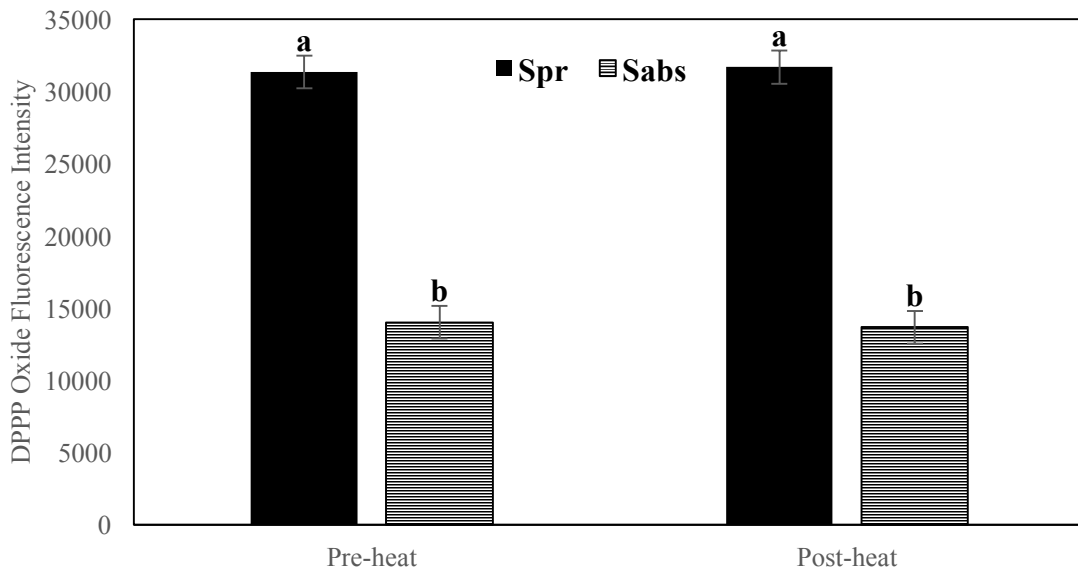

Figure 35. Lipid peroxidation of avocado Spr and Sabs O/W emulsions pre- and post- thermal trial. Values are the mean of all Spr and Sabs emulsions. Different letters indicate a significant difference (P<0.05) between means was detectable.
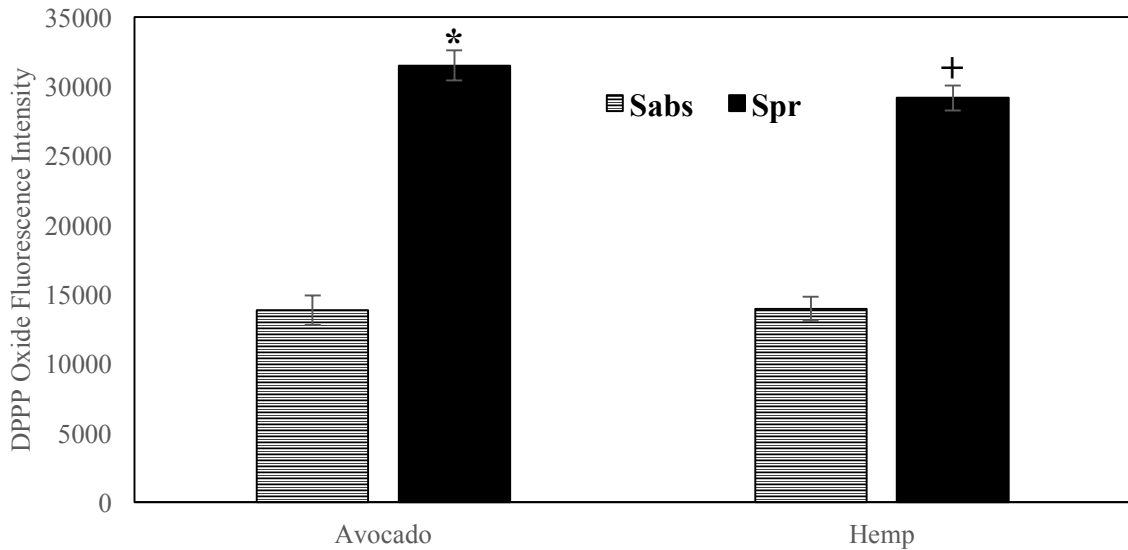
Figure 36. Lipid peroxidation of avocado Sabs and Spr O/W emulsions and hemp Sabs and Spr O/W emulsions for thermal trial. Values are the mean of all Spr and Sabs emulsions for avocado and hemp. The asterisk (*) denotes a significant difference (P<0.05) between avocado Sabs emulsions and avocado Spr emulsions means was detectable, the plus symbol (+) denotes a significant difference (P<0.05) between hemp Sabs emulsions and hemp Spr emulsions was detectable.

It can be noted the levels of lipid oxidation increased in emulsions between the end of the storage trial and start of thermal trial (after approximately 14 days passing). Levels of lipid oxidation in hemp Spr emulsions increased only slightly (by 3.34%), while Sabs emulsions increased by 31.34%. Lipid oxidation in avocado Spr and Sabs emulsions increased only slightly (by 2.20 and 5.12%). Levels of oxidation may have been rising as the manufacturer's indicated shelf life was approaching at the time of the thermal trial.

4.2.2 Protein Oxidation

Consistent with the storage trial, the level of protein oxidation was greater for hemp Spr emulsions, compared to Sabs emulsions, both pre- and post- heat treatment, although there was no significant change in oxidation for any hemp emulsions due to the heat treatment (Fig. 35). There was no significant difference in protein oxidation levels detected in hemp

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emulsions stored at 25℃ and 4℃. No difference in levels of protein oxidation were observed for avocado emulsions due to the presence of surfactant, different storage temperatures, or the heat treatment.
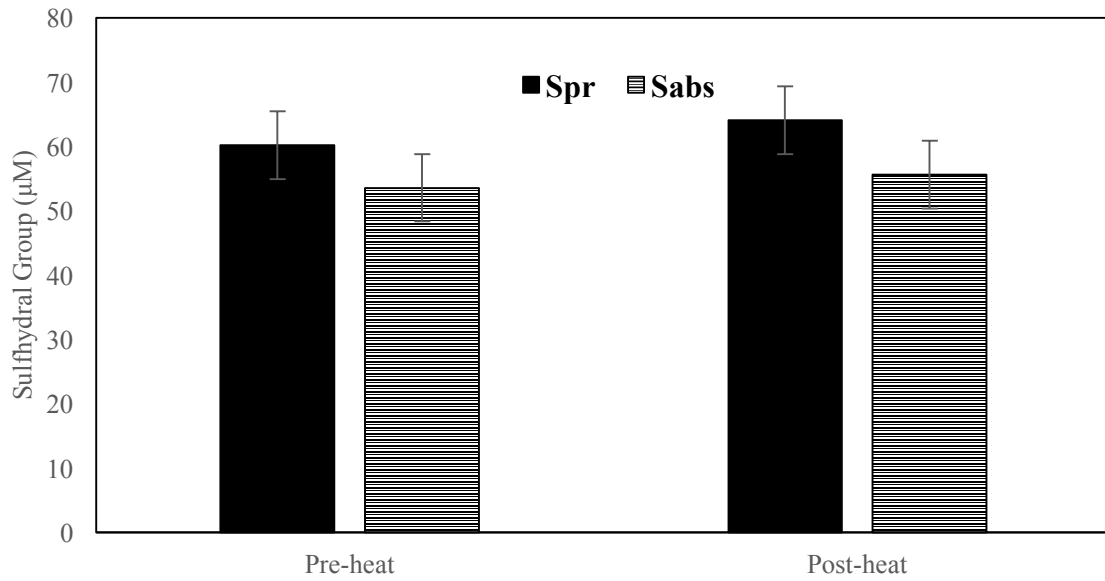
Figure 37: Quantification of sulfhydryl group content in hemp O/W emulsions pre-heat and post heat treatment in thermal trial (SEM 5.23). Values are the mean of all Spr and Sabs emulsions for avocado and hemp.

Chapter 5: Conclusion

Hemp seed oil has proven to be an agricultural commodity of industrial importance with potential for many food applications. However, its susceptibility to oxidation limits its utilization as a cooking oil and its incorporation into food products. This omega rich edible oil and its potential fortified food products should be stable, appealing, and convenient for the consumer.

The storage and thermal conditions tested did not accelerate oxidation in the emulsion samples. The emulsions were able to resist oxidative changes throughout the 42-day storage trial at 4℃ and 25℃. Exposure to 72°C also did not provoke further oxidation.

This micro-encapsulation method was successful at producing desirable physical properties of hemp O/W emulsions. The particle size and polydispersity of emulsions were lowered in emulsions with surfactant. Although the effective incorporation of hemp oil into aqueous food products can be achieved with positive emulsion qualities (consistency, homogenous, etc.), the oxidative stability may be sacrificed in return.

Contrary to the initial hypothesis, emulsions with surfactant did not better resist oxidation than those without. Lipid oxidation levels were found to be higher in emulsions with surfactant. It is possible the interface of the emulsions served as a build-up zone for radicals due to LEC sourced phospholipid oxidation and reactive iron ions, deteriorating the oil

content. LEC may have not effectively encapsulated the oil, leaving it vulnerable to reactive species. Also, the levels of protein oxidation detected in Spr emulsions compared to Sabs emulsions was higher than expected.

The choice of emulsifier is crucial for not only emulsion formation but also stability. Further studies should be performed to measure the oxidative effect of LEC and evaluate its further prospects for food encapsulation. LEC must be re-examined as a food grade emulsifier for sensitive oils with highly unsaturated FAs. A surfactant must not oxidize easily or promote oxidation of oil. There remain gaps in the development of an emulsion formulation suitable to encapsulate hemp oil to enable its use in fortified food products.

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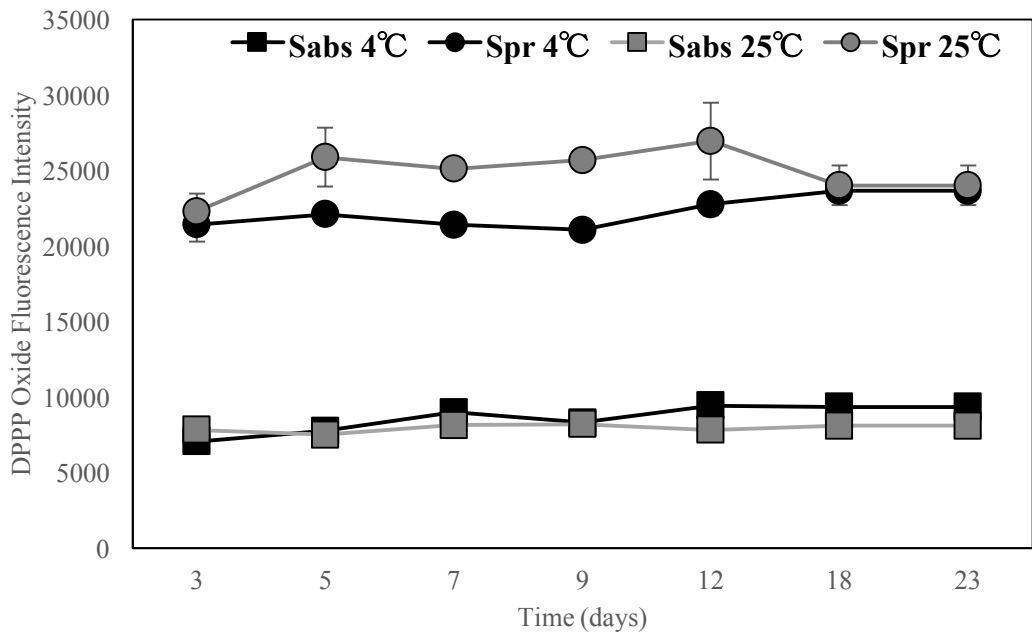
Appendix: Results of Preliminary Trial

Figure A. Lipid peroxidation of hemp Spr and Sabs O/W emulsions at 4℃ and 25℃, for duration of preliminary storage trial. Values are a mean of three emulsion batches on each day ± standard error.
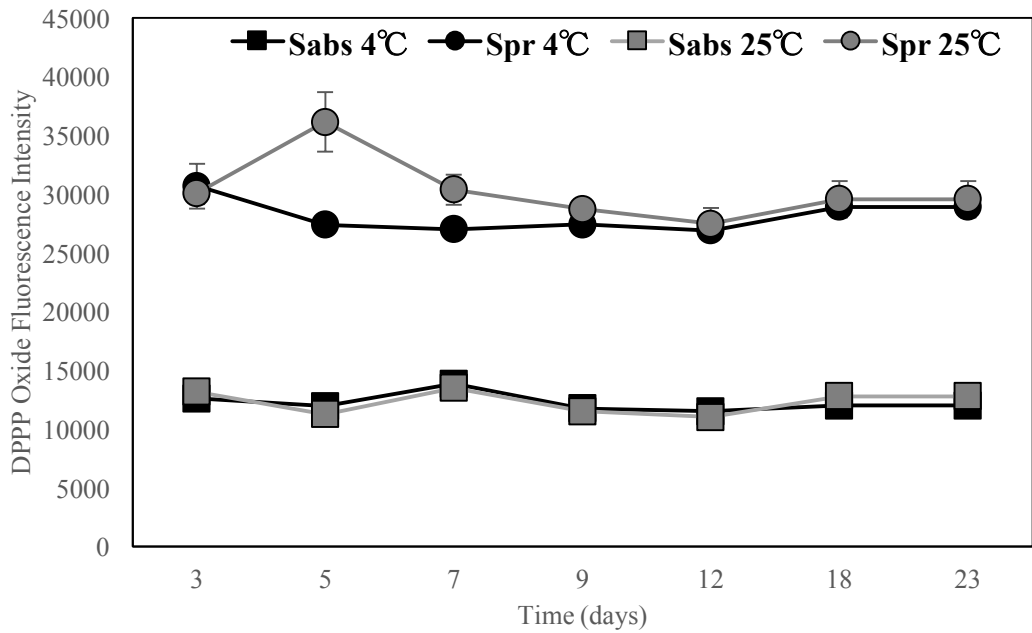

Figure B. Lipid peroxidation of avocado Spr and Sabs O/W emulsions at 4℃ and 25℃, for duration of preliminary storage trial. Values are a mean of three emulsion batches on each day ± standard error.
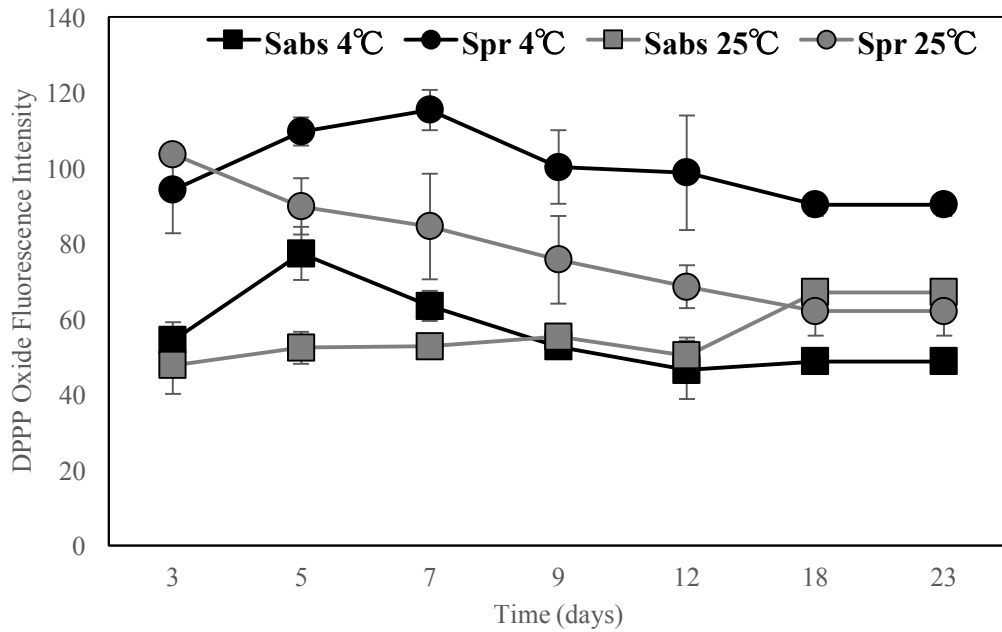
Figure C. Quantification of sulfhydryl group content in hemp O/W emulsions, stored at 4℃ and 25℃ for duration of preliminary storage trial. Values are a mean of three emulsion batches on each day ± standard error.
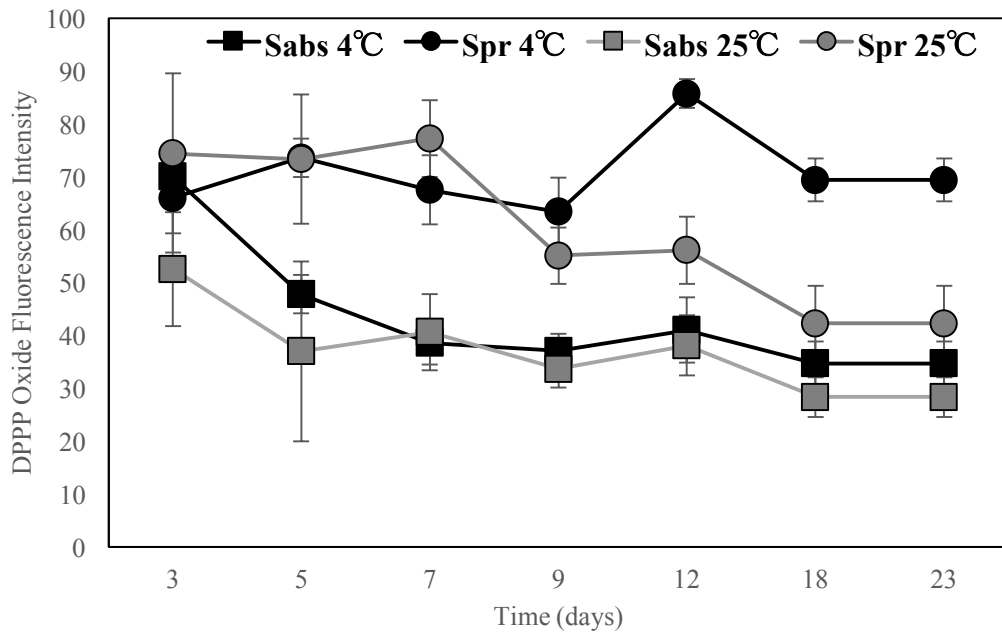

Figure D. Quantification of sulfhydryl group content in hemp O/W emulsions, stored at 4℃ and 25℃ for duration of preliminary storage trial. Values are a mean of three emulsion batches on each day ± standard error.