University of Alberta

Supercritical carbon dioxide processing for the extraction and delivery of flax bioactives

by

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Dedications

I am grateful to my supervisors for the support they have shown me throughout my work; not only financial, but scientific and emotional, as well. Despite the difficulties of being challenged, the rewards that result make the stuggle worthwhile.

To Blake, thank you for your patience and for allowing my work and stress to share our home for far too long.

Most of all, thank you to my parents who have been the strongest support network possible. If my self-confidence ever lacks, I know that they have more than enough confidence in my abilities and potential to overcome any deficit.

The ache for home lives in all of us,
the safe place where we can go as we are and not be questioned-
Maya Angelou

Abstract

Flax is an oilseed praised for high omega-3 oil, fiber and lignan content. Supercritical carbon dioxide ($SCCO_2$) can be used as a green solvent alternative to process flax bioactives.

$SCCO_2$ extraction was used to remove lignan Secoisolariciresinol Diglucoside (SDG) from full fat and defatted whole flax seeds and hulls and hydrolyzed whole seed. Temperature, pressure and addition of ethanol modifier had no effect on CO_2 loading of SDG. However, when seed was pre-hydrolyzed, significantly higher loading was obtained compared to other seed treatments, as hydrolysis reduced the size of SDG macromolecules and released free SDG.

$SCCO_2$ dried aerogels were formed from β-glucan and flax mucilage. Compared to 10% mucilage, 5% β-glucan aerogels had lower surface area (165.55 m^2/g vs 201.13 m^2/g) and maintained less of their hydrogel volume (37.62% vs 56.90%), but had a similar density (0.19 g/cm^3 for β-glucan and 0.16 g/cm^3 for mucilage). $SCCO_2$-dried aerogels showed significantly less volume shrinkage compared to air-dried gels and had a more uniform structure compared to freeze-dried gels.

Pregelatinized corn starch (PGS) and β-glucan aerogels were impregnated with bioactive lipids. The effect of processing conditions, including temperature, pressure and flow mode were dependent on the complexity of the lipid, as well as the partition of the lipid between the matrix and the $SCCO_2$ phase. Oleic acid had higher impregnation efficiency in PGS compared to flax oil because of higher purity and smaller molecular size and weight. For flax oil, its low solubility in $SCCO_2$ limited its impregnation in PGS. For β-glucan aerogels, depending on when oil is incorporated (before, after or during drying), different impregnation efficiencies were achieved. FTIR and SEM results suggest oil has an effect on the gelation of β-glucan.

SDG concentrate, Beneflax, was added to β-glucan and mucilage aerogels prior to $SCCO_2$ drying. When Beneflax was added prior to gelation, regardless of technique, SDG concentration was not different. However, when hydrogels were impregnated by soaking in 70% ethanol Beneflax solutions, SDG recovery from aerogels was significantly lower.

Overall, results suggest new uses for food-grade polymers using $SCCO_2$ processing, including the formation of aerogels and impregnation of flax bioactives.

Acknowledgements

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
ATR	Attenuated total reflectance
BET	Brauner Emmett Teller
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CO_2 DF WS	Ground seed defatted with SCCO2
AH	After hydration
BH	Before hydration
CO-RESS	Crystallization upon rapid depressurization of a supercritical solution
DENAB	4-(Diethylamino)-4'nitroazobenzene
DF H	Ground hulls defatted with petroleum ether
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DR 1	Disperse red 1
EPA	Ecoisapentaenoic acid
EtOH	Ethanol
FF H	Ground, full fat hulls
FM	Flax mucilage
FTIR	Fourier-transform Infrared Spectroscopy
GAS	Gas anti-solvent

GRAS	Generally recognized as safe
HMGA	3-Hydroxy-3-methyl glutamic acid
HPLC	High performance liquid chromatography
HY WS	Ground, hydrolyzed seed defatted with petroleum ether
LAR	Lariciresinol
LSD	Least significant differnce
MAT	Matairesinol
PG	Propyl gallate
PGS	Pregelatinized corn starch
PGSS	Particle formation from gas-saturated solutions/suspensions
PIN	Pinoresinol
PMMA	Polymethyl methacrylate
PUFA	Polyunsaturated fatty acid
RESS	Rapid expansion of supercritical solutions
SAS	supercritical anti-solvent
$SCCO_2$	Supercritical carbon dioxide
SDG	Secoisolariciresinol diglucoside
SECO	Secoisolariciresinol
SEM	Scannin electron microscopy
T DF WS	Ground seed defatted with petroleum ether
TBHQ	*Tert*-butyl hydroxyquinone
βG	β-Glucan

1. Introduction and Thesis Objectives

Flax is a nutraceutical crop of economic importance in Canada [1]. Flax seeds contain many beneficial components, including oil high in α-linolenic acid (ALA), lignans, and soluble and insoluble fiber. ω-3 Fatty acids, such as ALA, are essential fatty acids, and therefore must be obtained from the diet. Furthermore, ALA is an important precursor for longer chain polyunsaturated fatty acids, such as eicosapentaenoic acid (20:5 ω3) and docosahexaenoic acid (22:6 ω3). These fatty acids have been investigated extensively in terms of their role in human health [2]. Lignans are a group of phenolic phytohormones found in flax seed in high concentrations relative to other lignan containing plant materials [3]. Intake of lignan containing foods has been associated with a variety of physiological effects linked to health benefits, especially inhibition of hormonally linked cancers [4,5]. Both flax oil and flax lignans are susceptible to degradation, rendering them biologically inactive, and are typically extracted using large volumes of organic solvents at high temperatures.

Supercritical carbon dioxide ($SCCO_2$) is CO_2 pressurized and heated beyond its critical pressure and temperature, 7.4 MPa and 31 ^{o}C, respectively [6]. Under these conditions, $SCCO_2$ has properties in between those of a liquid and a gas, in terms of its density, diffusivity, viscosity and heat conductivity. Temperature, pressure and concentration of a co-solvent, or solvent modifier, can be altered to "tune" $SCCO_2$ solvent power. $SCCO_2$ can be used to extract non-polar compounds, similar to hexane. When polar co-solvents are added, $SCCO_2$ can also successfully extract more polar compounds, such as phenolics. With the

ability to tune the solvent power, and its non-toxicity, non-flammability, lack of solvent residue and environmentally favourable properties, $SCCO_2$ is an ideal solvent for many valuable food components.

The unique properties of $SCCO_2$ can also be exploited to develop different delivery forms for bioactive ingredients [7]. Delivery forms obtained through encapsulation or impregnation can protect components, which are susceptible to degradation as well as allow incorporation into complicated food matrices without separation. $SCCO_2$ can be used to increase the surface area to volume ratio, thus increasing the activity of bioactives, provide a protective coating, or to impregnate compounds of interest into a protective polymer matrix [7]. Extensive research has been conducted in the area of $SCCO_2$ polymer impregnation in areas other than food applications using synthetic polymers, which can be engineered to have specific properties beneficial to impregnation [8]. However there are many plant-based, food-grade polymers, which should not be overlooked. Polysaccharide polymers, such as various starch or soluble fiber preparations could potentially be used, alone or in combination with synthetic polymers, to impregnate various food grade compounds or nutraceuticals, including flax oil and lignans, for protection or controlled release. $SCCO_2$ can further be utilized to form porous polysaccharide polymer matrices, known as aerogels, which can then in turn be used as a delivery vehicle [9].

Although value-added processing of flax raw materials helps processors, it also increases the value of flax seed for Canadian farmers. This interest and potential increased revenue could not only improve the standard of living for

Canadian flax farmers, but also enhance innovation and research on flax nutraceuticals.

Recently, our research group has focused on using $SCCO_2$ in the value-added processing of flax bioactives, including utilizing encapsulation and impregnation techniques. While there is great potential to utilize $SCCO_2$ technology in this area, there is currently limited literature information on flax seed processing, beyond simply the extraction of lipids, indicating the necessity for further work. Therefore, the objectives of this thesis were:

- to determine the effect of temperature, pressure, level of ethanol addition and flax seed pretreatment on the CO_2 loading of flax lignan, secoisolariciresinol diglucoside (SDG), during $SCCO_2$ extraction of flax seed (Chapter 3),
- to determine the effects of $SCCO_2$ on pregelatinized corn starch (PGS) particle characteristics and to investigate the effects of $SCCO_2$ pressure and temperature on the impregnation efficiency of oleic acid and flax oil in PGS (Chapter 4),
- to form aerogels from barley β-glucan using $SCCO_2$ drying, freeze drying and air drying and to subsequently determine the effect of gel concentration and drying technique on the resulting characteristics of the aerogels (Chapter 5),
- to impregnate barley β-glucan aerogels with flax oil using $SCCO_2$ and to investigate the effects of point of oil addition, flow regime, impregnation

time and temperature and pressure on impregnation efficiency (Chapter 6), and

- to utilize flax mucilage and barley β-glucan aerogels for the delivery of SDG, and to compare the properties of the aerogels, as well as their final SDG contents (Chapter 7).

The results obtained should not only provide increased interest in flax bioactives, but also provide valuable insight into the behaviour of polysaccharide polymers in the presence of $SCCO_2$ in an effort to increase the use of renewable, biocompatible network forming polymers. Thus, this research could lead to new opportunities for novel processes and product development for flax bioactives based on supercritical technology.

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2. Literature Review[1]

2.1. Flax Seed

Flax, *Linum usitassimum,* is an oilseed crop which is used both as an industrial and an edible crop. Canada is currently the largest producer and exporter of flax seed, with 930,100 tonnes produced in 2009 - 2010 and exports mainly going to the United States of America, Europe, Japan and South Korea [1]. As an industrial crop, flax seed oil is used to produce linoleum, paints, surface coatings and oleo-chemical products, while its fiber is used to make linen and paper [2]. As an edible crop, the oil is extracted to produce a product high in ω-3 fatty acids, whereas the protein and fiber are mainly used as animal feed [2]. Flax seed is also added whole, or crushed, to a variety of consumer products, including breads, muffins and other baked goods.

Flax seed is recognized as a healthy addition to the diet due to its favourable composition. Typically, flax seed contains 40% oil, 30% dietary fiber, 20% protein, 4% ash and 6% moisture [2]. Extraction of flax oil yields a high quality protein meal, with an amino acid profile similar to that of soy protein [3], which can be used for a variety of purposes. In reviews by Oomah and Mazza [4] and Rabetafika *et al.* [3], flax protein is described as being potentially useful as an emulsifier and stabilizer in products such as sauces and ice creams, as well as a meat extender. The carbohydrate component of flax seed consists of insoluble, as well as soluble fiber, most well known as flax mucilage or flax seed gum. Cui *et al.* [5] found that flax seed contains approximately 8% gum, which is mainly

[1] Parts of this literature review are to be published as part of a book chapter, Sections 5.18.2 and 5.18.3; Temelli F, Saldaña MDA, Comin L. "Application of SFE in food processing" (Chapter 5.18) in "Comprehensive Sampling and Sample Preparation," J. Pawliszyn (ed) Vol 5. Elsevier.

composed of sugars such as *L*-rhamnose, *D*-xylose and *D*-galactose making up arabinoxylans and a pectin-like material. Flax seed mucilage shows weak-gel properties and can be used in food formulations in a similar way to gums like gum Arabic [5, 6]. In the following sections, flax lipids and lignans will be discussed in more detail.

2.1.1. Lipid Content

Flax seed is perhaps most well known as a source of essential fatty acids, more specifically the ω-3 fatty acid α-linolenic acid. Flax seed oil is one of the richest plant sources of α-linolenic acid (ALA). Typically 40 - 64% of the fatty acids, which make up the 40 - 52% (w/w) lipid component of flax seed are ALA [7]. Because of this, flax seed is the most prominent oil seed to be studied as a functional food [8].

ALA is a polyunsaturated fatty acid (PUFA), which is classified based on the presence of more than one double bond in its hydrocarbon backbone. ALA, an isomer of linolenic acid, has 3 double bonds, beginning at the third carbon position from the methyl terminal carbon, hence its designation as 18:3 ω3, as shown in Figure 2.1.

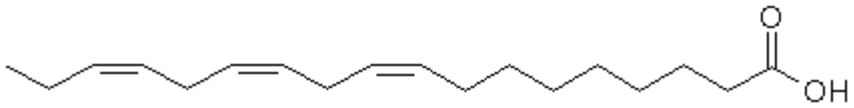

Figure 2.1: Chemical structure of α-linolenic acid

ALA is important because, as an essential fatty acid, it cannot be synthesized *in vivo*, or in the human body, and therefore must be obtained from dietary sources. However, ALA is also important as a precursor for longer chain PUFA. Through further desaturation and carbon chain elongation, once ingested and absorbed, ALA is metabolized into eicosapentaenoic acid (20:5 ω3), or EPA, and then further into docosahexaenoic acid (22:6 ω3), or DHA [9]. These two fatty acids have been investigated extensively in terms of their role in human health and are believed to play a role in many biological activities. They have been shown to be associated with decreased risk of cardiovascular disease, cancer, renal conditions, vision deficiencies, neurological disorders, fetal alcohol syndrome, attention deficiency and hyperactivity disorder, cystic fibrosis and reproductive abnormalities [10-14].

Another factor in ALA's nutritional popularity is its importance in the ω-6/ ω-3 ratio. ω-6 Fatty acids, with unsaturation beginning on the sixth carbon from the methyl terminal carbon, are found in many of the commonly consumed vegetable oils of the Western diet. The optimal ratio of ω-6/ ω-3 fatty acids is believed to be 1:1 to 3:1, as this is the ratio upon which humans evolved [10]. However, with the popularity of common and inexpensive vegetable oils in the Western diet, such as those from corn, as well as the trend towards feeding cereal crops to livestock, this ratio has changed to between 10:1 and 20:1 [10]. This ratio is important because the ω-6 and ω-3 fatty acids compete for a rate limiting enzyme, delta-6-desaturase. While ω-3 fatty acids are metabolized into EPA and DHA, ω-6 fatty acids are metabolized into arachidonic acid (AA) (20:4), and

docosapentaenoic acid (DPA) (22:5 ω-6) [11, 15]. Although both AA and EPA form eicosanoids, those formed by AA have the opposite effect from those formed from EPA. Eicosanoids from AA, at too high levels, can lead to thrombus formation, atheromas, and both allergic and inflammatory disorders [16]. Although reducing the ω-6/ ω-3 ratio back down to 1:1 could take the form of reducing the intake of ω-6 fatty acids, this is highly unlikely because of the prominence of ω-6 sources in the modern diet [10]. Therefore, it is most efficient to increase the intake of sources of ω-3 fatty acids, such as fatty fish, wild greens, and of course, flax seed.

The conversion efficiency of ALA to DHA, and even EPA is not 1:1; it depends greatly on absorption efficiency in the gastrointestinal tract, uptake and partitioning towards β-oxidation and incorporation into storage and structural pools [9, 17]. Therefore, it would seem most efficient to consume more products with DHA directly, or to supplement common foods with DHA, or even EPA. However, because of their chemistry, EPA and DHA are both highly susceptible to peroxidation, making them highly unstable [15]. Lipid peroxidation is the oxidative degradation of lipids, whereby free radicals remove electrons from the lipids forming lipid peroxides. Unsaturated fatty acids are the most susceptible, because the methylene groups, with their double bonds, have highly reactive hydrogens. EPA and DHA have 5 and 6 double bonds, respectively, or potential oxidation sites, whereas ALA has only 3.

As mentioned previously, flax oil is an excellent source of ALA, with proven health benefits of the long chain ω-3 fatty acids to be realized. The oil

itself is contained in the germ of the seed, which is covered by the shiny coat, typically dark brown in the case of higher ALA seeds (the low ALA seeds "Solin" tend to have a yellow coat). This hull contains phenolic compounds as well as other components, such as waxes, that act as natural protective barriers to oxidation. Because only ~40% of the seed is oil, a larger mass of whole seed needs to be consumed in comparison to consuming only the oil. While there are other beneficial entities in the whole seed, there are also some drawbacks to their consumption. Studies were conducted in which subjects were fed muffins made with whole flax seed, crushed whole flax seed and flax oil, and it was determined that those fed flax oil muffins had higher serum levels of ω-3 fatty acids [18]. In this same study, subjects eating the whole seeds reported cases of intestinal discomfort, including bloating and flatulence throughout the 12 week test period [18]. Although the oil muffins did cause some adverse effects initially, these effects seemed to be reduced with time [18].

Whole flax seed also contains some antinutritional factors, which would limit its use as a source of ALA, not to mention that it is not suitable to add to all formulations due its size, colour and flavour. The seed has a high fiber content, both soluble and insoluble, which may be viewed as negative by some consumers, due to its laxative and bloat-inducing effects. Other antinutritionals include phytic acid, cyanogenic glucosides, cadmium, trypsin inhibitors and linatine, an antipyridoxine factor [2].

Flax oil is not commonly used for food preparation, as it tends to be relatively more expensive than its cheaper counterparts, such as corn oil, and, as

mentioned previously, it is prone to oxidation and rancidity development. Flax oil can be obtained through cold-pressing, in which seeds are passed through a screw press to mechanically remove the oil. Flax oil can also be extracted using organic solvents, such as hexane, isopropanol or petroleum ether [4]. Supercritical carbon dioxide ($SCCO_2$) has also been proven successful in the removal of lipids from ground flax seeds [19]. With the laboratory extraction units utilized, recovery of flax oil was lower with $SCCO_2$, at the optimal extraction conditions of 70 ^oC, 55 MPa and a processing time of 3 h compared to that obtained with traditional methods. However, the level of ALA in the $SCCO_2$-extracted oil was still high [4, 19].

Once extracted, effort must be made to keep flax oil stable in order to prevent oxidation and rancidity, which can damage both the nutritional and organoleptic properties. In terms of packaging, flax oils tend to be packaged in dark, glass bottles or containers to prevent light from contacting the oil and promoting oxidation. Some oils may be packaged in opaque containers and the headspace may be flushed with nitrogen gas to remove oxygen [20]. But, once opened, such containers must be refrigerated to prevent any damage induced by heat.

Flax oils, especially those that are cold-pressed, contain natural phenolic antioxidant components, which protect the oil against oxidation [21]. Cold-pressed flax oil was found to contain phenolic acids such as *p*-coumeric, vanillic, *p*-hydroxybenzoic, caffeic and ferulic acids [21]. Flax oil contains approximately 95% triacylglycerols (TAG) and 5% minor components, including the phenolic

acids mentioned previously, tocopherols and other lipid components like sterols and phospholipids. Abouzaytoun and Shahidi [22] found that the oil, when stripped of these minor components, contained less ALA and total PUFA and more total volatiles. Although the non-stripped oil was still highly prone to oxidation, it was considerably less prone than the stripped oil. Łukaszewicz *et al.* [23] studied the fatty acid profiles of flax seed varieties as well as their susceptibility to oxidation. It was not the fatty acid profile, or the level of ALA in the oil that always determined the degree of oxidation but the endogenous antioxidants also played a role, further emphasizing the importance of these antioxidants in oil protection [23]. Of course, additional antioxidants can always be added to the oil prior to packaging, such as vitamin E or synthetic antioxidants, not only to aid those antioxidants that remain in the oil, but also to replace those that have been degraded or removed during processing.

2.1.2. Lignan Content

Lignans are phenolic, phytoestrogen compounds found in flax seed in high concentrations relative to other plant matter [24]. Although all lignans have a 2,3-dibenzylbutane backbone, there are several different forms, including matairesinol (MAT), pinoresinol (PIN), lariciresinol (LAR), secoisolariciresinol (SECO), 7-hydroxymatarisinol, arctigenin and syringaresinol, which differ in their degree of oxidation, substitution and degree of coupling [25-27]. In flax seeds, SECO is present as a glucoside, namely secoisolariciresinol diglucoside (SDG), which is linked to other SDG molecules by 3-hydroxy-3-methyl glutaric acid (HMGA) to form oligomers called lignan macromolecules, as shown in Figure 2.2 [28]. SECO

is the most efficient precursor of the biologically active mammalian lignans, enterolactone and enterodiol. Smeds *et al*. [24] found the SECO to be nearly 200 times more concentrated in flax seed than in the next most concentrated grain, wheat.

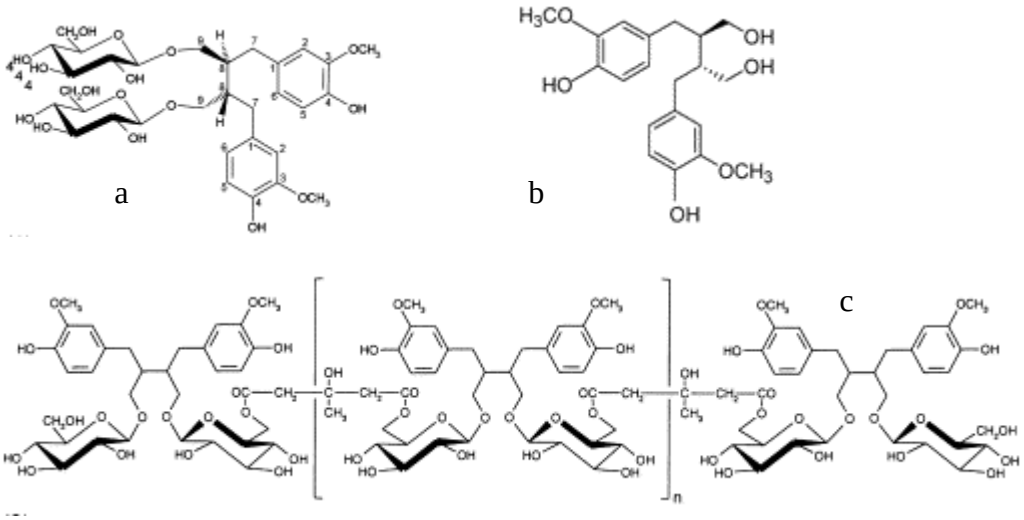

Figure 2.2: Chemical structures of : (a) (+)-secoisolariciresinol diglucoside (SDG), (b) (+)-secoisolariciresinol (SECO), and (c) SDG and HMGA oligomers in flax (n most commonly =3)[24, 28] (a) and (b) reprinted with permissions from Smeds, A. *et al*., (2007) Quantification of a broad spectrum of lignans in cereals, oilseeds and nuts. J Agric Food Chem 55:1337-1346. Copyright 2011 American Chemical Society. (c) reprinted from Journal of Chromatography A, 1012, Eliasson, C. *et al*., High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction, 151-159, Copyright 2011, with permission from Elsevier.

Lignan-containing plants have been used for hundreds of years in folk medicine, such as that of the Japanese and Chinese, to treat disorders like arthritis and ulcers [26]. The connection between lignans and their specific biological effects was not made until the end of the 20th century. SDG was first isolated and identified by Bakke and Klosterman in 1956 [29]. Since then, lignans have been

investigated, both *in vitro* and *in vivo,* and in both animal and human models. Their most studied physiological role is their inhibitory effects, at high levels, on hormonally linked cancers, such as breast and prostate, as well as colon and thyroid cancers [26, 30]. Dietary intake of lignans has also been linked to a reduction in the incidence of diabetes as well as to prevention of heart disease and other coronary events due to their ability to reduce oxidative stress [26]. In addition to these well studied relationships, it has been suggested that SDG has reno-protective and hepato-protective functions [26].

Lignan extraction methods traditionally involve solid-liquid extraction using large volumes of polar organic solvents. Samples are then hydrolyzed with acid, base or enzyme, or through feacal micro flora fermentation [30]. One example of enzymatic hydrolysis, which is a less common method, utilizes β-glucuronidase/sulfatase from the purified juices of *Helix pomatia* [31, 32]. These methods, however, are not considered optimal as they require long extraction times, are labor intensive and generate waste potentially harmful to the environment [33]. Recently, efforts have been made to use alternate protocols for the extraction of lignans from flax and other plant materials, including the use of pressurized water, microwave-assisted extraction and supercritical fluid extraction [33-38]. Lignan yields vary greatly between extraction methods and parameters employed and the use of different analytical techniques adds to variability in the results. According to a review by Muir [30], flax seed SDG levels reported in the literature ranged from 1 to 25.9 mg/g of whole seed.

2.2. Delivery of Bioactives

One method of protecting flax oil from degradation is microencapsulation. This technique, employed in the food industry for more than 60 years, is used to protect a variety of delicate substances, such as essential oils, proteins, flavours and fatty acids [39]. Microencapsulation consists of packaging a core material- solid, liquid, or gas- within a coating material. Capsules can take the form of a matrix where the core is dispersed within the coating material, or a shell where the core is grouped in the middle with the coating layer surrounding it. The coating material can be composed of a wide variety of food-grade materials, including carbohydrate polymers, proteins or carbohydrate-protein blends, surfactants or fats. Also, the size range of capsules can vary, with those being less than 100 nm deemed as “nano-capsules” and those greater than 100 nm “micro-capsules” [40].

If the correct shell or matrix material is selected, the capsule can act as a barrier to environmental conditions, including oxygen, moisture, heat, pH and light. However, encapsulation can serve many purposes beyond simply the protection of the core compound during processing and storage. It can also allow for the dispersion of an insoluble core ingredient within a solvent (i.e. water for food applications). Similarly, it can convert a liquid product, such as oil, into a dry powder product, which can easily flow [40]. In addition, if particular bioactive core ingredients are required to reach a particular part of the digestive tract without digestion, encapsulation can assist in controlling their release. If the shell or matrix is selected or engineered properly, then specific environmental factors at the target site, such as pH, shear, temperature or enzyme action, can trigger the

core release. Similarly, encapsulation can control the rate of release, as in the case of chewing gum flavours or “long-acting” medications, or prevent reactive species from making contact as in the case of leavening agents [39, 40]. If a core ingredient has an unpleasant flavour or odour that the manufacturer does not want to be associated with a product, the shell can also help mask these unpleasant properties.

There are a variety of different structures or forms that a microcapsule can take. For example, a single core capsule is the simplest construction of a core-shell capsule, in which a single layer or a single type of shell material coats the active core. This design can be further complicated by adding layers of different coating compounds, to make a multi-layer capsule. Such capsules may be effective if the environmental conditions are such that a single type of material cannot prevent core exposure or if more complicated timed release is required. Besides having multiple shell materials, a capsule can also contain multiple cores. A dispersed core capsule contains multiple particles of the core material dispersed or spread out within a matrix, such as a polymer gel.

Techniques employed to form microcapsules vary greatly and depend on the qualities of the shell or matrix and the core, as well as the intended function of the final material. Encapsulation techniques include spray drying or cooling, freeze drying or lyophilization, fluidized bed, extrusion, coacervation, co-crystalization and the formation of liposomes, emulsions, biopolymeric particles and glassy matrices [39, 41].

Spray drying is by far the most frequently used method of food grade encapsulation, as well as one of the oldest methods [42, 43]. Spray drying has been used extensively for ω-3 PUFA, from flax oil to fish oil and other marine lipids [44-50]. Although the process is technologically simple, there are many factors that must be considered when dealing with the starting materials, processing parameters and the resulting powders. Because ω-3 PUFA are hydrophobic, and the majority of dispersions and solutions used in the food industry are aqueous, an emulsion must be formed including the solvent, the coating material, the oil, and, in the majority of situations, an emulsifier. The size distribution of the emulsion droplets is critical in increasing the encapsulation efficiency and limiting the amount of surface oil. Ideal encapsulation requires that all the oil originally in the emulsion is encapsulated or embedded in the carrier material. Surface oil is not only prone to oxidation, causing off odours and flavours and possibly setting off a cascade of oxidation reactions, but it also lowers wettability and dispersability, which are two important quality parameters for food-grade powders [46]. Small and stable emulsion droplets are favoured. When using maltodextrin and either whey protein concentrate or modified starch, Hi-Cap 100, to encapsulate fish oil, Jafari *et al.* [46] found that the Hi-Cap 100 was a better emulsifier as the particles tended to have lower surface oil coverage than those particles, which included the whey protein concentrate. The technique for emulsion formation was also important, with microfluidization forming more favourable particles than ultrasound and Silverson rotor-stator emulsification [46].

Drusch *et al.* [51] attributed low particle stability to the composition and properties of the oil-water interface of the parent emulsion.

The properties of the surface material are also extremely important in terms of resulting particle stability, beyond just their role in forming the emulsion. The coating material can be, and has been, composed of a variety of ingredients, such as gelatin, maltodextrin, starch, plant gums, skimmed milk powder, whey proteins, soy proteins and modified cellulose [40, 42]. Some coatings, such as carbohydrates and other biopolymers, exhibit a type of phase transition, where above a certain temperature, the material becomes rubbery and sticky, but below that temperature, the material takes on a glassy form. It is very important that this transition temperature, referred to as the glass transition temperature, is high enough so that the resulting powder will exhibit a glassy state. In this state, the coating material is less permeable to oxygen, limiting oxidation reactions, and will allow the powder to flow freely, without sticking together. However, in a glassy state, coatings can also become more brittle and fragile, requiring extra care during processing and handling.

2.3. $SCCO_2$ Processing

CO_2 can be used as a solvent for plant components if its temperature and pressure are increased beyond their critical values, 31^oC and 7.4 MPa, respectively [52]. At conditions above the critical point of CO_2, it exists as neither a liquid nor a gas. $SCCO_2$ possesses favourable properties for mass transfer, as it has gas-like viscosity and diffusivity, but a liquid-like density [53]. $SCCO_2$ solvent power has been hypothesized to be a result of a structured first

coordination shell that prefers an equatorial configuration, as well as a nonzero instantaneous molecular dipole moment and an enhanced quadrupole moment [54]. There is a partial negative charge on the oxygen molecules and a partial positive charge on the carbon, and $SCCO_2$ can act as a Lewis acid or a Lewis base [55]. Therefore, site-specific interactions between CO_2 and solvent molecules can exist, and hydrogel bonding can occur with proton donor systems [55]. $SCCO_2$ is a favourable solvent, as it is non-toxic, non-flammable, inexpensive and easily separated from the extract upon depressurization [56, 57].

2.3.1. $SCCO_2$ Extraction

According to the $SCCO_2$ expert, Prof. Gerd Brunner, $SCCO_2$ extraction from solids is "by far the most important industrial application for supercritical fluids, primarily CO_2" [58]. Supercritical fluid extraction has been used for the extraction of oils and long chain alcohols (policosanols) from flax seed [19, 59]. The extraction of compounds of two different natures, non-polar oils, and polar phenolic compounds, is described in Section 2.3.1.1.

Different models exist for extraction of a solute from a matrix using $SCCO_2$ because of differences in matrix characteristics, flow distribution and flow direction [60]. However, most models consider the matrix as a porous sphere, including the shrinking-core model and the desorption-dissolution-diffusion model [61]. There are several stages in the extraction process. The first stage is known as the constant rate, or solubility controlled, stage, followed by 1 or 2 falling rate stages, which are controlled by diffusion and desorption [60, 62]. Extraction begins with diffusion of $SCCO_2$ into the pores of the matrix, and

adsorption of $SCCO_2$ on the fluid-matrix interface. The solute of interest must then dissolve or desorb into the $SCCO_2$ phase and then diffuse through the matrix where it is transported into the bulk fluid. The extraction rate will decline after solute freely available on the surface is depleted and those located in the interior parts of the solid particle require more time to reach the $SCCO_2$-matrix interface [62].

2.3.1.1. Processing Parameters

There are many processing and sample preparation variables involved in supercritical fluid extraction that will affect the final yield, including temperature and pressure, which control solubility. Therefore, temperature and pressure can be increased to alter the selectivity of the solvent. Generally, as pressure increases, so too does solubility, because the density of the solvent, and therefore its solvent power, is increased. Increasing density increases solubility because of intermolecular forces due to the tight packing of $SCCO_2$ molecules around the solute molecule [53]. The effect of temperature on the solubility behaviour is slightly more complex, as it depends on solute vapour pressure and solvent density changes. Below a certain pressure, an increase in temperature will reduce the solubility, whereas above this pressure, solubility is increased. This is due to the crossover of solubility isotherms. The pressure at which this cross-over occurs differs depending on the solvent used (e.g. the addition of modifiers, or co-solvents, to $SCCO_2$) and the solute properties. Maximum solubility is achieved at a temperature, which allows a compromise between the effects of both density and solute vapour pressure [53].

Solvent flow rate is another variable that will affect extraction yield. Although low flow rates will allow sufficient time for the solvent to become saturated, they make the process less time efficient. However, if the flow rate is too high, saturation will not be achieved and large quantities of CO_2 will be consumed. Extractions can be carried out over long or short time periods; however, time must be optimized to achieve maximum yield in the shortest possible time period. Other variables that will affect an extraction outcome include sample preparation steps, such as moisture content and particle size, as these affect mass transfer of solvent and solute to and from the plant matrix. When particle size is reduced through crushing or grinding, the outer layers of the particle matrix are damaged. Therefore, solute on these surfaces becomes easily accessible for the solvent during extraction, while solute in the core of the matrix has to diffuse to the particle surface, which is a much slower step due to the necessity to permeate the cell walls [60]. Effective extraction using supercritical fluids, therefore, requires extensive process optimization.

$SCCO_2$ is ideal for nonpolar entities, such as lipids, as it is nonpolar in nature. However, co-solvents, such as water and ethanol, can be added at low levels to alter the polarity of CO_2. Co-solvent addition is another process optimization variable. With the addition of these polar co-solvents, it has been shown that $SCCO_2$ is able to solubilise polar components such as phenolic SDG [37]. Co-solvents can assist in $SCCO_2$ extractions due both to their ability to increase solvent density and to the possibility of intermolecular interactions between the co-solvent and the solute of interest [57]. Also, co-solvents can cause

swelling of the matrix, which enhances mass transfer due to increased contact of $SCCO_2$ with the solute [63]. If present, intermolecular interactions, such as hydrogen bonding, between the co-solvent and the solute will lead not only to an increased solubility of the solute, but also to an increased selectivity of the solvent [57]. This increase in selectivity will decrease the need for additional isolation and purification steps necessary post-extraction. Hydrogen bonding has been found to occur with the use of ethanol, methanol and water [57]. If a co-solvent only has an effect on density, then only yield will be increases, and not selectivity [57]. The effect of a co-solvent, which must be miscible with the $SCCO_2$ phase, is dependent on its concentration [57]. Water and ethanol are generally considered to be safe for use as solvents in food-grade extractions. However, their presence does necessitate a separation step for their removal post-extraction. When water and ethanol are added to $SCCO_2$, electron donor-acceptor complexes form at an increased rate and the CO_2 shows significant deviations in its linear geometry [64, 65]. Also, in the presence of water, hydrogen bonding between the water and CO_2 molecules occurs, and the dipole moment of water is increased [64].

2.3.1.2. Oil Extraction

It is evident when reviewing the $SCCO_2$ literature that oils are the most common extraction targets. The extraction of specialty oils from plant matter has been reviewed several times recently [66-68]. Oils are mainly composed of non-polar triglycerides, but also contain mono- and diglycerides, free fatty acids, and other minor components. Specialty oils, such as those from nuts and seeds, are especially of interest, as they tend to be of high value, yet low volume. Their

value stems from their high concentration of bioactive components, which are known to confer health benefits, as well as from their unique sensory profiles [68]. Because of the low critical temperature of CO_2, this unique profile and bioactivity can be maintained more effectively, relative to high temperature solvent extraction methods.

Because they are mainly non-polar, edible oils are typically extracted with non-polar organic solvents such as hexane through solid-liquid extraction. However, these operations expose the oil to high temperatures both during the extraction and during the removal of any residual solvent. Hexane is also facing more stringent government regulations due to safety concerns - both environmental and in terms of human health [68]. In laboratory scale, Soxhlet extraction using hexane poses risks due to its flammability and toxicity. In terms of the extracted oil quality, hexane tends to be less selective than $SCCO_2$, and exposes the prized mono- and polyunsaturated fatty acids of specialty oils to adverse temperature conditions, causing degradation, such as oxidation, and rendering them biologically ineffective [67].

Considering the fact that triglycerides are non-polar components, oil extractions using $SCCO_2$ are, for the most part, performed without the use of a co-solvent. However, depending on the composition of the oil that is being targeted, as well as the nature of the interactions between the oil and the matrix, co-solvents, mainly ethanol, have been used in some recent studies [69-73]. Because the addition of ethanol to $SCCO_2$ increases the recovery of more polar species, ethanol has been used where oils high in antioxidants and unsaturated fatty acids

are targeted. For example, Mezzomo *et al.* [69] utilized ethanol at levels of 2 or 5 wt% to extract peach seed oil and showed that the addition of ethanol, especially at the higher level increased the phenolic content of the oil. As well, Sanchez-Vecente [70] found that the use of ethanol at 2.5 or 5 mol% increased the yield of oil from peach seeds. Mezzomo *et al.* [69] reported that when ethanol was used at the highest levels the oil yield obtained was similar to that of solvent extraction, where a yield of 24% oil was obtained with 5 wt% ethanol addition at 50 ^{o}C and 30 MPa compared to 25% oil with Soxhlet, while Sánchez-Vicente *et al*. [70] extracted 32% oil using $SCCO_2$ with 5 mol% ethanol and 48% with Soxhlet. When ethanol at levels of 5, 10 or 15 wt% was used in the extraction of oil from shiitake mushroom, the yield increased 6-fold in the presence of 15% ethanol compared to neat-$SCCO_2$ under the same conditions [73].

Studies on oil extraction generally show typical extraction patterns in terms of temperature and pressure dependence. As pressure is increased at a constant temperature, and $SCCO_2$ density increases, the extraction yields, and often the rate of the initial extraction period, increase as well. For safflower oil extraction, an increase in pressure from 22 to 28 MPa, at a constant temperature, increased lipid yield 3-fold [74]. Similarly, for coconut oil, increasing the pressure from 20.7 to 34.5 MPa increased yield by 95% [75]. In terms of oil quality, Passos *et al.* [76] found that changing extraction conditions did not affect the fatty acid or triglyceride profile of $SCCO_2$ extracted grape seed oil. Increases in pressure resulted in higher phenolic contents of peach almond oil [69]. In contrast,

decreasing the pressure resulted in cantaloupe seed oil with higher anti-radical activity [77].

Cross-over of solubility isotherms was exhibited in the majority of oil extractions, with cross-over pressure differing, depending on the oil source and composition. For shiitake oil extraction, the cross-over pressure was between 15 and 20 MPa and for Inca peanut it was between 30 and 40 MPa [73, 78]. Some studies, however, did not reveal cross-over behaviour, possibly because the pressure and temperature ranges investigated were not broad enough. For instance, for safflower oil, it was found that decreases in temperature increased extraction rate and total yield within the pressure range of 22 to 28 MPa [74]. This would indicate that a cross-over pressure may exist above 28 MPa, where an increase in temperature would result in an increase in extraction yield. It is important to note that cross-over behaviour refers to solubility isotherms and not to total yield. This is often confused. If allowed to continue to completion, extraction curves (yield vs. time or amount of CO_2 used) at differing extraction temperature and pressures would eventually merge asymptotically approaching 100% recovery. For instance, it was reported that, for pomegranate seeds, the increase in yield with temperature above 32 MPa was due to cross-over behaviour [79]. For virgin coconut, cross-over behaviour is reported at pressures just above 28.3 MPa [75].

Several studies attempted to optimize the CO_2 flow rate to be used in an extraction. Flow rate is, however, found to have mixed effects. Increasing the flow rate had a significant positive effect on the oil yield from pomegranate seeds

[79]. For yellow horn oil and prickly pear seed oil, increasing flow rate increased yield only to a certain level, above which further increases had no significant effect [80, 81]. These levels corresponded to 12 and 10 kg CO_2/h for the yellow horn and prickly pear seed, respectively. On the other hand, although increasing the CO_2 flow rate increased the yield during the fast extraction period for sesame, at the completion of the 2 h extraction there was no significant difference in yield [82]. In contrast to the above studies, Zhang *et al.* [83] found that increasing the flow rate from 5 to 15 mL CO_2/min (basis not indicated) decreased yield. These results indicate that the effects of flow rate depend on the solubility or saturation of the $SCCO_2$ with the oil, and the availability or ease of mass transfer of the oil to the $SCCO_2$ phase. Increasing the flow rate decreases the contact time between the supercritical solvent and the solid matrix and thus saturation limit may not be reached.

Material preparation was found to have an effect on $SCCO_2$ extraction of edible oils. Generally, a reduction in particle size caused either an enhancement in yield and/or rate, or had no influence on yield. For sesame and safflower seeds, decreasing particle size increased overall yield, while for Inca peanut and yellow horn, changes to particle size did not result in significant effects [74, 78, 81, 82]. Zhang *et al.* [83]and Passos *et al.* [84] both applied unique pretreatments in their studies with almonds and grape seeds, respectively. When almonds were pretreated by autoclaving, the oil yield increased by 6.44% compared to non-autoclaved almonds [83]. Autoclaving increased the availability of the oil to mass transfer, while having no effect on the fatty acid profile of the extract. When grape

seeds were treated with an enzyme cocktail, including cellulase, hemicellulase, pectinase and protease, yield of grape seed oil was increased from 11.5% for untreated seeds to 16.5% for treated seeds [84]. Once again, the increase in yield suggests that the treatment liberates more oil from the matrix, allowing higher loading of the $SCCO_2$.

For the most part, solvent extraction, such as Soxhlet, has been reported to result in higher edible oil yields when compared to $SCCO_2$ under the conditions investigated. For instance, when the $SCCO_2$ extraction of flax seed oil at 50 ^oC and 30 MPa is compared to solvent extraction, solvent extraction resulted in a 38.8% yield, while $SCCO_2$ yield was only 35.3% [85]. However, increasing the extraction pressure, temperature and solvent-to-feed ratio should result in higher yields. $SCCO_2$ extraction did, however, result in a higher yield than expression of oil by screw press, which only yielded 25.5% oil. Although many studies reported no significant difference in terms of the fatty acid profile of solvent-extracted vs. $SCCO_2$-extracted oils, for both flax seed and yellow horn, $SCCO_2$ extracts had higher concentrations of poly- and monounsaturated fatty acids [81, 85]. $SCCO_2$ extracts of pomegranate seed oil also contained higher tocopherol levels, suggesting that $SCCO_2$-extracted oil may have more natural antioxidants present, and therefore may be of higher quality [79].

It is recognized that $SCCO_2$ may not be the ideal supercritical solvent to use for lipid extractions, and that propane would be more efficient due to higher lipid solubility in propane. However, due to obvious safety issues and the GRAS (generally recognized as safe) status of CO_2, propane is not commonly utilized in

the extraction of edible oils. When the extraction of sesame oil using supercritical propane and $SCCO_2$ was compared [86], propane did result in a faster extraction and it was concluded that propane was, indeed, a better solvent in terms of lower pressure needed and time.

2.3.1.3. Extraction of Phenolic Compounds and Lignans

Phenolics are a group of diverse compounds, which are distributed ubiquitously throughout plant cells. Characterized by an aromatic ring substituted with at least one hydroxyl moiety, phenolics are secondary metabolites, which are formed when the plant experiences stress, such as infections or wounds. For example, resveratrol is produced in plants, like the grape, in response to a fungal infestation [87]. Derived from phenylalanine and tyrosine, phenolics include simple phenols, phenolic acids derived from both cinnamic and benzoic acids, coumarins, flavonoids, stillbenes, tannins, lignans and lignins [88].

Apart from their protective functions in plants, phenolic compounds serve a wide array of desirable functions within food systems, and within the human body, once ingested. Because of their structures, phenolic compounds act as antioxidants and can protect fats and oils, both in processed and unprocessed foods, from deterioration. Such deterioration would result in rancid odours and flavours and the potential formation of toxic by-products, leading to decreased consumer acceptability. Phenolics can protect foods from free radicals and can quench singlet oxygen. Natural antioxidants are gaining growing interest in the food industry, as they can replace synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and

tert-butyl hydroquinone (TBHQ). These synthetic antioxidants are falling out of favour with consumers and producers alike, as the push for “natural”, traceable food ingredients and the concerns about the safety of synthetic compounds increase.

Within the human body, phenolic compounds can protect vital biological molecules, such as DNA, lipids and proteins from damage, reducing the risk for cancer and other degenerative diseases [89]. Many individual phenolics are suggested to have unique health properties beyond just their action as antioxidants. For instance, caffeic acid phenethyl ester and 3,5-diprenyl-4-hydroxycinnamic acid from propolis have been shown to inhibit colon cancer cells [90]. Also, resveratrol, found in grapes and red wine, has been associated with improving cardiovascular health, among many other benefits [87].

Because of their diverse structures, beyond the inclusion of the phenolic ring, the solubility behaviour of phenolic compounds is also greatly varied. Phenolics can be highly polymerized, as is the case with tannins, or they can be complexed with other molecules, such as carbohydrates and proteins, increasing their molecular weight and limiting their solubility. Phenolics, which exist within the cell wall tend to be joined with other cell components of higher molecular weight and are therefore less soluble than those, which exist within the cell vacuole [88, 91].

As expected, extraction techniques for phenolics depend greatly on the chemical nature of the target compound(s). Because they are relatively polar, more polar solvents are typically used, including alcohols like ethanol, methanol

and propanol, water, acetone, ethyl acetate, and dimethylformamide [88]. Mixtures of solvents are also quite commonly used. Due to the strong associations, which are possible between phenolics and other cellular components in the plant or food matrix, long extraction times, typically ranging from 1 to 24 h, and elevated temperatures are required. Alkaline, acid or enzymatic hydrolysis may be necessary to liberate the target molecules from the matrix or to remove associated compounds, such as carbohydrates, liberating the free phenolic.

$SCCO_2$ as a solvent preferentially solubilizes non-polar, low molecular weight compounds and is not well suited for phenolic extraction, especially those phenolics, which are highly polymerized or associated with other cellular components. Solubility of most phenolics is very low in $SCCO_2$, regardless of temperature and pressure. To improve the solubility of relatively polar compounds, co-solvents are heavily relied upon and GRAS solvents, such as water and ethanol, are most frequently used. Diaz-Reinoso *et al.* [89] suggested that 2% ethanol (basis not indicated) in $SCCO_2$ will assist in the extraction of low molecular weight phenolics, 5% will extract catechins and epicatechins, 10% will extract epicatechin gallate and 15% will aid in proanthocyanin extraction. Depending on the complexity and structure of the target compound, increasing the concentration of ethanol will decrease the selectivity of $SCCO_2$. If the starting material is high in lipids, removal of these lipids, possibly with neat $SCCO_2$, prior to utilizing $SCCO_2$ plus a co-solvent may be beneficial for the recovery of phenolics and increase the ease of analysis.

Despite the low solubility of phenolics in $SCCO_2$, such extractions have been investigated quite extensively, especially in recent years. Solid-liquid extractions, which are traditionally utilized for phenolic compounds have several drawbacks. As discussed previously, there are concerns about the solvent residues in the extract, which are removed typically using heat. The heat involved during extraction and subsequent solvent removal in traditional solvent extractions can lead to degradation of the phenolic compounds through oxidation, eliminating their beneficial properties. $SCCO_2$ does not leave behind any solvent residues, especially if a co-solvent is not used. However, addition of co-solvents like ethanol adds to the challenge of solvent removal following extraction. $SCCO_2$ extraction can be performed using mild temperature conditions and minimize phenolic degradation, allowing the retention of their biological activity.

Based on a review of the $SCCO_2$ extraction of phenolics, it is evident that the most important processing parameter is the use of a co-solvent. Although ethanol is the most popular co-solvent, water and ethyl acetate were also used [92, 93]. Generally, an increase in ethanol concentration resulted in an increase in the extraction rate and yield of phenolic compounds. In some studies, however, it was evident that an increase in ethanol level was only useful in increasing the phenolic yield up to a certain level. After this level, further increases in ethanol did not significantly increase yields. For example, with the extraction of flavonoids from propolis, it was found that increasing ethanol from 2 to 5 wt% increased yield, while further increases to 7 wt% resulted in no further significant improvement [94]. Not only did ethanol addition increase yield, but it also had effects on the

antioxidant activity of rosemary phenolics. Carnosol, carnasoic acid, caffeic acid and *p*-coumeric acid were all extracted using $SCCO_2$ modified with 7% ethanol (basis not indicated), and the addition of ethanol not only significantly enhanced extract yield, but it also increased activity [95]. However, studies on antioxidant activity were limited for laboratory scale extractions and extractions had to be performed on a pilot scale to generate sufficient quantities needed for various assays. Ethyl acetate was not as effective as ethanol as a co-solvent, both at 10 wt% levels, for the extraction of phenolics from guava seeds [92]. Furthermore, although ethyl acetate addition enhanced the recovery of 3,5-deprenyl-4-hydroxycinnamic acid from propolis compared to neat-CO_2, it also decreased purity, necessitating further clean-up or purification steps, which are obviously not desirable. Although the benefit of $SCCO_2$ over solvent extraction, in terms of the volume of solvent used and the necessity of removing residual solvent may be questionable when ethanol and other co-solvents are required in extraction, there is still a benefit, despite co-solvent use. In the extraction of phenolics from rice wine lees, it was found that the use of ethanol increased phenolic yield over the use of neat-CO_2 [96]. Although the amount of phenolics in the $SCCO_2$ extract was only 43% of that obtained in Soxhlet extraction using ethanol, only 10% of the amount of ethanol used in Soxhlet extraction was used for the modification of $SCCO_2$.

Temperature during $SCCO_2$ extraction of phenolics is usually kept low, so as to prevent the degradation of functional groups. Therefore, most studies were performed at temperatures below 60 ^oC, although some studies also evaluated

higher temperatures. Once again, similar to lipids, phenolic compounds displayed cross-over behaviour of their solubility isotherms. In general, the cross-over pressure was between 20 and 25 MPa. For stillbenes and flavones from pigeon pea, phenolic fractions from guava seeds and phenolic fractions from cashew nut liquid, below this pressure range increases in temperature decreased yield, while above this range, the yield increased with temperature [92, 97, 98]. In terms of pressure, most studies reported that increases in pressure increased solubility and therefore extraction yields of phenolics and antioxidants. However, an increase in pressure also decreases selectivity and therefore reduces the purity of extracts, which has implications in terms of their biological activity [99, 100].

Multi-stage extractions, or cycles of pressurization-depressurization, are more prevalent with $SCCO_2$ extractions of phenolics and polar compounds compared to lipid-soluble compound extractions. For example, rosemary, sage, thyme, hysopp and winter savory phenolics were extracted using a two-stage extraction [101, 102]. In all cases, the first stage was performed at a lower pressure than the second stage. For the herbs rosemary, sage, thyme and hyssop, the first extraction was performed at 40 ^{o}C and 11.5 MPa, and was intended to remove essential oils prior to the 100 ^{o}C and 35 MPa extraction of phenolic antioxidants [101]. The antioxidant activity of these extracts was comparable to that of the synthetic antioxidant BHA. For winter savory, the first stage was performed at 40 ^{o}C and 9 MPa, while the second stage was at 50 ^{o}C and 25 MPa [102]. The second stage was more efficient than the first in terms of vanillic acid, protocatechuic acid and (+)-catechin extraction. Multiple pressurization-

depressurization stages were used to enhance the extraction yield of phenolic compounds from cashew nut shell liquid [98]. It was found that these pressure cycles facilitated the removal of compounds from the shells, improving yield. The enhancement of extraction yield resulting from pressurization-depressurization cycles is well noted in the literature, which makes such a $SCCO_2$ treatment a good step prior to extraction.

Literature reveals minimal information on the use of $SCCO_2$ in relation to the extraction of lignans from flax seed. It is primarily used as a preparatory step to remove the lipid fraction prior to a solvent extraction and hydrolysis. Harris and Haggerty [93] investigated $SCCO_2$ modified with tetrahydrofuran, water and acetic acid to extract lignans and phenolic acids from flax seeds. This work, however, was done in an attempt to develop a rapid assay procedure to demonstrate stability and was not intended to extract the lignans for further use in food systems since tetrahydrofuran is not a food-grade solvent. Also, no indication of process optimization procedures was given and details of extra protocols were not provided.

Although $SCCO_2$ has not been utilized to any great extent to extract lignans from flax seed specifically, it has been used to extract lignans and related phenolics from plants such as *Schisandra chinensis* [34, 37]. Sovova *et al.* [37] examined the effects of temperature (40, 50 and 60^{o}C), pressure (20, 25 and 27 MPa), solid feed (4.4 to 5.5 g), CO_2 flow rate (0.4 to 0.8 g/min) and ethanol concentration in $SCCO_2$ (2 or 4 ± 1 wt%) on the extraction yield of lignans from the stems and leaves of *Schlisandra chinensis*. The solubility of lignans in the

modified $SCCO_2$ was 0.4 to 0.6 g/100 g CO_2 [37]. The optimum extraction temperature and pressure were 50^oC and 27 MPa, respectively, with 2 to 4% ethanol addition increasing the rate, but not the yield of extraction [37]. Choi *et al.* [34] varied the temperature (40 to 80^oC) and pressure (13.6 to 34 MPa) of $SCCO_2$, without the use of a polar co-solvent. The optimal extraction conditions were 60 ^oC and 34 MPa, although temperature and pressure changes had only a minor influence on yield. Of the five lignans investigated, schisandrol A and B and schisandrin A, B and C, those with a hydroxyl group were less soluble than those with only a hydrogen attached to their main ring [34]. This is because the H moiety is less polar, and therefore more soluble in the nonpolar solvent, than the component with the OH constituent. At the optimum conditions, schisandrin B had the greatest yield, and total lignan yield was 80% of that obtained by methanol extraction, but superior to those obtained using chloroform-methanol, *n*-hexane and petroleum ether [34]. It is important to note that although studies show that methanol addition improves polar component extraction; it also tends to extract more unwanted polar compounds with the lignans. The chemical structure of SDG has hydroxyl groups (Fig. 2.2), indicating that its extraction may be increased with the addition of a polar co-solvent [28].

2.3.2. Delivery of Bioactives Using Supercritical Technology

2.3.2.1. Particle Formation Technologies

A significant advantage of $SCCO_2$ technology from a post-extraction processing point of view is the ability to form small particles from the extract, on the micro or even nano scale. There are several methods of particle formation

using $SCCO_2$, including rapid expansion of supercritical solutions (RESS), supercritical anti-solvent (SAS) processes and particle formation from gas-saturated solutions or suspensions (PGSS) [103]. Particles formed can then be further encapsulated or coated to alter or enhance their functionality.

The basis of $SCCO_2$ particle formation is the solubility differential between the extraction chamber and the expansion or particle formation chamber. In the extraction chamber, the solubility of the solute in the solvent (CO_2) is very high due to the high pressure and temperature combination employed to reach the supercritical state. However, the expansion chamber is at a much lower pressure, often atmospheric, and the solution is no longer in the supercritical state. CO_2 makes a transition from the supercritical state, with high solvent power, to a gaseous state, which has relatively very low solvent power for the higher molecular mass solute. Therefore, the solution expands, the gas dissipates and the solute is precipitated out.

Particle formation from supercritical solutions has been well reviewed in literature, including publications by Weidner [104], Jung and Perrut [103], Knez and Weidner [105] and Shariati and Peters [106]. This technology has primarily been used for polymer formation and pharmaceutical delivery systems, as it enables small, controlled particle size without solvent contamination.

2.3.2.2. $SCCO_2$ Impregnation

Because of the unique properties of $SCCO_2$, it is becoming a popular solvent for polymer processing. By exposing polymers to $SCCO_2$, it has been found that they can undergo substantial changes, including a reduction in

viscosity, interfacial tension and glass transition temperature and an increase in permeability and swelling. The effects of $SCCO_2$ on polymeric materials have been reviewed previously [107-109].

By combining the solvent power of $SCCO_2$ and its effects on polymeric compounds, $SCCO_2$ impregnation can be achieved. In the supercritical impregnation process, a polymeric material is first contacted with $SCCO_2$, then $SCCO_2$ containing the target solute for impregnation is introduced to the $SCCO_2$ plasticized or porous polymer. The small size of CO_2 molecules allows for increased diffusion of the solute. Once the impregnation process has been carried out, the $SCCO_2$ is then released, trapping solute molecules, which remain in the polymer [109]. In such a process, the affinity of the solute to $SCCO_2$ vs. the polymer or partitioning of the solute between the two phases is obviously very important.

There are two common methods of impregnation known as dynamic and static methods. In the flow mode or dynamic impregnation, the amount of solute, or active component, impregnated depends on the solubility of the solute in the flowing $SCCO_2$ phase and the partition coefficient of the solute between the $SCCO_2$ and polymer matrix, similar to chromatography. If the solute is highly soluble in the $SCCO_2$ phase at the conditions of operation, as was the case for trifusal in polymethyl methacrylate (PMMA) at 20 MPa and 35 ^{o}C, then much of the solute will be carried out of the cell with the $SCCO_2$ [110]. The second method is a stagnant, or static, approach, where the cell is sealed for a defined

time period, allowing diffusion of the solute into the matrix, prior to a slow depressurization.

Just as in solubility or extraction processes, the temperature, pressure and the use of a co-solvent determines, in part, the impregnation efficiency of compounds in a matrix. This is again due to the solubility of the compounds in $SCCO_2$ vs. their affinity to the carrier material. In most studies, pressure is kept quite low, relative to extraction processes. Costa *et al.* [111] found that the impregnation efficiency of flurbiprofen into soft contact lenses was most efficient at pressures below 11 MPa, although the process was still feasible at higher pressures. Similarly, for organic biocides, as pressure was increased from 8 to 15 MPa, the retention ratio in a sawdust packed column was decreased [112]. Because CO_2 density decreases with temperature, increasing temperature from 40 to 50 ^{o}C, at relatively low pressures, also increased biocide retention [112]. The effects of increasing pressure and temperature were also found for Avicil PH 101 pellets impregnated with ibuprofen [113].

While the solubility of the solute in $SCCO_2$ is important for impregnation efficiency, so too is the nature of the polymer itself. While physical entrapment of a solute can occur, polymer-solute chemical interactions also take place. Kazarian *et al.* [110] found that, for azo dyes disperse red 1 (DR1) and 4- (diethylamino)-4’ nitroazobenzene (DENAB), the impregnation efficiency of DR1 in PMMA was greater than that in DENAB due to hydrogen bonding between DR1 and PMMA but not between DENAB and PMMA. For contact lenses formed from different polymers, it was found that not only did ethanol addition increase the solubility of

flurbiprofen in $SCCO_2$ but that ethanol also interacted with hydroxyl groups on the contact lens polymers increasing free volume, swelling, plasticization and diffusion [111].

2.4. Aerogels

While $SCCO_2$ is able to swell and plasticize polymers, as well as impregnate solutes within them, as described above, it can also modify polymer gels creating new structures with unique properties. Aerogels, with a bulk density range of 0.004 - 0.5 g/cm^3, are formed when the pore liquid of a polymer gel is removed and replaced with air, without a significant change in the structure of the original pores [114]. When wet gels are dried conventionally, under atmospheric pressure with the addition of heat, the evaporation of the pore liquid causes capillary forces, which act on the pore walls causing them to collapse. Dry gels end up with a high density due to significant shrinkage.

In the 1930's, Kistler discovered that when the pore liquid is removed under supercritical conditions, much of the original pore structure and polymer arrangement could be maintained [115]. In the supercritical state, there is a reduction in surface tension, leading to diminishing capillary forces. The solvent, now a supercritical fluid, can easily be replaced with air upon depressurization. Supercritical drying is certainly not the only method of producing an aerogel, as other methods, such as freeze drying and vacuum drying have also been investigated [114].

Since Kistler's discovery of a new use for supercritical fluids, aerogels have been formed using a variety of inorganic and organic polymer gels, with

SiO_2 being one of the most popular choices. Aerogel formation has been reviewed previously, including their chemistry, drying, properties and uses [114, 116-121].

2.4.1. Polysaccharide Gels

Given the ability of many polysaccharide polymers to form a gel, it is possible for a renewable, food-grade aerogel to be produced using a gelling polysaccharide. While silica is often used for drug delivery applications and is biocompatible, it is not biodegradable, a characteristic polysaccharides possess. Polysaccharide polymers are chains of homogeneous or heterogeneous sugar monomers, which can be linear, in the case of cellulose or amylose, or branched, as is the case in amylopectin. In an aqueous environment, many polysaccharides will form a physically or chemically cross-linked network, trapping water between the polymer chains.

The soluble fiber fractions of many grains are composed of gelling polysaccharides, which are currently underutilized, but could be extracted for use in aerogel formation. Flax, as mentioned previously, contains a mixture of polymers known as mucilage, which have weak gelling properties [6]. β-Glucan, part of the soluble fiber fraction of barley and oats, will also form a gel with water [122]. Exploitation of such polymers for use in aerogels will not only provide a use for potential waste-stream components, but will also add further value to important Canadian crops.

Several polysaccharide gels have been investigated in terms of their potential for successful aerogel formation and they will be described in more detail below.

2.4.1.1. Starch Aerogels

Starch is one of the most well-known gelling polysaccharides. Starch is composed of glucose chains, in the form of linear amylose and branched amylopectin. The gelation of starch begins when water and heat cause leaching of amylose, melting of the crystalline regions of amylopectin, and swelling of the native granule upon gelatinization. Water becomes trapped within the network of both amylose and amylopectin and upon cooling and retrogradation, the mixture sets to form an opaque gel. The gelatinization temperatures, as well as the final gel properties, vary depending on starch source, granule size and amylose to amylopectin ratio.

Starch gels are relatively new in terms of their use as an aerogel base; however, they have been successfully utilized with supercritical drying [123-125]. Starch gels are usually dried at relatively low supercritical temperatures and pressures, less than 15 MPa and around 40 ^{o}C, after exchanging much of the water with ethanol. Starch requires high concentration aqueous solutions, relative to other aerogel polymers, to allow for enough mechanical stability to maintain its shape, volume and network characteristics, with concentrations of around 12.5 to 25% (w/w) [124, 125]. Maio *et al.* [124] found that as starch concentration was increased from 15 to 25%, surface area and total pore volume of the resulting aerogels decreased. The network structures, which were found to be entangled and dendritic in nature, became denser as concentration increased.

Mehling *et al.* [125] examined the properties of aerogels of different starch sources, namely potato and a maize starch, Eurylon7 amylomaize. Shrinkage,

both during solvent exchange and $SCCO_2$ drying, was dependent on starch type, with potato starch shrinking 77% in volume during ethanol exchange, with negligible shrinkage during the $SCCO_2$ stage, and amylomaize starch shrinking 62% during ethanol exchange and 68% overall. Amylomaize aerogels had greater surface area overall, with 90.3 m^2/g compared to 72.5 m^2/g for potato aerogels and whereas increased retrogradation time increased surface area for potato aerogels, amylomaize was not affected [125].

2.4.1.2. Marine Polysaccharides

Alginate, agar and carageenan, obtained from seaweed, and chitin, from the shells of marine organisms, have been used as a platform for aerogel formation [126-131].

In agar, the agarose component allows for gelation, forming a 3-D network of ordered double helices connected by junction zones [126]. Brown *et al.* [126] studied the effects of sucrose concentration, CO_2 flow rate and depressurization rate on the $SCCO_2$ drying of 2% (w/v) agar hydrogels at 20 MPa and 50 ^oC. This study is unique compared to other polysaccharide aerogel studies in that, rather than converting the polysaccharide hydrogel into an ethanol based alcogel prior to $SCCO_2$ drying, ethanol was introduced at 6 mol% as a co-solvent during drying. It was found that ethanol addition was able to reduce the drying time from 180 min with no ethanol addition to 130 min when CO_2 flow rate was 3 L/min. However, addition of ethanol led to foaming on the aerogel surface, possibly due to the enhancement of the interaction between $SCCO_2$ and the agar polymers [126]. Interestingly, it was found that at the levels examined, 1 - 3 L/min and 0.4 - 1.6

MPa/min for flow rate and depressurization rate, respectively, neither flow rate nor depressurization rate had a significant effect on aerogel structural characteristics. This was likely due to the inflexibility of cross-linked chains. The addition of sucrose at a 10% level reduced volumetric shrinkage of the gels, due to the increase in mechanical stability that sugar provided [126].

Of all the polysaccharides used for aerogel production, alginate is the one most commonly selected. Alginate is composed of (1−4) linked β-d-mannuronic and α-l-guluronic produced by brown algae, and is able to form a gel at very low concentrations, in the range of 1 - 2% [130]. Alginate aerogels show consistently high Brunauer Emmett Teller (BET) surface areas, up to 590 m^2/g, which is much higher than that found for carrageenan gels (200 m^2/g) [129, 131]. Scanning electron micrographs reveal a dendritic network of alginate fibrils, which increase in packing density upon drying but not in shape and connectivity [130]. Similar to starch, nitrogen adsorption isotherms of type IV were obtained, indicating that alginate networks are mesoporous [129, 130]. Alginate polymers include carboxylic acid groups, requiring the use of divalent or trivalent cations to reduce electrostatic repulsion between polymer chains and induce cross-linking [132]. Alnaief *et al.* [131] examined the effect of gelation method, using both a diffusion method and internal setting method, and found that the much slower internal setting method resulted in a higher surface area and a more homogenous gel internal structure. In contrast to starch aerogels [124], increasing the initial alginate concentration resulted in improved surface areas, pore volumes and pore sizes of alginate aerogels [131].

Chitin can be obtained from the exoskeletons of marine organisms, such as mollusks, arthropods and crustaceans, as well as fungal and algal cell walls [132]. Chitosan is obtained by deacetylation of chitin, and is a linear copolymer of linked β (1-4) glucosamine. Unlike most polysaccharide aerogels produced, which use ethanol as an exchange solvent and are therefore suitable for use in edible applications, Tsioptsias *et al.* [128] used methanol and isopropanol for 0.9 and 1.9% chitin aerogels. Also, much higher pressures and temperatures were investigated, with 8 - 30 MPa and 40 - 80 ^{o}C being used, respectively. Aerogel surface area and porosity increased with increasing pressure and decreasing temperature, with the highest surface area, 363 m^2/g, obtained at 30 MPa and 40 ^{o}C [128]. This surface area is similar to that obtained by Quignard *et al.* [129] for acetylated β-chitin microspheres (330 m^2/g).

2.4.2. Aerogel Impregnation

By combining several $SCCO_2$ processes, high-value, functional materials can be formed. Because of their high porosity and low density cross-linked network structure, polymer aerogels can be impregnated with high value compounds for enhanced delivery. Adsorption on aerogels can prevent agglomeration and stabilize drugs, which have very low water solubilities.

Aerogels can be loaded during sol-gel transformation or during a post treatment after drying [133]. Because of the delicate network properties of aerogels, impregnations involving liquid solvents would result in loss of functionality, among other setbacks. Gels are typically added to a high pressure cell in their dried state and physically separated from the compound of interest,

using filter paper or metal grates. $SCCO_2$ becomes saturated with the solute compound and makes contact with the aerogel where it is then adsorbed. Adsorption can be static, where low depressurization rates are utilized, or the compound can be deposited inside the pores of the gel via crystallization upon rapid depressurization of the supercritical solution, also called CO-RESS [134]. When static adsorption is employed, saturation of $SCCO_2$ with the solute must be avoided to prevent crystallization, which limits final drug loading. However, drug loading has been found to increase with increasing concentration in the $SCCO_2$ phase [134, 135]. For naphthalene, the maximum loading was 0.35 mmol/kg without any crystallization, while 60 mmol/kg exhibited visible crystallization. When the CO-RESS process was used, crystals of up to 50 μm size could be achieved, in the case of benzoic acid, with adsorbed solute acting as sites for nucleation [134].

Functional groups of aerogel polymers can be modified to change the aerogel surface chemistry. Several studies have examined loading abilities for water insoluble drugs of hydrophobic versus hydrophilic aerogels [133-135]. For microazole, ketoprofen, naphthalene [135], griseofulvin [133] and benzoic acid [134], hydrophilic silica aerogels had significantly higher loading compared to their hydrophobic counterparts. It was hypothesized that these drugs have high adsorption in the aerogels due to available hydroxyl groups, which provide active sites for hydrogen bonding [135]. For benzoic acid, when silica aerogel bulk density was 0.76 g/m^2, loading was 15% compared to only 9.3% for hydrophilic and hydrophobic gels, respectively [134]. Differential scanning calorimetry was

performed on the gels, which further revealed greater interaction between the drug and hydrophilic gels compared to hydrophobic gels. For starch, simply altering the type of starch had a significant impact on drug loading. Starch aerogels with higher BET surface areas and smaller pore sizes, and thus increased capillary forces, such as those formed from Eurylon7 amylomaize, had almost double the drug loading compared to those made from potato starches [125].

Release of a solute from a loaded aerogel has been found to depend on the chemistry of the gel. Mehling *et al.* [125] found that, for ibuprofen, aerogels made from potato starch had much slower release than those made from Eurylon7 amylomaize. Amylomaize aerogels were also found to collapse quickly during drug release, while potato starch was much more stable, indicating that it is possible to control the release rate by altering polymer composition [125].

2.5. Conclusions

As a result of its unique properties, $SCCO_2$ has remarkable potential for the value added processing of agricultural by-products, including those from flax seed. By combining multiple $SCCO_2$ techniques, such as extraction, aerogel formation and impregnation, bioactive materials can be delivered and used in food and other products in a unique way, without the use of damaging processing conditions and non-renewable, non-food grade, potentially toxic organic solvents.

2.6. References

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3. Supercritical Carbon Dioxide Extraction of Flax Lignans[2]

3.1 Introduction

Flax is an oilseed grown primarily in cool climates, such as the Canadian Prairie Provinces [1]. An important aspect of flax's increasing popularity is its high nutritional content. In its 2006 Flax Monograph, Health Canada recognizes flax seed as a source of essential fatty acids, especially α-linolenic acid and soluble and insoluble fiber [2].

Flax lignans are phenolic compounds linked to many health benefits, including cancer prevention [3, 4]. In flax seed, the predominant lignan, secoisolariciresinol (SECO) is present as a glucoside, namely secoisolariciresinol diglucoside (SDG), which is linked to other SDG molecules by 3-hydroxy-3-methyl glutaric acid (HMGA) to form oligomers called lignan macromolecules (Fig. 2.2, Section 2.1.2) [5, 6].

Since the discovery of their physiological value, lignans have been extracted from flax seeds and other plants. Once extracted, lignans can be added to food or taken in a concentrated form, in an attempt to take advantage of their functionality and benefits. Traditionally, solvent extraction using alcohols like ethanol or acetone has been used to recover lignans [7] due to the polar nature of these phenolic compounds. This extraction is either combined with or followed by hydrolysis of the lignan macromolecule using acid, base or enzyme. Use of large quantities of organic solvent, such as methanol, requires their removal, which usually involves high temperatures.

[2] A version of this chapter has been published as Comin LM, Temelli F and Saldaña MA 2011. J Am Oil Chem Soc. 88: 707-715.

Recently, efforts have been made to use alternate protocols for the extraction of lignans from flax seed and other plant materials, including the use of pressurized low-polarity water [8], microwave-assisted extraction [9, 10] and supercritical fluid extraction [11, 12].

Supercritical fluid extraction has been used previously for the extraction of oil and policosanols from flax seed [13, 14]. Supercritical carbon dioxide ($SCCO_2$) is a favourable solvent for natural product applications because it is non-toxic, non-flammable, inexpensive and easily separated from the extract upon depressurization [13, 15]. While $SCCO_2$ is ideal for the extraction of non-polar compounds, such as lipids, solvent modifiers including water and ethanol can be added at low levels to alter the polarity of the CO_2.

Literature reveals limited information on the use of $SCCO_2$ in regards to the extraction of lignans from flax seed. It is primarily used as a preparatory step to remove the lipid fraction prior to solvent extraction and hydrolysis of lignans. Harris and Haggerty [16] investigated $SCCO_2$ modified with tetrahydrofuran, water and acetic acid to extract lignans and phenolic acids from flax seeds. This study [16], however, was not intended to extract the lignans for further use in food systems since tetrahydrofuran is not a food-grade solvent nor did it attempt process optimization.

Although $SCCO_2$ has been used to extract lignans and related phenolics from plants such as *Schisandra chinensis*, with and without polar modifiers [11, 12], it has not been utilized to any great extent to extract lignans from flax seed specifically. Therefore, the objectives of this investigation were: a) to determine

the effect of temperature, pressure and level of ethanol addition on the CO_2 loading of SDG during $SCCO_2$ extraction of flax seed, and b) to determine the effect of flax seed pretreatment on the CO_2 loading of SDG. It is hypothesized that due to the large size and polarity of the SDG macromolecule, CO_2 loading will be the greatest when pressure and temperature levels are selected to maximize density, when ethanol is included as a co-solvent and when more free SDG is made available through flax seed pretreatments.

3.2. Materials and Methods

3.2.1 Materials

Industrial yellow flax seed was generously donated by Agricore United (Winnipeg, MB, Canada) and stored at -20 ^{o}C until needed. All solvents and reagents used were of analytical grade.

3.2.2. Sample Preparation and Pretreatments

Once removed from cold storage, seeds were ground in a coffee grinder (Philips Model HD5112, Markham, ON, Canada) for 30 sec. Ground seed batches were then mixed to homogenize and to ensure uniformity in terms of particle size. Solvent defatting was performed over 7 h using a Goldfisch apparatus (Labconco Co., Kansas City, MO, USA) with petroleum ether. Samples were placed in the fume hood overnight to ensure that all residual solvent was removed by evaporation. Where samples were defatted by $SCCO_2$, the protocol of Bozan and Temelli [13] was followed at 70 ^{o}C and 55 MPa.

Dehulling was performed using a Buhler MLU 202 Flour Mill (Markham, ON, Canada) with the break rolls engaged (front and back break rolls set at a gap

of 0.12 and 0.14 mm, respectively) and the reduction rolls blocked. The collected, crushed seed was sorted by a gravity table (Westrup, Slagelse, Denmark) in an attempt to collect a sample of hulls as pure as possible. Small pieces of residual meal were removed manually. Hulls were then ground as previously mentioned.

For ground, defatted seed, which was hydrolyzed prior to $SCCO_2$ extraction, ground seed, defatted using solvent method described above and 0.5 N NaOH in 70% ethanol were combined in a 1:5 (w:v) ratio and placed in a 60 ^{o}C water bath for 3.5 h with vortexing at 30 min intervals (personal communication with Dr. A. Muir, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada). Concentrated glacial acetic acid was used for neutralization. Samples were dried using a roto-evaporator, and then placed under a gentle flow of nitrogen to remove all remaining solvent. Hydrolyzed seed was then re-ground as previously mentioned.

3.2.3. Traditional Lignan Extraction

As a comparison to $SCCO_2$ extraction, SDG content of the starting material was determined using a 2 step solvent extraction method [17] with further modifications to combine extraction and hydrolysis (personal communication with Dr. A. Muir, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada). Two g samples along with 10 mL of 0.5 N NaOH in 70% methanol were placed in a screw top test tube. Samples were then placed in a shaking water bath at 65 ^{o}C for 3.5 h and neutralized as previously mentioned. Samples were then refrigerated until use (4 ^{o}C). Prior to HPLC injection, samples were centrifuged and the supernatant was passed through a 40 µm syringe filter tip

(Millipore, Cork, Ireland). Solvent extractions were performed in duplicate. $SCCO_2$ extraction residue samples were analyzed using the same methodology. Modifications to the extraction protocol were made for samples, both starting material and residue, which were pre-hydrolyzed. These samples were not re-exposed to alkali conditions, but were instead just incubated in the 70% methanol solution.

3.2.4. $SCCO_2$ Extraction

A laboratory scale supercritical fluid extraction system (Fig. 3.1) (Newport Scientific, Inc., Jessup, MD, USA) used in the extraction of oil from flax seed previously [13] was used for the determination of CO_2 loading of SDG. Four g total of sample was loaded into a 25 mL basket, which was placed in the 300 mL extraction cell. Carbon dioxide of 99.95% purity (Praxair, Edmonton, AB, Canada) was pressurized using a diaphragm compressor with a maximum rating of 69 MPa. Pressure was controlled (± 1 MPa) by a back pressure regulator. The extraction vessel was heated with a heating jacket and the temperature controlled using a thermostat (±1 ^{o}C). Where ethanol was used as a co-solvent, liquid ethanol was pumped into the pressurized CO_2 stream using a separate co-solvent pump (Gibson 305, Middleton, WI, USA). The flow rate of the CO_2 was 1 L/min, as previously determined to be appropriate for flax oil extraction using the same system [13], measured at ambient conditions by a dry gas meter and controlled by a heated micro-metering valve. Ethanol was removed upstream of the gas meter using a cold trap situated after sample collection.

Glass collection vials were connected to the depressurization valve and held in a refrigerated bath (-20 ^{o}C). Sample collection tubing was washed using minimal (~5 mL) volumes of ethanol. Residual ethanol was removed from the samples by purging gently with nitrogen gas. Sample weights were determined gravimetrically. Collected samples were stored at -20 ^{o}C until ready to be analyzed by HPLC.

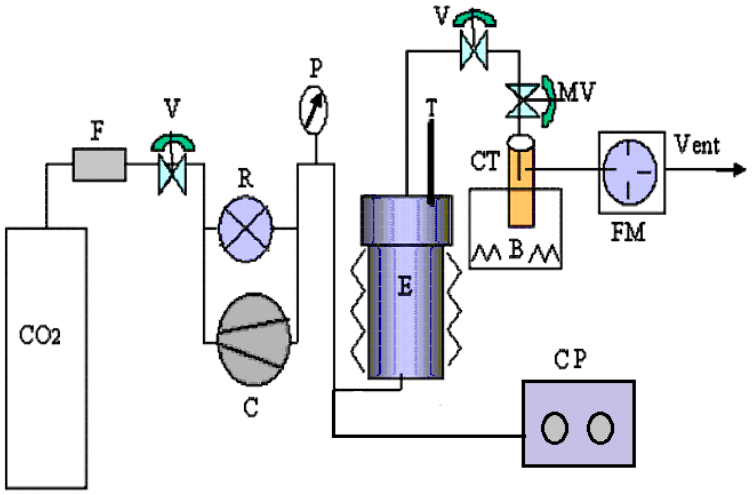

Figure 3.1: Supercritical extraction system: F: filter, V: on/off valves, C: compressor, R: back-pressure regulator, P: pressure gauge, E: extraction vessel with heater, T: thermocouple, MV: micrometering valve, CT: collection tube, B: cold bath, FM: gas flow meter, CP: co-solvent pump.

3.2.5. Experimental Design

Two sets of extractions were performed. Extract samples were collected as a function of time, and analyzed using HPLC to generate extraction curves. The effects of temperature, pressure and level of modifier addition were studied in the first set. Defatted, ground whole flax seed was loaded into the extractor as

described above. CO_2 was compressed to pressures of 35, 40 or 45 MPa and temperature was controlled at 40, 50 or 60 ^oC. Ethanol was introduced at 0, 10 or 20 mol%. Extractions were carried out for a total extraction time of 6 h and samples were collected at 20 min intervals for the first hour, 30 min intervals for the second hour and then once every hour after that, for a total of 9 samples.

The effect of different pretreatments was studied in the second set of extractions. The temperature, pressure and solvent composition found in the first set of extractions to result in the greatest CO_2 loading of SDG were utilized for the extraction of SDG from ground seed defatted with petroleum ether (T DF WS), ground seed defatted with $SCCO_2$ (CO_2 DF WS), ground hydrolyzed seed, defatted with petroleum ether (HY WS), ground hulls defatted with petroleum ether (DF H), and ground full-fat hulls (FF H). Extractions were performed in triplicate for 4 h. Samples were collected at intervals similar to those in the first set of extractions and analyzed by HPLC. Sample preparations can be seen in Figure 3.2.

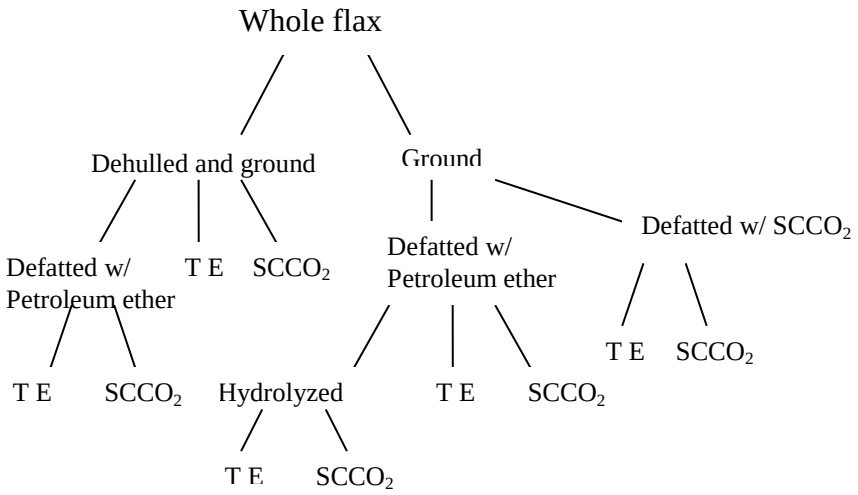

Figure 3.2: Sample treatment steps for traditional and $SCCO_2$ extractions (T E= traditional lignan extraction, $SCCO_2$ E= $SCCO_2$ lignan extraction).

3.2.6. HPLC Analysis

Starting material and $SCCO_2$ extraction residue analysis were performed using a Varian Prostart 210 HPLC (Varian, Palo Alto, CA, USA) equipped with a Waters 486 Tunable Absorbance Detector (Waters, Milford, MA, USA) set at 280 nm, as per the analysis protocol [17] modified by Muir (personal communication with Dr. A. Muir, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada). The column used was a C 18- 5 μm, 4.6 mm x 150 mm reverse phase column (Supelco, Sigma-Aldrich, St. Louis, MO, USA) and the mobile phase was a mixture of 0.05% trifluoroacetic acid in HPLC grade water (A) and 0.05% trifluoroacetic acid in HPLC grade acetonitrile (B). The gradient, t= 0 min A= 99%, t= 0.5 min A=99%, t= 5 min A= 60% t= 6 min A= 99%, was run at 1.5 mL/min for a total time of 15 min. Samples were compared to HPLC grade SDG standards (ChromoDex, Irvine, CA, USA) dissolved in 70% methanol. Quantification and SDG standard curves were established using Galaxie Version 1.9 software (Varian, Palo Alto, CA, USA) in the range of 0.01 to 2 mg SDG/mL of 70% methanol.

For $SCCO_2$ extracts from the first set of extractions focusing on process conditions, post-extraction hydrolysis was necessary due to the potential existence of SDG polymers in the extract; thus samples were solubilised in 4 mL of 0.5 N NaOH in 70% methanol directly in the extraction vials, and left in a shaking water bath for 3.5 h at 60 ^{o}C. They were then neutralized with concentrated glacial acetic acid, centrifuged and analysed by HPLC under conditions slightly modified from those above. The solvent gradient was stretched out to t= 0 min A= 99%, t=

0.5 min A= 99%, t= 8 min A= 75%, t= 10 min A= 0%, t= 13 min A= 0%, t= 14 min A= 99%, t= 23 min A= 99 for a total run time of 23 min.

Extracts from the second set of extractions focusing on pretreatments were prepared similarly to those from the first set with several modifications. First, samples were solubilized in only 2 mL of 0.5 N NaOH in 70% methanol in an attempt to improve quantification. Also, extracts from pre-hydrolyzed ground seeds were soaked in 70% methanol but were not re-hydrolyzed. The gradient was slightly changed to reflect column and sample changes to t= 0 min A= 99%, t= 0.5 min, A= 99%, t= 12 min A= 75%, t= 14 min A= 0%, t= 17 min, A= 0%, t= 18 min, A= 99%, t= 23 min, A= 99%. New standard curves were generated using this method, with concentrations in the range of 0.0001 to 0.01 mg SDG/mL of 70% methanol.

3.2.7. Statistical Analysis

A 3 variable Box-Behnken design was used for the investigation of pressure, temperature and solvent modifier effects, with 5 replications at the center point. The order of all experiments was randomized. Design-Expert 7.1.6 software (Stat-Ease, Minneapolis, MN, USA) was used to establish the Box-Behnken design, to conduct an analysis of variance (ANOVA) and to validate the model. Extraction curves based on the amount of SDG extracted (μg) vs. g CO_2 were established for each extraction run. The slope of the initial linear portion of the extraction curves was determined, which represented the SDG loading in CO_2 and used as the response variable. The conditions (temperature, pressure and

solvent composition), which yielded the steepest initial slope (CO_2 loading) at α=0.05 were considered optimum.

Extraction curves were again generated for the extractions of SDG from the flax seed with different pretreatments and an ANOVA of the results was performed using the General Linear Model procedure of SAS Statistical Software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Multiple comparison of the means was performed by a Least Significant Difference (LSD) test at the α=0.05 level.

3.3. Results and Discussion

3.3.1. Effect of Temperature, Pressure and Ethanol Addition

Figure 3.3 shows typical HPLC chromatograms for the SDG standard, $SCCO_2$ extract fraction, and the residue. Based on the quantification of SDG by HPLC, extraction curves were generated for each run, and a typical curve is presented in Figure 3.4 as the amount of SDG extracted vs. the amount of CO_2 used.

In general, the slope of the initial linear portion of an extraction curve is reported as “solubility” when the starting material is a pure component or as “apparent solubility” for complex mixtures, like plant materials, as long as the equilibrium requirements are met. The equilibrium requirements are to pump CO_2 at low enough flow rates to allow sufficient contact time and to have enough solute present to saturate the CO_2 [18]. In this study, the CO_2 flow rate was maintained at 1 L/min (measured at ambient conditions), which should provide sufficient contact time to reach equilibrium based on previous studies [19]. On the

other hand, the saturation level for SDG in CO_2 is not known. As well, SDG is mainly present as a macromolecule in the flax seeds, so any free SDG would be available only at very low levels. Considering these limitations, the slope of the linear portion of extraction curves is reported as "CO_2 loading" rather than "apparent solubility".

The CO_2 loading of SDG in $SCCO_2$ modified with ethanol varied from 0.087 to 0.55 μg/g CO_2 (Table 3.1). There were no significant differences ($p > 0.05$) between the CO_2 loading at each condition. As well, there were no significant ($p > 0.05$) interactions between temperature, pressure and solvent composition. Choi *et al.* [11] investigated the effect of temperature and pressure on the yield of lignans schisandriol A and B and schisandrin A, B and C after 30 min of extraction. They found that there was no significant effect of temperature and pressure on the yield of these compounds. Their "optimum" condition was at 60 ^{o}C and the highest pressure investigated, 34 MPa.

In the case of the quantity of SDG in residue samples (Table 3.1), the interaction between modifier level and temperature was significant ($p \leq 0.05$), with the quantity of SDG in the residue decreasing with increasing ethanol level and temperature (Fig. 3.5). The residue which would have the least amount of SDG remaining after $SCCO_2$ extraction, according to the model generated was obtained at 7.1 mol% ethanol, 37.9 MPa, and 60 ^{o}C with 1.8% SDG (w/w) and the residue, which would have the most SDG remaining was at 0 mol% ethanol addition, 45 MPa, and 50 ^{o}C with 2.7% SDG (w/w).

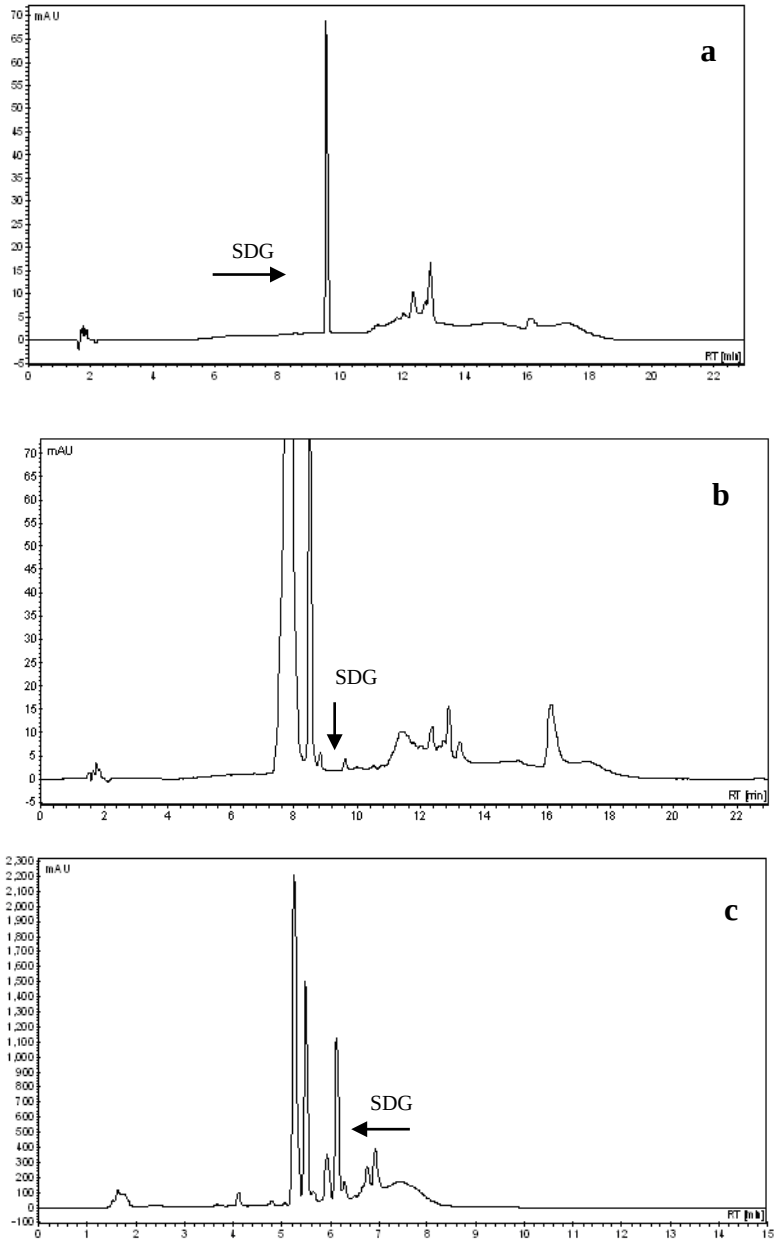

Figure 3.3: Sample HPLC chromatograph: a) SDG Standard (0.09 mg/mL of 70% methanol), b) $SCCO_2$ extract fraction collected between 40 - 60 min at 45 MPa, 50 ^oC and 0 mol% ethanol and, c) residue from $SCCO_2$ extraction at 45 MPa, 50 ^oC and 0 mol% ethanol.

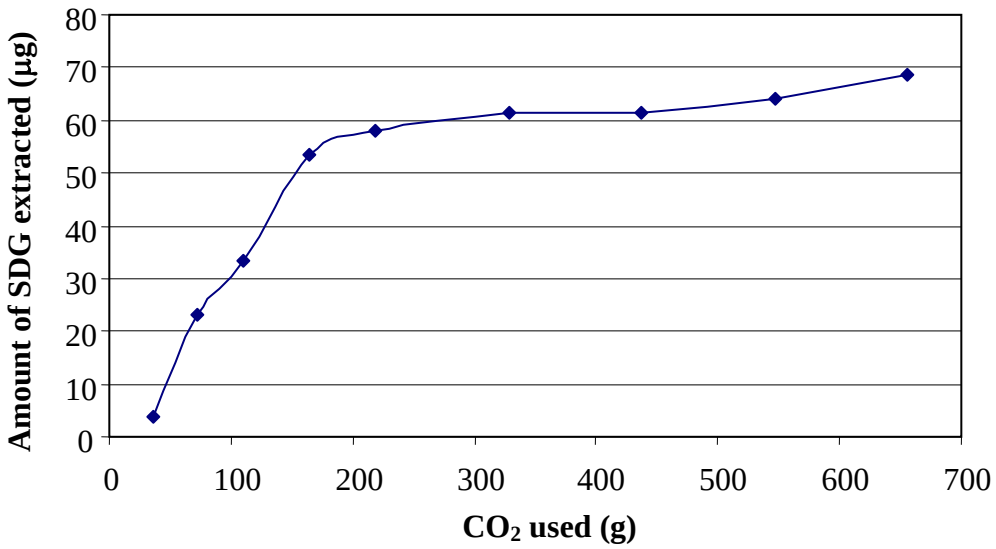

Figure 3.4: Extraction curve for SDG as a function of CO_2 mass at 35 MPa, 60 ^{o}C and 10 mol% ethanol.

Table 3.1: SDG extraction from 4 g of flax seed using $SCCO_2$ modified with ethanol.

Ethanol Addition (mol%)	**Pressure (MPa)**	**Temperature (^oC)**	**CO_2 loading of SDG (µg /g CO_2)[1]**	**Amount of SDG in $SCCO_2$ extract at 6 h (µg SDG)[1]**	**SDG in residue (% w/w)[1,2]**
10	40	50	0.38	70.4	1.6
20	40	40	0.12	46.0	2.2
0	40	60	0.18	54.3	2.7
10	40	50	0.39	44.7	2.1
10	35	60	0.34	68.6	1.6
0	40	40	0.13	27.4	2.4
10	40	50	0.35	46.9	1.6
10	40	50	0.25	107.2	2.1
10	35	40	0.24	28.9	2.3
10	45	60	0.55	84.7	1.9
20	40	60	0.11	33.6	1.4
0	35	50	0.087	10.9	2.4
10	40	50	0.11	21.9	1.4
20	35	50	0.23	42.2	2.3
0	45	50	0.22	56.7	2.5
10	45	40	0.25	44.3	2.1
20	45	50	0.13	44.9	2.1

[1] Mean ± standard deviation for CO_2 loading, amount of SDG in extract and in residue are 0.30 ± 0.12 µg/g CO_2, 58.2 ± 32.3 µg and 1.8 ± 0.31%, respectively, for the center point, based on 5 replications.
[2] SDG content of starting material = 2.2% (w/w).

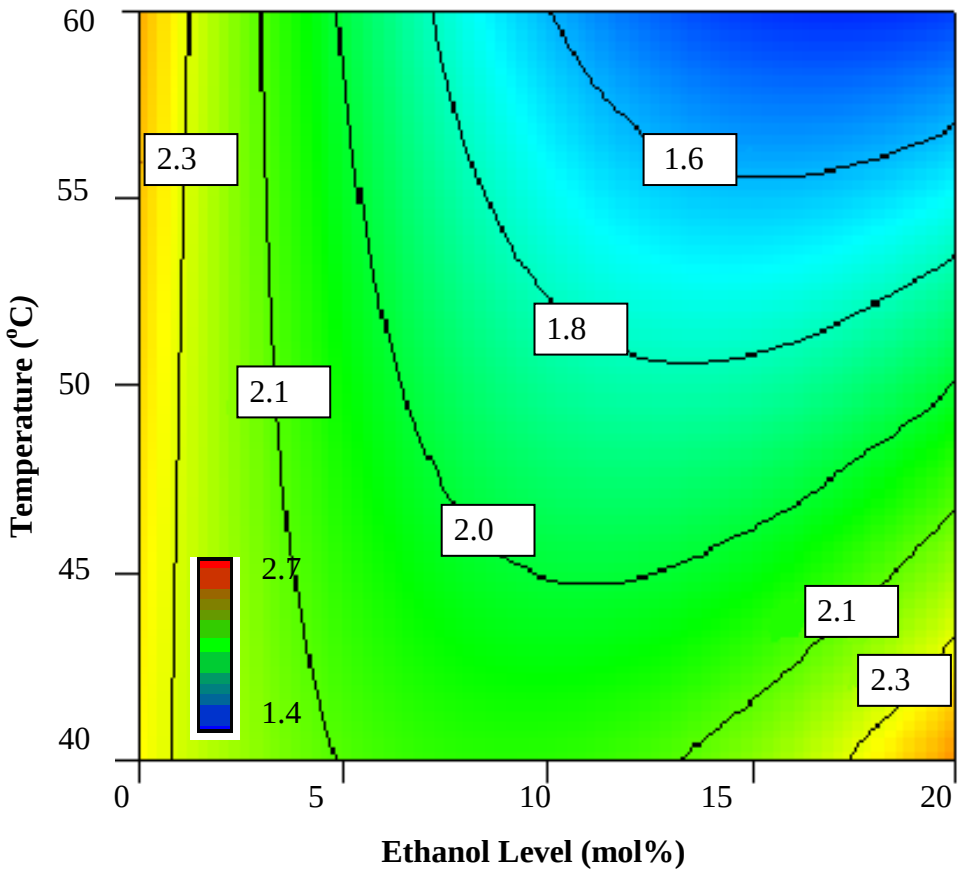

Figure 3.5: Quantity of SDG in residue (% w/w) at 37.9 MPa.

Because SDG is a polar compound, it was expected that increases in ethanol would increase the CO_2 loading of SDG; however, this effect was not significant ($p > 0.05$). At high ethanol concentrations, such as 20 mol% ethanol, it is likely that not all ethanol was in the supercritical state and dissolved in CO_2. Typically, an increase in pressure also has a positive effect on the solubility of compounds in $SCCO_2$ due to increased CO_2 density and thus higher solvent power. There are several reasons why there may be no evident trends in CO_2

loading and that significant optimization conditions could not be established through these experiments. First, SDG typically occurs in a polymer form in flax seeds, and is linked to other SDG molecules via HMGA to form a lignan macromolecule, with most commonly 5 SDG molecules and 4 HMGA molecules [5, 6]. Recent work has revealed that the macromolecule may also contain *p*-coumaric acid glucoside, ferulic acid glucoside, caffeic acid glucoside and herbacetin diglucoside [20]. Because the macromolecule has a much greater molecular mass than SDG alone, it would not be solubilized to the same degree that molecular SDG could be. The values reported reflect the SDG released from all soluble forms, including the larger macromolecules. Choi *et al.* [11] and Lojkova *et al.* [21] found much higher extraction efficiencies in their studies with lignans from *Schisandra chinensis* with recovery of 80% after 30 min of $SCCO_2$ extraction compared to methanol extraction for fruits, and 96% from seeds and 26% from fruits after 60 min of $SCCO_2$ extraction, compared to petroleum ether/methanol extraction, respectively. This is most likely due to differences in the structures and associations of these lignans compared to those in flax. *Schisandra chinensis* contains free lignans with much lower polarities; therefore, they are likely to have much higher affinities to $SCCO_2$ than do flax lignans.

Another contributing factor to error was the difficulty in maintaining constant pressure and flow rate when ethanol was incorporated into the system, especially at the maximum level of 20 mol%. Manual control of the micrometering valve becomes difficult when relatively large quantities of ethanol have to pass through the small opening around the stem upon pressure drop down

to atmospheric level. The flow meter used was a dry gas flow meter; if the ethanol in the exhaust stream was not completely condensed in the cold trap between the outlet and the flow meter, it may have caused problems with the flow meter reading. Lojkova *et al.* [21] also found flow difficult to control, especially during the beginning of the extraction due to the high concentration of easily extractable lypophilic compounds. Because of the importance of flow rate in the determination of CO_2 loading, as mentioned previously, fluctuations can cause variation.

The next set of extractions, investigating the effect of pretreatments, required an "optimum" set of conditions to be selected, regardless of statistical significance. The statistical software generated an optimum CO_2 loading of 0.49 μg/g CO_2 at 7.8 mol% ethanol, 45 MPa and 60 ^oC. Figure 3.6 illustrates that, although not statistically significant, when modifier addition is fixed at 7.8 mol% ethanol, the CO_2 loading of SDG increases with both temperature and pressure. Statistically, however, any of the conditions tested could have been selected because the model was not significant.

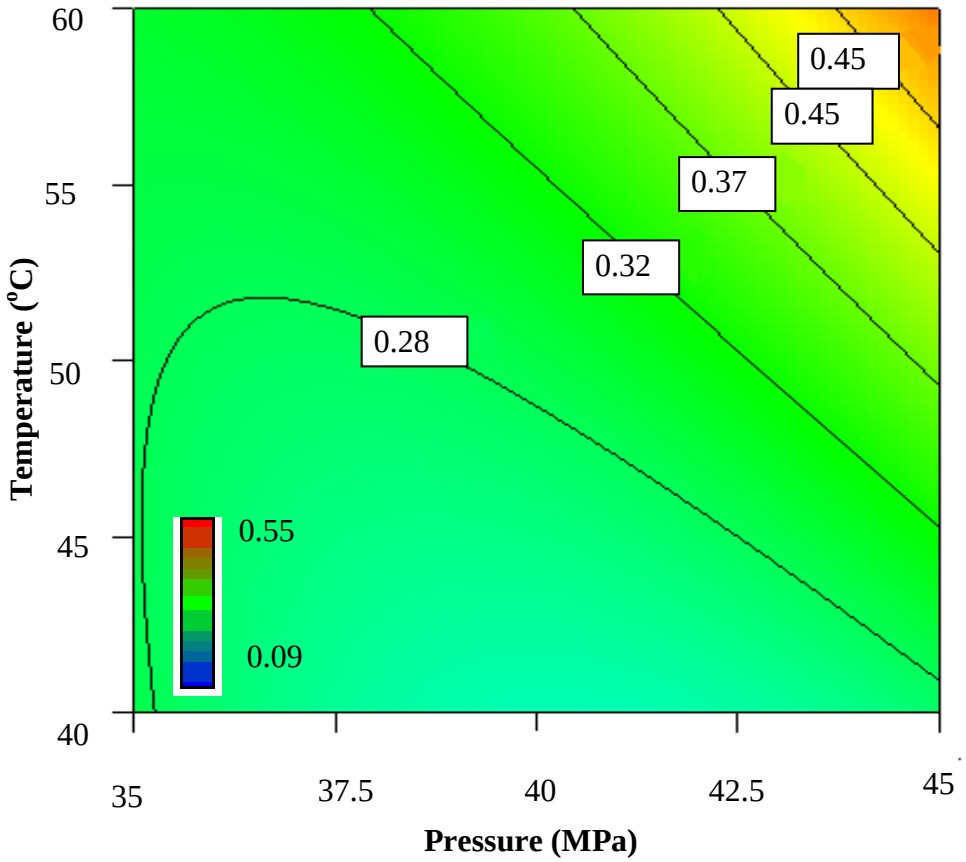

Figure 3.6: CO_2 loading of SDG (μg/g CO_2) with 7.8 mol% ethanol.

3.3.2. Effect of Sample Pretreatment

Based on the findings from the investigation into the effects of process conditions, it was predicted that the maximum CO_2 loading of 0.49 μg SDG/g CO_2 from whole, ground flax seed defatted by petroleum ether could be achieved at 7.8 mol% ethanol, 60 ^{o}C and 45 MPa. As observed in Table 3.2, this was not the case experimentally. The CO_2 loading at these conditions was actually 0.16 μg/g CO_2. One reason was that the model generated previously, which was used

to predict the maximum CO_2 loading, was not found to be significant. Regardless, because the extraction conditions were kept constant for the pretreatment portion of the study, it should be possible to determine the optimal starting material to maximize CO_2 loading based on relative comparisons. Again, it is important to point out that it is the CO_2 loading that is reported, not solubility. For the samples which underwent alkali hydrolysis prior to $SCCO_2$ extraction, it is likely that the CO_2 loading would be approaching "apparent solubility"; however, confirmation of "apparent solubility" requires further research.

The CO_2 loading of SDG obtained from T DF WS, CO_2 DF WS, FF H and DF H were similar ($p > 0.05$) (Table 3.2). This may be due to the high variability between replicates of each treatment, as parameters such as flow rate and pressure were difficult to control precisely in the presence of ethanol. However, the CO_2 loading obtained from the above mentioned treatments were significantly ($p \leq 0.05$) less than that from HY WS, which was more than 13 times greater. The alkali treatment hydrolyzed the large SDG macromolecules into smaller macromolecules and free SDG. Because solubility in $SCCO_2$ depends on molecular mass and size, the relatively smaller molecules in the hydrolyzed sample, as expected, resulted in higher CO_2 loading.

Table 3.2: CO_2 loading of SDG from different preparations of flax seed in $SCCO_2$ modified with 7.8 mol% ethanol at 60 ^oC and 45 MPa.

Treatment[1]	**CO_2 loading (µg SDG/g CO_2)**[2]
T DF WS	0.16 ±0.08[a]
CO_2 DF WS	0.06 ±0.05[a]
FF H	0.29 ±0.16[a]
DF H	0.12 ±0.1[a]
HY WS	3.80 ±0.6[b]

[1]T DF WS = ground seed defatted with petroleum ether, CO_2 DF WS= ground seed defatted with $SCCO_2$, FF H= ground full-fat hulls, DF H= ground hulls defatted with petroleum ether, and HY WS =ground hydrolyzed seed, defatted with petroleum ether
[2]Mean ± standard deviation based on triplicate extractions (n=3).
[a,b] Means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$).

It was expected that the CO_2 loading of SDG from the flax seed hulls would be significantly higher than that from the whole seed, considering that SDG is mostly concentrated in the hulls [22]. However, this was not the case. The increased complexity of the hull matrix, such as the existence of waxes and mucilage [16], in comparison to the matrix of the meal may have contributed to this outcome. Diffusion of $SCCO_2$ and transfer of SDG through the hulls was likely less efficient compared to that in the meal, as more barriers to $SCCO_2$ permeability exist. SDG may also exist in larger polymers in the hull than in the meal, and these larger polymers would be less soluble in $SCCO_2$ compared to smaller polymers and individual compounds. As well, SDG polymers may be more tightly bound in the hull matrix. So, despite the higher concentrations, the CO_2 loading of SDG from hulls was not significantly greater. It was expected that the defatted hulls would result in a higher yield of SDG from traditional extraction

than the full fat hulls, considering that hulls contain approximately 18.3% fat when extracted using petroleum ether [23], but this was also not seen (Table 3.3), possibly due to matrix changes or component degradation during defatting.

Table 3.3: Average material balance for SDG extractions (6 h) for 4 g of each flax seed treatment using $SCCO_2$ + 7.8 mol% ethanol at 60 ^oC and 45 MPa

Treatment[1]	**SDG in starting material (mg)**[2]	**SDG in residue (mg)** [2]	**Total SDG in extract (µg)** [2]	**SDG gained after $SCCO_2$ extraction (µg)** [2]
T DF WS	60.7 ±7.2^c	60.8 ±20.6^c	15.9 ±5.4^a	106 ± 92.9
CO_2 DF WS	54.7 ±5.7^b	54.8 ±15.1^b	6.1 ±3^a	9.5 ±7.7
FF H	90.6 ±11.4^e	90.6 ±26.1^e	21.6 ±13.1^a	7.2 ±8.7
DF H	80.8 ±6.7^d	80.7 ±21.7^d	19.4 ±15.3^a	5.1 ±13.7
HY WS	37.1 ±3.0^a	36.8 ±7.3^a	171.8 ±36.1^b	-125.3 ±609

[1] T DF WS = ground seed defatted with petroleum ether, CO_2 DF WS= ground seed defatted with $SCCO_2$, FF H= ground full-fat hulls, DF H= ground hulls defatted with petroleum ether and HY WS =ground hydrolyzed seed, defatted with petroleum ether
[2]Mean ± standard deviation based on triplicate extractions (n=3)
[a-e] Means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$)

Due to lengths of tubing and fittings of the extraction system, and the necessity to rinse these parts to recover all possible extract, some of the target component, in this case SDG, may have been lost. An SDG mass balance was completed for each extraction, assuming that the final mass of residue was equal to the mass of the starting material minus the mass of collected extracts and the mass of the fraction potentially lost. Results of the mass balance calculations are presented in Table 3.3. With the exception of the HY WS, more SDG was

recovered after $SCCO_2$ extraction (from the residue and the extract) compared to the amount in the starting material, although this difference was in the μg range (Table 3.3). During $SCCO_2$ extraction, the dense CO_2 diffuses into the matrix of the flax sample, expanding the sample. Upon depressurization the CO_2 dissipates from the sample, resulting in an irreversible opening of the matrix. Therefore, after the $SCCO_2$ extraction, the SDG in the residue is more accessible to traditional extraction used for analysis purposes than the starting material is and the resulting quantity is relatively greater. This phenomenon has been observed and taken advantage of in oil extraction. Dong and Walker [24] illustrated that canola flakes "exploded" through the $SCCO_2$ depressurization process showed improvements in subsequent oil extractability. Potentially, rapid pressurization/depressurization could be a beneficial pretreatment prior to traditional SDG extraction to enhance recovery.

Through traditional extraction used for analysis, significantly more SDG was extracted from the hulls than from the whole seed (Table 3.3). The alkali treatment was able to not only hydrolyze the SDG macomolecules, but also loosen the cell wall components, which previously limited the availability of the SDG. For the HY WS, less SDG was recovered from the residue after $SCCO_2$ extraction compared to the starting material. This indicates that, although the effect of the $SCCO_2$ making the SDG more available to traditional extraction was most likely also present in these samples, there was a net loss. These extracts tended to be much stickier as a result of the hydrolysis, and this could have led to the increased deposit of extract on tubing, which was not possible to rinse off and recover by

ethanol. Interestingly, the amount of SDG extracted using traditional methods from the hydrolyzed starting material was significantly ($p \leq 0.05$) less than that from the other treatments. These samples were treated similarly to those in a traditional extraction, with the exception of the use of food-grade ethanol rather than methanol and the solvent was evaporated off before the sample was reground. For HPLC analysis, the hydrolyzed seed was soaked in 70% methanol for the same time and at the same temperature as in a traditional extraction. As previously mentioned, the alkali serves to not only hydrolyze the SDG macromolecule, but may also hydrolyze or solubilize other cellular constituents, which limit SDG availability for dissolution in the $SCCO_2$ phase. When the hydrolyzed sample was dried, even though cellular damage had already been caused by the alkali, the hardening of the sample may have created new barriers to availability. When the sample was soaked in 70% methanol to prepare for HPLC, there was no alkali added to help in breaking up the newly formed matrix.

3.4. Conclusions

$SCCO_2$ extraction resulted in an extremely low quantity of SDG (maximum 0.0027%) compared to traditional extraction (2.2%). Level of ethanol addition, temperature and pressure of extraction did not have a significant effect ($p > 0.05$) on CO_2 loading of SDG. The amount of SDG extracted and CO_2 loading were significantly ($p \leq 0.05$) enhanced by the pre-hydrolysis of the starting flax material. Additionally, although all of the $SCCO_2$ extracts did not contain high levels of SDG, it was apparent that flax seed samples that had undergone pressurization and depressurization in the presence of $SCCO_2$ had

increased recovery of SDG using traditional extraction methods. Therefore, $SCCO_2$ is not recommended for flax SDG extraction.

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4. Impregnation of Flax Oil in Pregelatinized Corn Starch Using Supercritical Carbon Dioxide[3]

4.1. Introduction

Supercritical carbon dioxide ($SCCO_2$) is a solvent with unique properties, which can be exploited for processing of sensitive nutraceuticals and biopolymers. In terms of food ingredients, $SCCO_2$ has been used to extract high value compounds, including bioactive lipid components [1-3]. More recently, however, significant developments have been made in particle formation aspects especially for drug delivery purposes [4] and to a limited extent for the delivery of nutraceuticals [5, 6].

Impregnation of biopolymers is just one technique for compound delivery. Impregnation using $SCCO_2$ has been investigated and reviewed, including its use in impregnation of drugs, dyes, organo-metallic complexes, monomer and initiators, and wood biocide impregnations [7, 8]. However, its food product related applications are limited. When held under supercritical conditions in the presence of CO_2, glassy polymers swell and become less viscous, with the $SCCO_2$ acting as a plasticizer and reducing the glass transition temperature of the polymer. If the solute is sufficiently soluble in $SCCO_2$, it is then able to diffuse with the fluid into the polymer. If the partition coefficient permits, the solute will remain in the polymer or upon depressurization, as the solubility of the solute in the solvent is reduced, the solute will become trapped within the polymer matrix. Starch, especially corn or maize starch, is a common food ingredient available in

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large quantities and at a low cost, which has found uses beyond just human nutrition, including in paper and textile products, viscosity modifiers in oil drilling, cosmetics, adhesives and as a substrate for ethanol production [9]. One of its many uses has been as a delivery vehicle for high value food extracts, including oils [10] and flavourings [11, 12]. Starch has also been processed using $SCCO_2$ where it has been shown that, when starch and water are heated in $SCCO_2$ environment, the gelatinization temperature of starch is lowered significantly [13, 14]. This effect is believed to be due to the enhanced diffusion of water and the increased plasticity of starch in the supercritical fluid environment. Starch gels have also been dried using $SCCO_2$, producing aerogels, which can be used as drug carriers or scaffolds for tissue replacement [15, 16]. $SCCO_2$ extrusion has been applied to starch, producing cross-linked starch [17] and microcellular foams [18, 19].

Bioactive lipids, such as flax oil, are often encapsulated or protected in food polymers and other materials to ensure their delivery to the target location and prevent degradation, to preserve their beneficial properties [20]. Techniques for lipid protection and delivery vary greatly depending on the intended use and desired properties of the product. Spray drying is by far the most popular technique and has been used for oils high in ω-3 fatty acids [21-23]. However, complex coacervation [24] has recently been used to protect flax oil. Impregnation of lipids, including flax oil, in starch, however, has not yet been investigated under $SCCO_2$ conditions.

The objectives of this study were to determine the effects of $SCCO_2$ on pregelatinized corn starch (PGS) particle characteristics and to investigate the effects of $SCCO_2$ pressure and temperature on the impregnation efficiency of oleic acid and flax oil in PGS. If lipids, for example oleic acid as a model lipid and flax oil are able to diffuse into the starch granules in the presence of $SCCO_2$ and become trapped upon depressurization, then there may be potential to use lipid-impregnated pregelatinized starch as a delivery vehicle for various bioactives.

4.2. Materials and Methods

4.2.1. Materials

Pregelatinized corn starch, prepared by spray drying, Ultra-Tex 1, was kindly donated by National Starch (Bridgewater, NJ, USA), and cold-pressed flax oil was kindly donated by Bioriginal Food and Science Corp. (Saskatoon, SK, Canada). Oleic acid (97%), hexane, acetone and n-propanol were all obtained from Fisher Scientific (Fairlawn, NJ, USA). Nile red fluorescent dye (Technical grade, N3013) was obtained from Sigma-Aldrich (Oakville, ON, Canada).

4.2.2. Characterization of PGS Particles

PGS sample (5 g) was loaded into a 25 mL basket, and placed inside the high pressure cell of the supercritical fluid extraction system previously described (Section 3.2.4). The system was pressurized with CO_2 to either 15 or 30 MPa and heated to 40, 60 or 80 ^{o}C. After these conditions were held for 8 h with no CO_2 flow, the temperature was lowered to 40 ^{o}C and the pressure was released through a heated micrometering valve at 1 L/min (measured at ambient conditions). All treatments were performed in triplicate.

$SCCO_2$ treated PGS samples were analyzed using scanning electron microscopy (SEM) (Zeiss EVO MA 15, Carl Zeiss Canada Ltd., Toronto, ON, Canada) and a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, Brea, CA, USA) and compared to a control PGS sample, which was not treated with $SCCO_2$. For SEM analysis, a thin layer of the sample was applied to a sample mount using double-sided carbon tape, and sputter coated with gold using a Xenusput XE200 (Edwards High Vacuum, Crawley, UK). Analysis of each sample was performed in duplicate. For laser diffraction particle size analysis, particles were analyzed in dry powder form, which was fluidized in air.

4.2.3. Impregnation of PGS with Oleic Acid and Flax Oil

Impregnation experiments were performed both under static and dynamic conditions. Five g of PGS, 2 g of glass beads and 5 g of lipid, either oleic acid or cold-pressed flax oil, were placed into the supercritical system described earlier. For the static impregnations, once again, the system was pressurized to 15 or 30 MPa and heated to 40, 60 or 80 ^{o}C. After a static hold of 8 h, the temperature was lowered to 40 ^{o}C and the pressure was released through a heated micrometering valve at 1 L/min (measured at ambient conditions). Conditions, which resulted in the highest level of impregnation, for both oleic acid and flax oil, under static mode were selected for the dynamic mode with continuous CO_2 flow (1 L/min, measured at ambient conditions) throughout the 8 h impregnation. In dynamic mode experiments, the lipid was placed upstream of the starch, with a stainless steel frit (2 µm) separating them. Upon depressurization, the extracted lipid was removed using a cold trap and exhaust CO_2 was vented off. In all cases, residual

lipid remained behind the frit, upstream of the PGS, after the 8 h period. In addition, to represent a more traditional, organic solvent impregnation method, lipid and starch were mixed and stirred vigorously in 50 mL of hexane for 8 h at ambient temperature and pressure. After 8 h, the mixture was filtered to remove the hexane and placed under a gentle flow of nitrogen to dry. All treatments were performed in triplicate and samples were stored in opaque vials at room temperature and under nitrogen headspace until further analysis.

4.2.4. Characterization of Impregnated Particles

Impregnated samples were analyzed for both surface lipids and total lipids. For surface lipids, 0.5 g sample (excluding glass beads) was placed and spread out evenly on a glass fiber filter paper in a Buchner funnel and placed on a clean, dry, pre-weighed side-arm flask. The samples were then washed with 10 mL of cold hexane delivered by a glass transfer pipet. Samples were filtered and dried under vacuum to ensure that all residual solvent was removed. Once dry, the side-arm flask was weighed again and the surface lipid % was calculated as: [(Final mass of flask - Initial mass of flask) / (Mass of starch+lipid sample)] * 100%.

Total lipids were determined according to methods previously used [25, 26] that were modified slightly for this study. A sample (0.1 g) was placed in a 25 mL centrifuge tube, sealed with a rubber o-ring, together with 10 mL of 75% (v/v) n-propanol. These samples were then placed in a boiling water bath for 4 h, with vortexing every hour. The samples were then filtered into a pre-weighed, clean, dry side-arm flask under vacuum and the residual starch was washed with 10 mL hexane to ensure the removal of all lipids. The samples were then dried under a

gentle flow of nitrogen at atmospheric temperature and the side-arm flask was weighed again. The total lipid % was calculated using the same formula as above. Impregnated lipid % was determined by subtracting the surface lipids from the total lipids. Surface and total lipid analysis were all performed in duplicate for each sample.

In an attempt to visualize the distribution of lipids in the starch granules after $SCCO_2$ processing, both oleic acid and flax oil were stained with a fluorescent dye and the supercritical treatment under the conditions which resulted in the highest level of lipid impregnation was repeated. To stain the oil, a solution of Nile red in acetone was prepared (10 mg Nile red / 200 mL acetone). Then, 20 mL of this solution was added to 50 mL of each lipid and the acetone was removed using a rotovap for a final concentration of 0.02 mg Nile red / mL of lipid. The starch granules impregnated with the stained lipid were then viewed at approximately the middle of the granule using a laser scanning confocal microscope (LSM 510 Laser Scanning Confocal Microscope, Carl Zeiss Canada Ltd., Toronto, ON, Canada). For samples where surface lipids were removed, a small amount of sample was placed on a glass slide and covered with a drop of water, while samples with surface lipids remaining did not have water added.

4.2.3 Statistical Analysis

Analysis of variance of the results was performed using the General Linear Model procedure of SAS Statistical Software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Multiple comparison of the means was performed by a least significant difference (LSD) test at the α=0.05 level.

4.3. Results and Discussion

4.3.1. Effect of Temperature and Pressure on PGS

The mean particle diameter for the pregelatinized starch granules before treatment with $SCCO_2$ was 85.99±4.75 µm. Comparison of particle size before and after $SCCO_2$ treatment under different temperature and pressure conditions is presented in Figure 4.1. There was no apparent trend in the changes in the mean particle diameter of pregelatinized starch after $SCCO_2$ processing. There was no significant difference ($p > 0.05$) between the means of each treatment and no significant effects of temperature, pressure or the interaction between them. Therefore, there is no significant impact of $SCCO_2$ treatment and pressurization/depressurization, at the levels studied, on the particle size.

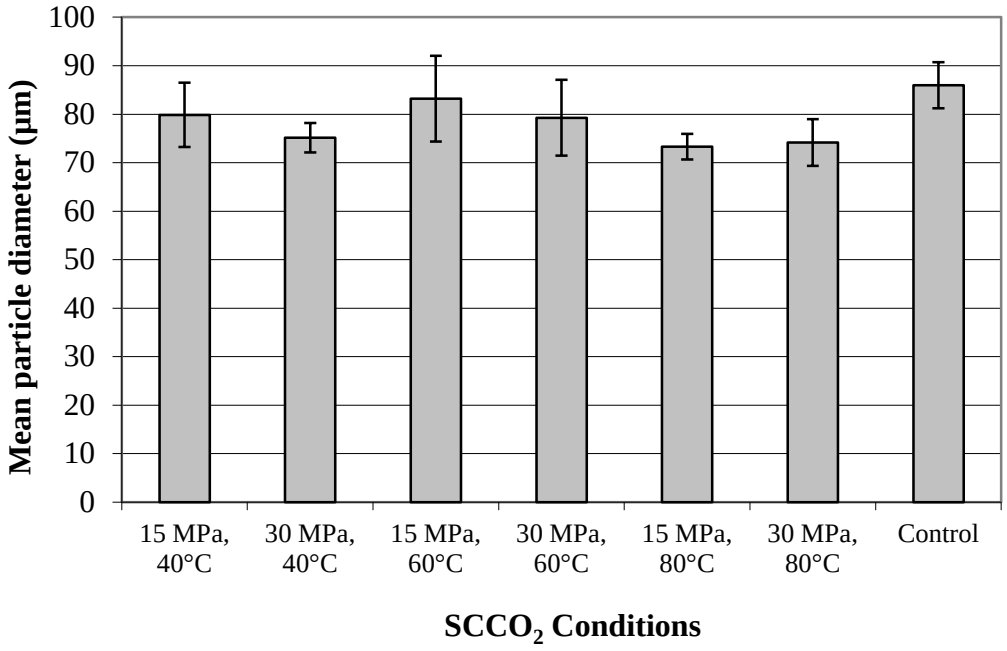


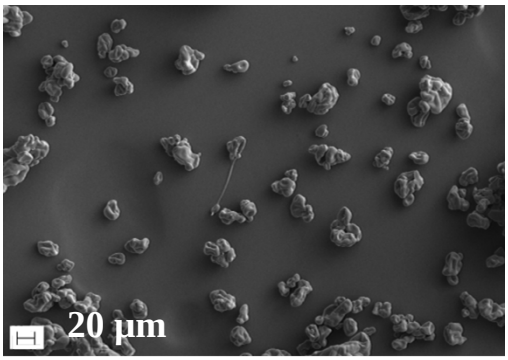
Figure 4.1: Mean particle diameter of pregelatinized starch processed with $SCCO_2$ at different temperature and pressure conditions (n=3).

According to the SEM images (Fig. 4.2), the starch granules, which were pregelatinized and then spray dried, were clumped together in agglomerates as a result of their processing. The $SCCO_2$ pressurization-depressurization could have affected the starch granules in several ways. First, the diffusion of $SCCO_2$ into the porous starch granules may have swelled the granules. Upon depressurization, the $SCCO_2$ rapidly exits the granule and the extraction chamber as it returns to its gaseous state. This process has been shown to "damage" particles, creating abscesses, which may alter the mean particle diameter [27, 28]. The depressurization rate plays an important role in terms of the extent of damage to the particles. In this study, the depressurization rate was controlled at a rate of 1 L/min. In addition, attempts were also made to determine the extent of possible swelling of a bed of PGS in a capillary tube placed in a $SCCO_2$ phase monitor equipped with a 10 mL cell with quartz windows (SFT Phase Monitor II, Supercritical Fluid Technologies, Newark, DE, USA) under different temperature and pressure conditions to test the hypothesis that starch granules may swell as CO_2 diffuses into them. No appreciable swelling of the bed was observed within the limitations of this system. Due to the porous nature of pregelatinized starch, the extent of the swelling/exploding effect of the $SCCO_2$ was difficult to estimate. However, since there is no damage apparent in the SEM images, this may be promising for developing applications focusing on impregnation, since any further damage to the granule structure may enhance oxidation of air-exposed lipids. Alternately, the forces involved in pressurization-depressurization may have pushed the agglomerates apart, into single granules or smaller aggregates.

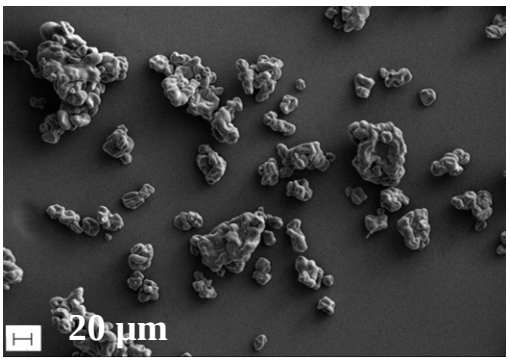
Although the SEM images in Figure 4.2 suggest that the agglomerates were reduced in size, the laser diffraction analysis of mean particle diameter does not show this effect to be significant (Fig. 4.1) or to follow a trend. Particle size determination has been shown to be method specific with different techniques yielding different results {{807 Hassellöv,M. 2008; 806 Holzer,L. 2009}}. Fluidization of the powder during particle size analysis may have contributed to breaking up of aggregates, especially in the control sample. On the other hand, sample preparation and mounting for SEM analysis may have led to the appearance of sample aggregation or clumping {{807 Hassellöv,M. 2008}}. Further analysis is therefore necessary, possibly employing different techniques or utilizing different particle size standards.

Previously, it was found that native corn starch and jet cooked oat bran product, Nutrim-OB, defatted with $SCCO_{2,}$ did not show any morphological or X-ray diffraction pattern changes compared to their unprocessed counterparts [29, 30]. Nutrim-OB, which would be pregelatinized by the jet-cooking process, and therefore similar in nature to the pregelatinized starch used in this study, showed lower amorphous amylose-lipid thermal transition onset melting temperatures after $SCCO_2$ lipid extraction, compared to unprocessed samples [30].

a: 15 MPa, 40 ^{o}C



b: 30 MPa, 80 ^{o}C



c: control

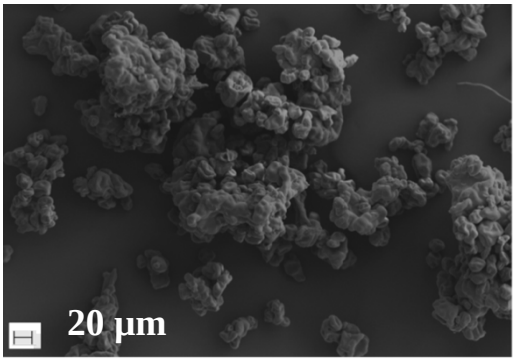


Figure 4.2: Scanning electron microscopy images (200X mag) of pregelatinized corn starch treated with $SCCO_2$: a) 15 MPa and 40 ^{o}C, b) 30 MPa and 80 ^{o}C and, c) control.

4.3.2. Effect of Temperature and Pressure on Impregnation of PGS with Oleic Acid and Flax Oil

At the end of the impregnation experiments, the starch-oil mixtures removed from the $SCCO_2$ cell appeared to have less surface or free lipids, or to be more homogeneous than the mixtures that were originally loaded into the cell, which may indicate that $SCCO_2$ has deposited the oil inside the granules. This is despite the fact that only very small amounts of lipid (an average of 0.22 g for oleic acid and 0.10 g for flax oil) were removed throughout depressurization, due to the slow depressurization rate employed (data not shown). Statistical analysis showed that there was a significant difference ($p \leq 0.05$) in the amount of oleic acid impregnated at the various conditions tested (Figs. 4.3 and 4.4). The effects of both temperature and pressure were significant, but there was no interaction between them. The percentage of oleic acid impregnated at 40 ^{o}C was significantly greater than that at 60 ^{o}C, but not at 80 ^{o}C. In terms of pressure, the percentage of impregnated lipids decreased as pressure increased from 15 to 30 MPa. The greatest level of impregnated lipids was 11.4% of the total mass, obtained at 40 ^{o}C and 15 MPa.

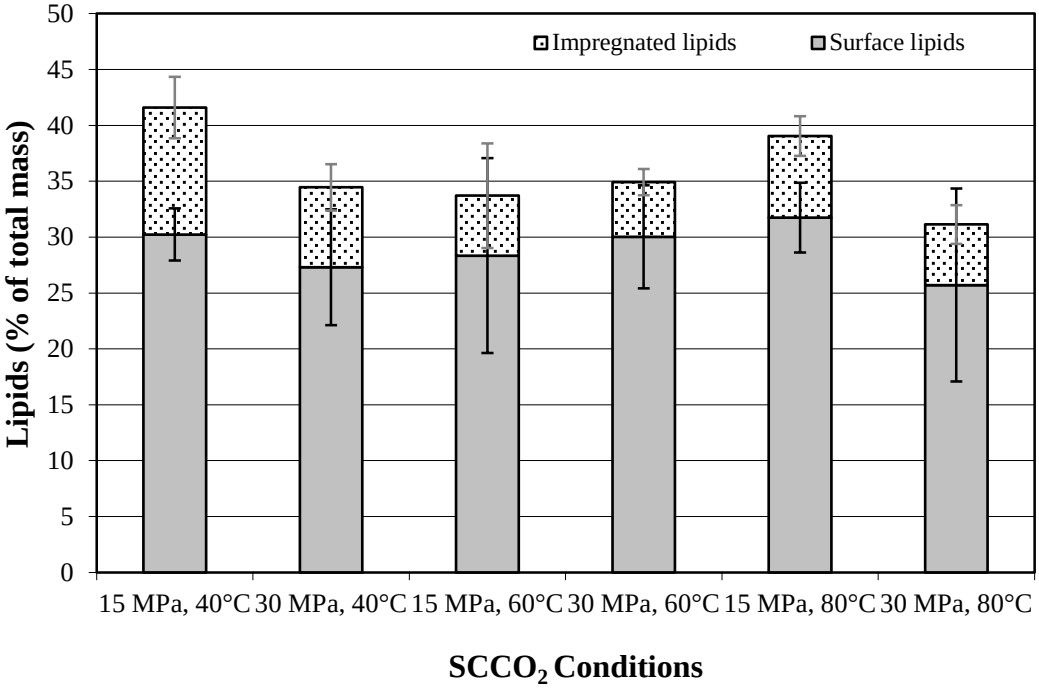


Figure 4.3: Oleic acid, as a % of total particle mass, impregnated in and on the surface of pregelatinized starch granules using $SCCO_2$ under different conditions (n=3).

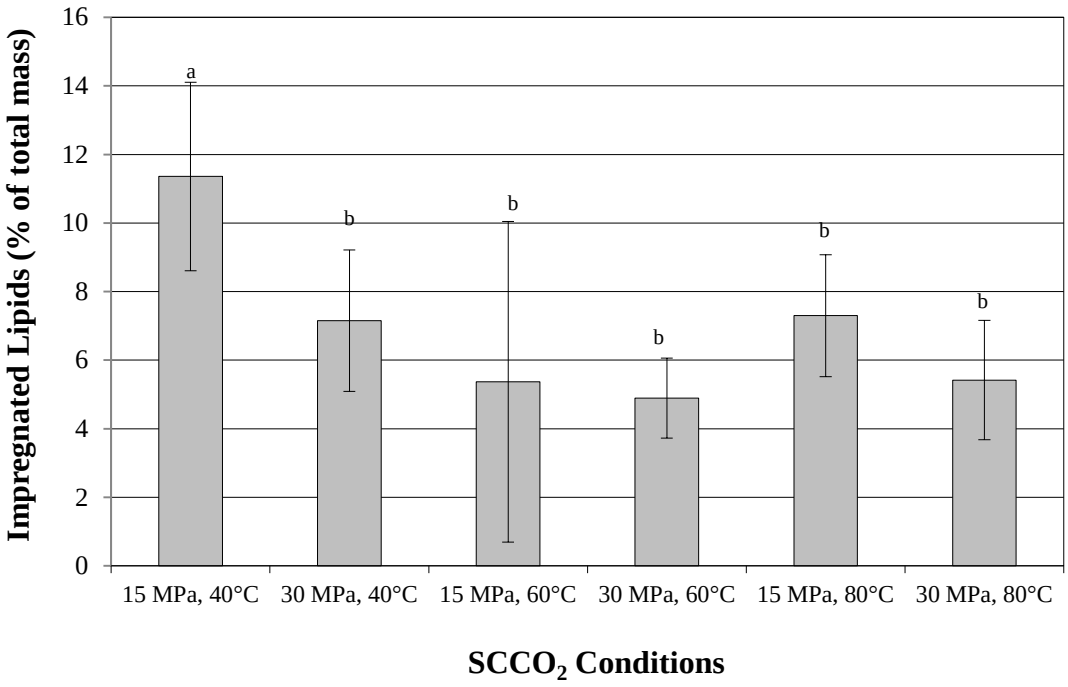


Figure 4.4: Oleic acid, as a % of total particle mass, impregnated in pregelatinized starch using SCCO2 under different conditions. Means with the same letter are not significantly different at $p > 0.05$ (n=3).

The effect of increasing pressure is consistent with the results of Cosijns *et al.* [31] for the impregnation of ibuprophen in Avicel PM 101 and NaCl porous pellets. It was found that increasing temperature and pressure decreased drug deposition because the interactions between the supercritical solvent and the drug were stronger than the interactions between the drug and the pellet under these conditions. Upon initial depressurization, there is potential for increased extraction of the drug as well, if the solubility of the solute in the solvent is high. Increasing pressure decreased the impregnation efficiency of flurbiprofen in P(MMA-EHA-EGDMA) as well, since the partition coefficient between the drug and matrix became less favourable [32]. The effect of increasing temperature,

however, would depend on the cross-over pressure of the solute solubility isotherms. At low pressures, increasing temperature decreases solute solubility, while at high pressures, the opposite is true; the exact conditions of such a cross-over are unique to the solute in question [33].

For the impregnation experiments using flax oil, there were no significant differences ($p > 0.05$) between the amounts of oil impregnated under each condition tested (Figs. 4.5 and 4.6). The impregnation efficiencies obtained with oleic acid (4.89 - 11.86%) tended to be relatively higher than those for flax oil (0.51 - 6.60%) (Figs. 4.4 and 4.6). Impregnation of lipids in PGS relies on solubility behaviour and mass transfer properties. First, the lipid must be dissolved in $SCCO_2$ to make contact with the PGS bed. Many factors will contribute to the extent of lipid solubility in $SCCO_2$, including the molecular weight, polarity and vapour pressure of the lipid solute, as well as the density of the $SCCO_2$ phase. As a fatty acid, oleic acid has a lower molecular weight and higher vapour pressure than flax oil, which is composed mainly of triglycerides. These differences translate to a higher solubility of oleic acid in $SCCO_2$ compared to flax oil at constant temperature and pressure.

Güçlü-Üstündag and Temelli [34] utilized literature data to correlate the solubility behaviour of various lipids in $SCCO_2$ using Chrastil's equation, $\ln c = k \ln d + a/T + b$, where c is the solubility of a solute in the solvent (g/L), d is the density of the pure solvent (g/L), T is the experimental temperature (K), k is the number of molecules in the solvato complex, a is dependent on the total heat of reaction and b is dependent on the molecular weights of the solute and solute and

the association constant. Thus, the solubilities of oleic acid and triolein, acting as a model system for the flax oil, can be estimated at the conditions used for impregnation in this study. At 15 MPa and 40 ^{o}C, the lowest temperature and pressure conditions, which resulted in the highest impregnation of oleic acid, the solubility of oleic acid in $SCCO_2$ is estimated to be 6.91 g/L, 4.6 times greater than that of triolein, 1.50 g/L, at the same conditions. For both oleic acid and triolein, as pressure increases at constant temperature solubility increases. However, the impact of temperature at isobaric conditions depends on vapour pressure. At each temperature and pressure used for impregnation, the estimated solubility of oleic acid is greater than that for triolein, or flax oil. It has been reported that increased impurities will affect solubility, often negatively, while introduction of double bonds changes values only slightly, indicating that the solubility of flax oil, which is a mixture of numerous triglycerides and other minor components, is possibly even less than that of pure triolein [34].

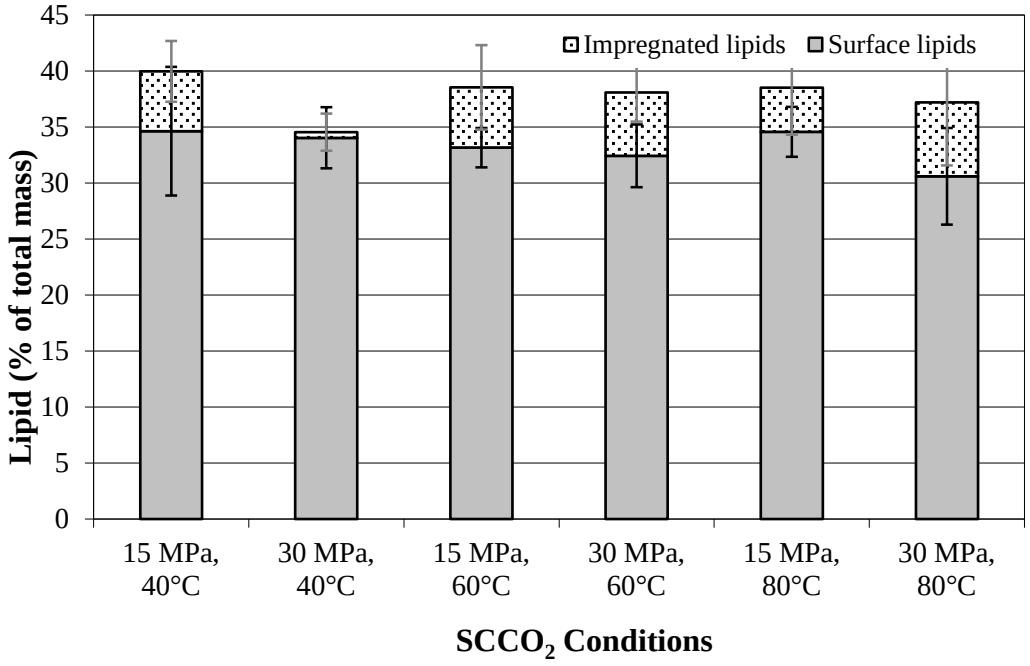


Figure 4.5: Flax oil, as a % of total particle mass, impregnated in and on the surface of pregelatinized starch using $SCCO_2$ under different conditions (n=3).

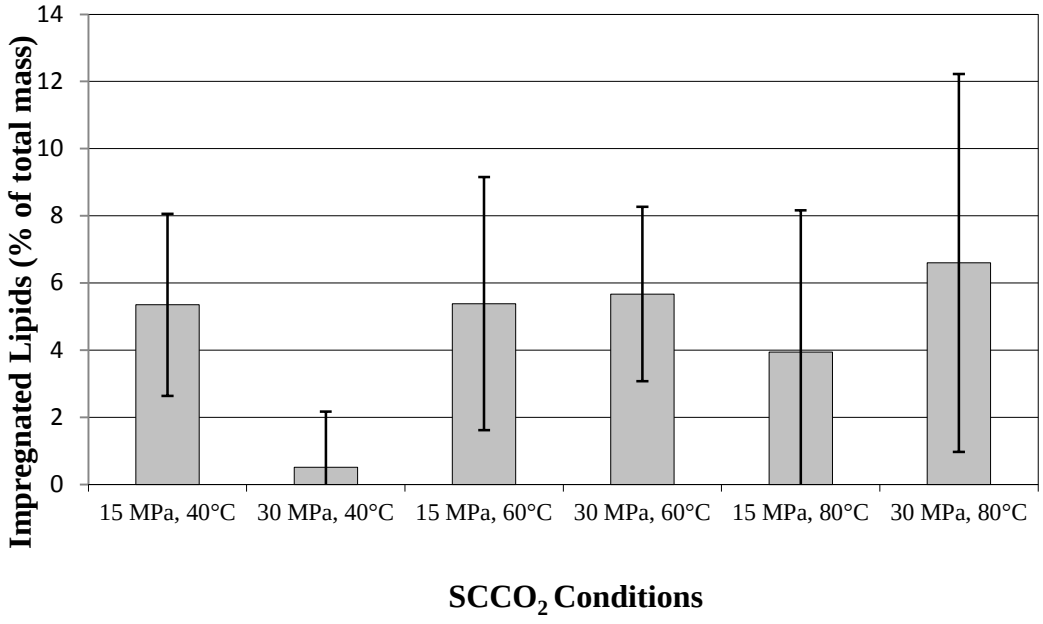


Figure 4.6: Flax oil, as a % of total particle mass, impregnated in pregelatinized starch using $SCCO_2$ under different conditions (n=3).

Once the oil is dissolved in $SCCO_2$, the mixture contacts and diffuses through the PGS particles. The diffusion coefficients for lipids have been estimated using supercritical fluid chromatography and the Taylor-Aris peak broadening technique [35]. Diffusion coefficients are affected by temperature and pressure, as well as position and number of double bonds. The diffusion coefficient of lipids decreases with increasing pressure and increases with increasing temperature. At the conditions studied, 36 MPa and 50 ^{o}C, the diffusion coefficient for oleic acid was $3.57\pm0.18 \times 10^{-9}$ m^2/s, while that for triolein was only $1.91\pm0.09 \times 10^{-9}$ m^2/s. This difference influences the impregnation efficiencies of oleic acid vs. flax oil.

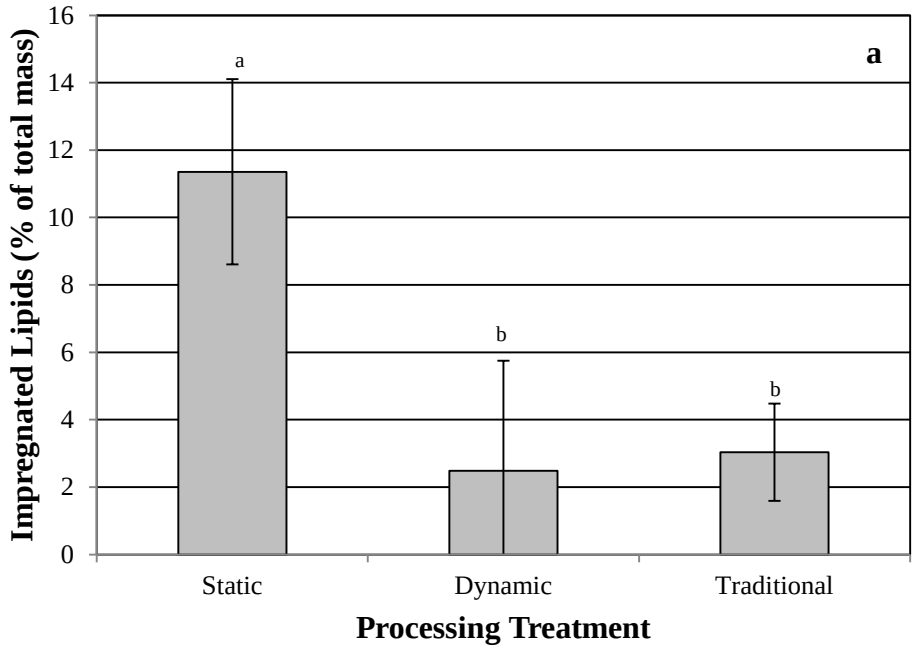
Taking into consideration the solubility and diffusion information as summarized above for oleic acid and triolein, it is expected that the highest impregnation efficiencies for both lipids would be a result of the highest solubility and largest diffusion coefficient. For solubility, these conditions occur at 30 MPa and 40 ^{o}C and in terms of diffusion at 15 MPa and 80 ^{o}C. For oleic acid, the difference between the highest temperature and the lowest temperature was not significant at constant pressure and impregnation efficiency significantly increased as pressure decreased. This suggests that at 40 and 80 ^{o}C, solubility did not limit impregnation, and therefore, conditions which favoured high diffusion were optimum. At 15 MPa, the solubility of oleic acid at 60 ^{o}C is estimated to be less than that at 40 or 80 ^{o}C, suggesting that perhaps in this case, solubility did limit impregnation. For flax oil, neither temperature nor pressure had a significant effect. While not significant, the highest impregnation efficiency was obtained at

30 MPa and 80 ^{o}C, suggesting that both low solubility and diffusivity play a limiting role in impregnation.

To be impregnated, the lipid would have to remain inside the starch granule. Lipids could deposit themselves into the granules and remain there due to a higher affinity to the starch than to the $SCCO_2$. During the pressure hold, the lipid could form a complex with the starch components, such as amylose, thereby remaining in the granules. This may be the case with the oleic acid forming an amylose-lipid complex, but it is unlikely with the flax oil, due to the larger size and structure of the triglyceride molecules [36]. Further studies, determining the time it takes for oleic acid and flax oil dissolved in $SCCO_2$ to pass through the packed bed of PGS may add not only to the understanding of mass transfer mechanisms involved in PGS impregnation, but also to the study of possible interactions. Other spectroscopy techniques have been used previously to determine interactions between lipids and starch, such as Fourier-transform infrared spectroscopy or differential scanning calorimetry and may be beneficial for assessing the interactions between oleic acid and flax oil with PGS in future studies [37, 38]. Alternately, the lipids may be deposited into the granules during the depressurization step. As the pressure and temperature drops within the cell, $SCCO_2$ loses solvent power and the lipids precipitate out of the fluid. Whichever lipid components were in the starch granules at the time of depressurization would precipitate and remain within the starch granules.

Comparing the impregnation efficiencies of the three techniques tested, static $SCCO_2$, where the pressure was held for 8 h, dynamic $SCCO_2$, where

continuous flow was maintained, and a “traditional” organic solvent impregnation, once again, impregnation efficiency depended greatly on the type of lipid used. For oleic acid, impregnation efficiency was significantly greater ($p \leq 0.05$) for the static method than it was for the dynamic and traditional methods (Fig. 4.7a). However, for flax oil, high variations within replications of the same method, especially in the case of dynamic impregnation, resulted in no significant difference ($p > 0.05$) between the three techniques even though there was a similar trend of static method resulting in higher impregnation efficiency (Fig. 4.7b). In the case of dynamic impregnation, to contact the starch placed after the stainless steel filter frit, the lipid must be solubilized and carried by the $SCCO_2$. Lower solubility, in the case of the flax oil, would result in less oil contacting the starch at any one time. Dynamic impregnation may have also resulted in a lower impregnation of oleic acid, in relation to the static method, due to a decrease in residence time. Even though flow could encourage mixing, increasing mass transfer, it also decreases contact time of the lipid with the starch. Given insufficient affinity of the lipid for the starch, the oil would stay in the $SCCO_2$ phase and simply be carried out of the system. Difficulty in maintaining a steady flow over the 8 h period, mainly due to the presence of lipids in the small opening of the depressurization valve, may have resulted in the high variation observed. For the traditional method, because diffusion in the hexane medium is much slower than that in $SCCO_2$, there was less mass transfer of lipids, especially for flax oil, from the organic phase into the starch. This method, of course, also has the downside of utilizing an organic and non-food grade solvent.



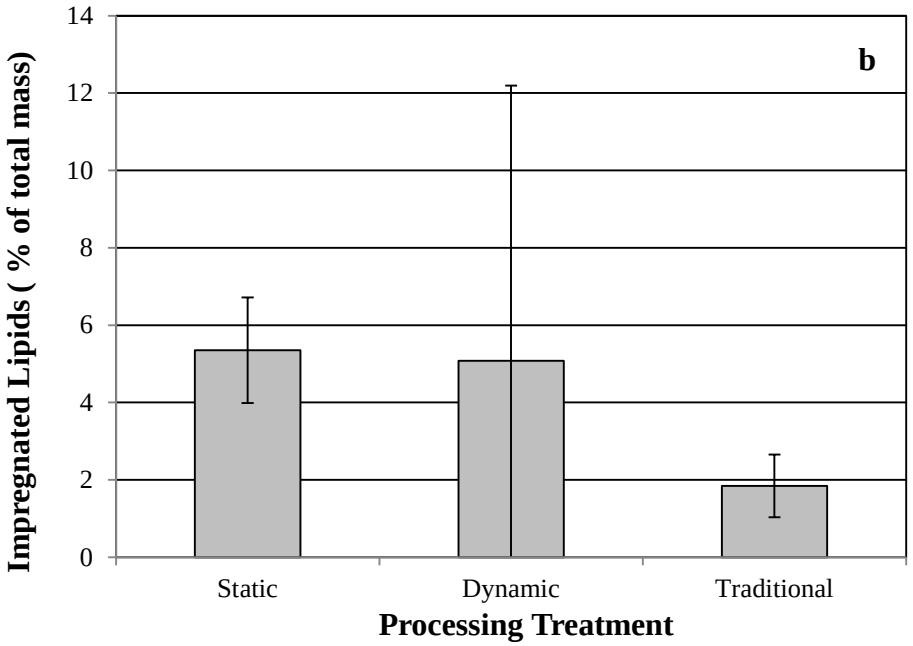


Figure 4.7: Lipids, as a % of total particle mass, impregnated in pregelatinized starch using different techniques, a) Oleic acid, b) Flax oil. Means with the same letter are not significantly different at $p > 0.05$ (n=3).

The structure of the pregelatinized starch caused some difficulty in the distinction between surface lipids vs. impregnated lipids. Pregelatinized starch is produced by gelatinizing the native starch in the presence of water and heat, and then drying. The gelatinization process loosens the organization of the amylose and amylopectin molecules in the starch granule, where amylose leaches out of the granule and the amylopectin crystals melt. Several methods of surface washing were attempted prior to the hexane wash described above was chosen. SEM and confocal laser scanning microscope images of PGS impregnated with oleic acid and flax oil under different conditions are presented in Figures 4.8 and 4.9, respectively, before and after surface wash. Even though no distinction could be made due to processing temperature and pressure, a layer of lipid can be seen on the granules, especially those with flax oil, prior to the surface wash (Figs. 4.8 a, c, e, g and 4.9 a, c). After the surface wash (Figs. 4.8 b, d, f, h and 4. 9 b, d), this layer is no longer there and the granules resemble those of the control. Although it is possible that some of the impregnated lipids were also removed during this surface wash, it is assumed that those on the surface were mostly removed. Although pregelatinized starch was used rather than native starch because it was originally hypothesized that it would swell to a greater extent and allow for greater diffusion of $SCCO_2$ and oil, perhaps the use of native starch would reduce the difficulty in separating surface lipids and impregnated lipids. Obviously, if the impregnated starch were to be used as a delivery vehicle for lipids, the reduction of the surface lipids would be desirable. Surface lipids are unprotected from oxygen and therefore highly prone to oxidation. “Washing” the

starch with neat $SCCO_2$ after the impregnation process may help to remove surface lipids, as long as the conditions, including flow rate, are mild enough not to disturb the lipids impregnated within the particles. It has been suggested that such an approach would decrease experimental error by reducing variation in solute condensation during depressurization [39]. Because the washing step could remove solute, which is already impregnated within the carrier matrix, using an inert gas to wash the sample bed was also suggested [39]. Stability of impregnated lipids is an essential parameter and storage studies testing for the extent of oxidation have to be performed prior to the utilization of starch as a delivery vehicle.

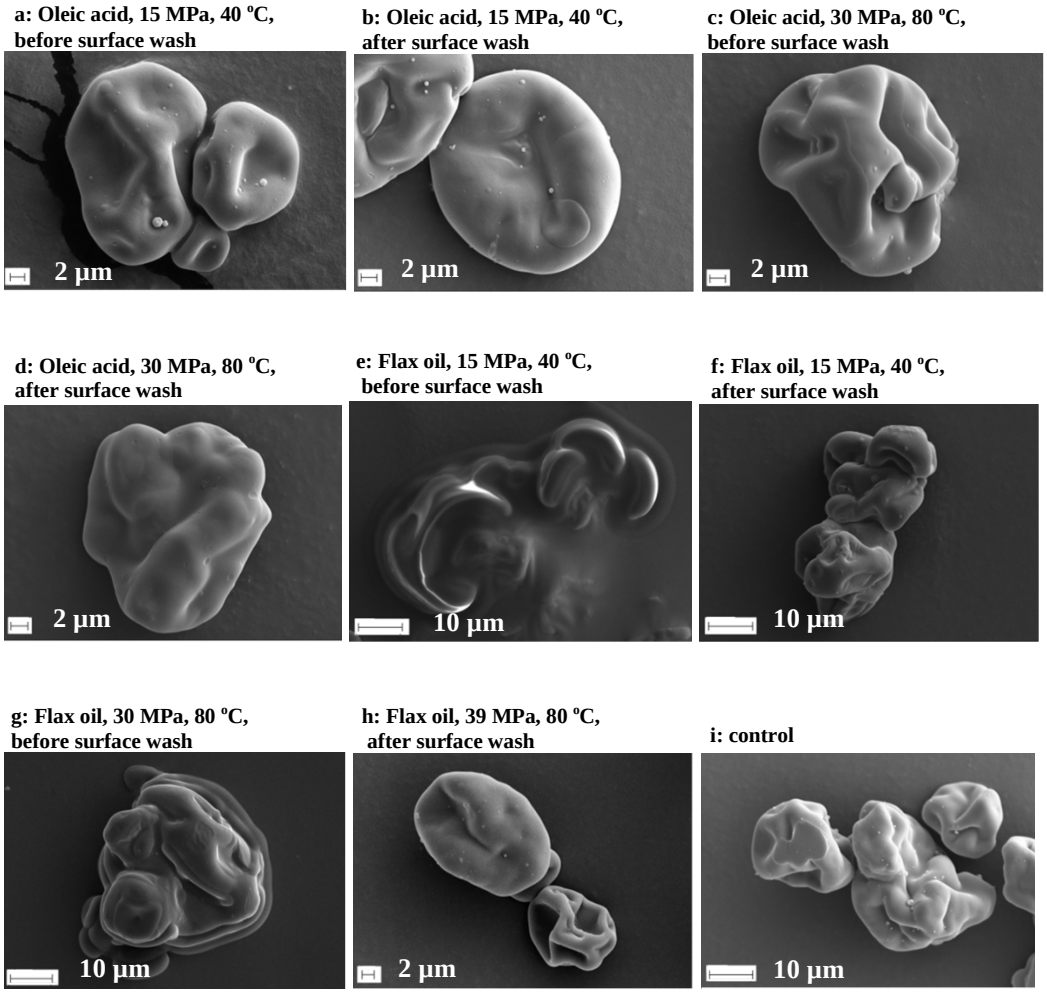


Figure 4.8: Scanning electron microscope images of pregelatinized corn starch a) oleic acid, 15 MPa, 40 ^{o}C prior to surface wash, 2.43kX mag, b) after surface wash, 2.68kX mag, c) oleic acid, 30 MPa, 80 ^{o}C prior to surface wash, d) after surface wash, 2.83kX mag, e) flax oil, 15 MPa, 40 ^{o}C, prior to surface wash, 1.45kX mag, f) after surface wash, 1.49kX mag, g) flax oil, 30 MPa, 80 ^{o}C prior to surface wash, 1.4kX mag, h) after surface wash, 2.01kX mag and, i) control, 1.48kX mag.

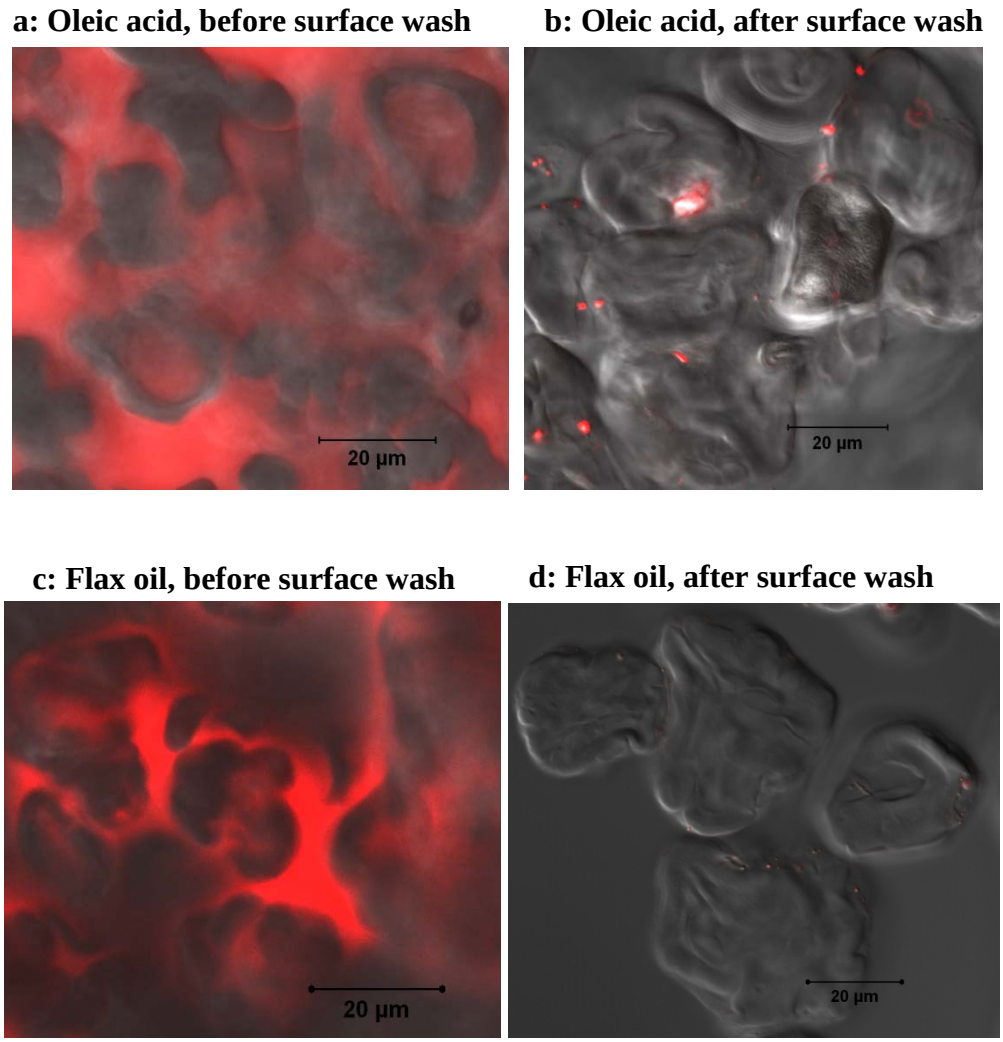


Figure 4.9: Confocal laser scanning microscope images of oil on the surface of, and impregnated in pregelatinized starch at 15 MPa and 40 ^{o}C a) Oleic acid prior to surface wash, b) Oleic acid after surface wash c) Flax oil prior to surface wash, d) Flax oil after surface wash.

4.4. Conclusions

Pregelatinized starch was used as the carrier to impregnate lipids such as oleic acid and flax oil using $SCCO_2$. It was found that simple lipids of lower molecular weight and higher purity, such as oleic acid, are impregnated with higher efficiency than more complex lipid mixtures, such as flax oil, mainly due

to the higher levels of solubility and mass transfer properties of simple lipids. For oleic acid, temperature, pressure and impregnation method had a significant effect on the amount of lipid that could be deposited within the pregelatinized starch particles. When solubility did not limit impregnation, conditions which favoured higher diffusivity prevailed. For flax oil, however, it is likely that both low solubility and diffusivity played a limiting role in its impregnation under the conditions investigated. The results suggest that supercritical fluid impregnation of biopolymers is a potential means for nutraceutical delivery. However, for liquid lipids, prone to oxidation, further improvements are possible and necessary.

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5. Barley β-Glucan Aerogels via Supercritical Carbon Dioxide Drying[4]

5.1. Introduction

Aerogels, formed by the replacement of the liquid solvent in a gel with air, have become popular due to their many unique and beneficial properties. Aerogels, especially the most commonly investigated silica-based gels, have very low densities, as low as 3 times the density of air, high specific surface areas, porosities and shock absorption, and low thermal conductivities, indices of refraction and sound propagation [1-3]. These properties make aerogels useful in a variety of applications, including, but not limited to, radiation detectors, electronics, thermal insulators, filter materials, heat storage devices, storage media for gases in fuel cells, carriers for catalysts and dust scavengers [1, 2, 4]. Originally discovered by Kistler in the 1930's [4], aerogels can be dried to remove their pore solvent in several ways. For example, freeze drying or lyophilization removes the solvent using sublimation, producing a cryogel. On the other hand, supercritical carbon dioxide ($SCCO_2$) has become a popular drying agent in recent years [5-7]. If miscible, $SCCO_2$ reduces the surface tension of the pore solvent and dries the gel with relatively less cracking and deformation than that prevalent in thermal, vacuum or freeze drying [2].

Aerogels are typically prepared using inorganic compounds, such as SiO_2 [7, 8]. While silica is the most widely used and studied aerogel building block [5, 9], organic aerogels have been prepared using materials such as cellulose [8, 10-

[4] A version of this chapter has been submitted for publication to Carbohydrate Polymers.

12]. The majority of aerogels investigated, including those from cellulose, are either composed of or require during processing materials which are not biodegradable, biocompatible and renewable and thus cannot be used in edible applications. Many other non-cellulosic, food-grade polysaccharides form hydrogels and are therefore excellent candidates as alternative aerogel building blocks. Thus far, aerogels have been created using chitin [13, 14], alginate [6], agar [15] and starch [16-18].

β-Glucan is a polysaccharide polymer composed of β-*D*-glucopyranosyl units joined by β(1-3) and β(1-4) glycosidic linkages. It is found in grains, especially oats and barley, at typical concentrations of 3 - 7% of grain weight [19, 20]. β-Glucan has received much attention for its health promoting properties, including reducing plasma cholesterol and postprandial serum glucose levels, mainly due to its high viscosity. Based on numerous clinical trials demonstrating its beneficial effects, the US Food and Drug Administration has approved a health claim that soluble fiber from oats and barley can reduce the risk of heart disease [21, 22]. It has also been suggested that it can be used as a thickener and stabilizer in food products and biomedical applications, and it has been used to make biodegradable, edible films [23, 24]. Burkus and Temelli [25] found that, when combined with water, β-glucan formed a gel at concentrations greater than 5% (w/w) with strength greater than that of corn starch gel at the same concentration. This gelation ability makes the β-glucan polymer a potential candidate for aerogel formation, which could be used for edible applications, such as carriers for drugs or nutraceuticals. It is important to note, however, that gelation ability depends on

the molecular weight of the β-glucan used. Lazaridou *et al.* [26] examined the gelation of various molecular weight fractions of oat β-glucan. It was found that while samples an average molecular weight of 35 to 140 kDa would form gels, samples with a higher molecular weight of 250 kDa, showed no tendency to gel under similar conditions, likely due to decreased mobility of longer chains [26].

The main goal of this study was to form aerogels from barley β-glucan at several concentrations using $SCCO_2$ drying, as well as air drying and freeze drying. The specific objective was to determine the effect of gel concentration and drying technique on the resulting characteristics of the aerogels. It was hypothesized that if a β-glucan hydrogel can be formed, then it should be possible to dry the gel using $SCCO_2$ to obtain a low density aerogel.

5.2. Materials and Methods

5.2.1. Hydrogel Formation

Low-molecular weight barley β-glucan concentrate was obtained at a pilot plant as described by Burkus and Temelli [19]. The average molecular weight of β-glucan was 198,000 Da, determined by using intrinsic viscosity measurements [19]. The β-glucan concentration was 83.3% (dry matter basis) as characterized previously [19]. The remaining portion of this concentrate was composed of 1.20±0.17% starch, 1.49±0.02% protein, 4.13±0.11% ash and 0% free glucose and lipids with other non-starch carbohydrates making up the balance [19].

The method of Burkus and Temelli [25] was used to form 5, 6 and 7% (w/v) hydrogels from the β-glucan concentrate, corresponding to 4.2, 5 and 5.8% pure β-glucan, respectively. β-Glucan concentrate was added to 50 mL of distilled

water and stirred to disperse and hydrate. The mixture was then covered with Al foil to reduce water loss, heated with constant stirring until boiling, and then allowed to boil for 5 min. After boiling, the temperature was reduced to 75 ^{o}C and the mixture was stirred for an additional hour. The mixture was then spooned into 5 or 10 mL plastic syringes, with the tips removed, covered with parafilm to prevent moisture loss and allowed to set overnight at ambient conditions. Five mL syringe-molds were only used in the case of 7% gels dried directly from the hydrogel stage, using $SCCO_2$ + ethanol as a co-solvent. In this case, gels were removed from the mold, with a diameter of 1 cm, and cylinders of 1 cm in length were cut. For all other gels, 10 mL syringe molds, with a diameter of 1.4 cm were used and cylinders of 2 cm in length were cut. Smaller cylinders were required for the gels dried using $SCCO_2$ + ethanol to fit into the $SCCO_2$ cell, as the ethanol-solvent exchange step was eliminated, also eliminating the shrinkage incurred during this process.

5.2.2. Gel Drying

5.2.2.1. Air-Dried and Freeze-Dried Gels

Five, 6 and 7% hydrogels underwent a solvent exchange process to replace the water with ethanol. Gels were placed in 50 mL baths with increasing concentrations of 20, 40, 60 and 80% (v/v) ethanol with a residence time in each bath of 1 h and then in a 50 mL 100% ethanol bath overnight. Conversion of hydrogels to alcogels occurred under ambient conditions. Alcogels were then measured, weighed and allowed to dry, uncovered, under ambient conditions overnight. Once all solvent had been removed, as evidenced by a constant weight

being reached, samples were once again measured and weighed. All experiments were done in triplicate. Moisture content of the gels was determined by drying them overnight in a 105 ^{o}C convection oven.

For freeze dried samples, cut gels were arranged on stainless steel sheets, covered with aluminum foil, and frozen at -18 ^{o}C overnight. The frozen samples were then freeze dried (Virtis Co., Gardiner, NY, USA) over a 24 h period at a pressure of 85 Pa and a shelf temperature of -3 ^{o}C. All experiments were done in triplicate.

5.2.2.2. $SCCO_2$-Dried Gels

Five, 6 and 7% hydrogels samples intended for drying with pure-$SCCO_2$ underwent a solvent exchange process as described previously (Section 5.2.2.1) to convert hydrogels to alcogels at ambient conditions. Seven percent gels to be dried using $SCCO_2$ + ethanol as a co-solvent were not subjected to the ethanol solvent exchange process. Gels were then measured and weighed, and placed into a 25 mL stainless steel cell, with a diameter or 1.4 cm, which was reduced in volume to approximately accommodate twice the length of the alcogel, using a stainless steel frit (2 µm pore size) divider. The remainder of the cell was filled with 3 mm glass beads. The cell was sealed at both ends using stainless steel filter frits and placed into a supercritical extractor, described in Chapter 3 (Section 3.2.4). The cell was pressurized with CO_2 (99.9% purity, Praxair, Edmonton, AB, Canada) to 15 MPa and the temperature inside the cell was held at 40 ^{o}C. Once the desired pressure and temperature had been reached, the conditions were held for 1 h, at which point CO_2 flow was started and regulated at approximately 1 L/min

(measured at ambient conditions) for 4 h using a heated micro-metering valve. Drying time of 4 h was selected based on preliminary studies, ranging from 0.5 to 24 h, indicating that 4 h of drying resulted in consistently dried gels. After 4 h of $SCCO_2$ flow, the CO_2 compressor and tank were turned off and the system was allowed to depressurize at a maximum rate of 1 L/min until tank pressure of 6.2 MPa was reached, at which point the flow was increased to minimize depressurization time. Samples were held in zipper lock sealed bags overnight to allow any CO_2 inside the aerogels to dissipate before being measured and weighed.

Gels dried with $SCCO_2$ with an ethanol co-solvent were placed in the extractor at the hydrogel stage. Ethanol was pumped into the $SCCO_2$ prior to entry into the extraction cell, using a separate HPLC pump (Model 1330, Brio-Rad Laboratories Ltd., Mississauga, ON, Canada) at a flow rate of 0.3 mL/min for 5 h, at which point ethanol flow was stopped and only $SCCO_2$ was delivered for an additional hour.

5.2.3. Aerogel Characterization

Density at each stage of the process, hydrogel, alcogel and aerogel, was approximated by dividing the mass of the gel by its volume. From the measurement of dimensions, changes in volume at each stage were also calculated.

Internal and external network structure was assessed using Scanning Electron Microscopy (SEM) (Zeiss EVO MA 15, Toronto, ON, Canada). Samples were first carefully scored around their circumference using a razor blade before

being gently cut in half and mounted with either the external or internal surface facing upwards, then sputter coated with gold (Xenosput XE200, Edwards High Vacuum, Crawley, UK) prior to microscopic analysis.

Pore size, volume and Brauner-Emmett-Teller (BET) surface area of 5, 6 and 7% $SCCO_2$-dried aerogels were determined using nitrogen adsorption and desorption isotherms (Quantachrome Autosorb 1MP AS1-MVP-9, Boynton Beach, FL, USA). Samples were cut at 0.5 cm with a mass of approximately 0.08 g. Samples were then heated to 115 ^{o}C for approximately 4 h under vacuum to degas, prior to analysis. For multi-point BET surface area, adsorption characteristics at a relative pressure of $p/p_o<0.5$ were evaluated, where p and p_o represent sample pressure and saturated vapor pressure, respectively. For pore size distribution, desorption characteristics at a relative pressure of $p/p_o \geq 0.35$ were evaluated, as per Mehling *et al.* [17].

To evaluate the colour of the aerogels, *L*, *a*, *b* colour scores were measured using a Konica Minolta colourimeter (CR410, Mississauga, ON, Canada). Samples were placed on a solid black colour tile and measurements were taken 3 times, turning the sample 90 ^{o} each time, and the average was reported.

5.2.4 Statistical Analysis

Analysis of variance of the results was performed using the same methodology described in Chapter 4 (Section 4.2.3). Where comparisons between only 2 samples were made, a *t*-test was performed at the $\alpha = 0.05$ level.

5.3. Results and Discussion

Formation of an aerogel first requires the formation of a cross-linked polymer network surrounded by a liquid solvent. β-Glucan of sufficiently low molecular weight to allow mobility of polymer chains in solution form a physically cross-linked 3-D network, with inter and intra-chain hydrogen bonds [27]. Long chains of consecutive β(1→ 4) linkages associate similar to cellulose and act as junction zones [28]. Although high molecular weight β-glucans form high viscosity solutions and provide more potential cross-linking locations, their mobility is less, preventing gel formation [26].

5.3.1. Effect of Drying Technique on Aerogel Formation

Both freeze drying and $SCCO_2$ drying of the gel yielded a low density 5% aerogel (0.17 and 0.20 g/cm^3, respectively), with densities similar to those of 8% chitin, 0.171 - 0.175 g/cm^3 [13], but lower than those reported for 12.5% potato and 12.5% Eurylon7 starch, 0.46 and 0.34 g/cm^3, respectively [17]. The air-dried aerogels had a significantly higher density (0.67 g/cm^3) than those resulting from the other two drying methods ($p \leq 0.05$) (Table 5.1). Although the freeze-dried whole samples have a low density, this may be misleading because when the gels were cut in half for SEM imaging the freeze dried samples had a hollow void in the center, while the other aerogels were homogenous. The difference in the packing of β-glucan polymers can be seen in the SEM images of the interior of the aerogels (Fig. 5.1 b, d and e). While the $SCCO_2$-dried aerogels showed a more spongy network formation, this was not present in the air-dried or freeze-dried samples, in which a more continuous, uniform surface was observed. The ability

of $SCCO_2$ to swell polymers is well known [29] and it is possible that during drying $SCCO_2$ diffused between the polymer chains, creating a physical barrier to the dense packing of polymers.

Table 5.1: Properties of 5% β-glucan aerogels prepared using different drying techniques

Treatment	Density (g/cm^3)	Aerogel Volume (% of hydrogel volume)	Aerogel Mass (% of hydrogel mass)	Aerogel volume (% of alcogel volume)	Aerogel mass (% of alcogel mass)	Colour Score		
						L	*a*	*b*
Freeze Dried	0.17 ±0.02[a]	38.77 ±3.81[a]	5.73 ±0.02[a]	----	-----	61.35 ±1.29[a]	0.55 ±0.04[b]	0.62 ±0.03[a]
Air Dried	0.67 ±0.15[b]	10.86 ±1.55[b]	6.50 ±0.24[b]	22.93 ±2.34[a]	18.14 ±0.83[a]	62.14 ±2.33[a]	0.85 ±0.08[c]	1.94 ±0.15[b]
$SCCO_2$ Dried	0.20 ±0.004[a]	37.62 ±3.26[a]	6.28 ±0.12[a]	77.28 ±14.48[b]	17.56 ±0.68[a]	64.16 ±0.53[a]	0.43 ±0.04[a]	0.61 ±0.12[a]

[a-c] Values within columns followed by the same superscript letter are not significantly different at $p > 0.05$.

$SCCO_2$ drying without shrinkage, deformation and cracking requires a pore liquid with a sufficiently low critical temperature and pressure or high solubility in $SCCO_2$ to prevent the existence of a liquid-gas interface. Water has a high surface tension, high critical temperature and pressure and a low solubility in $SCCO_2$ compared to solvents like ethanol and acetone. Capillary forces created by water are strong and can result in a damaged aerogel [2, 30]. While water is not a preferred solvent of gel formation intended for $SCCO_2$ drying, polysaccharides are not soluble in solvents, which are highly miscible in $SCCO_2$ and will precipitate in ethanol. Once a polysaccharide gel is formed, water is exchanged

for a more favourable solvent by storing or washing the gel in ethanol/water mixtures of increasing ethanol concentrations. The mechanism for removal of ethanol from the gel via $SCCO_2$ has been proposed in detail elsewhere [5].

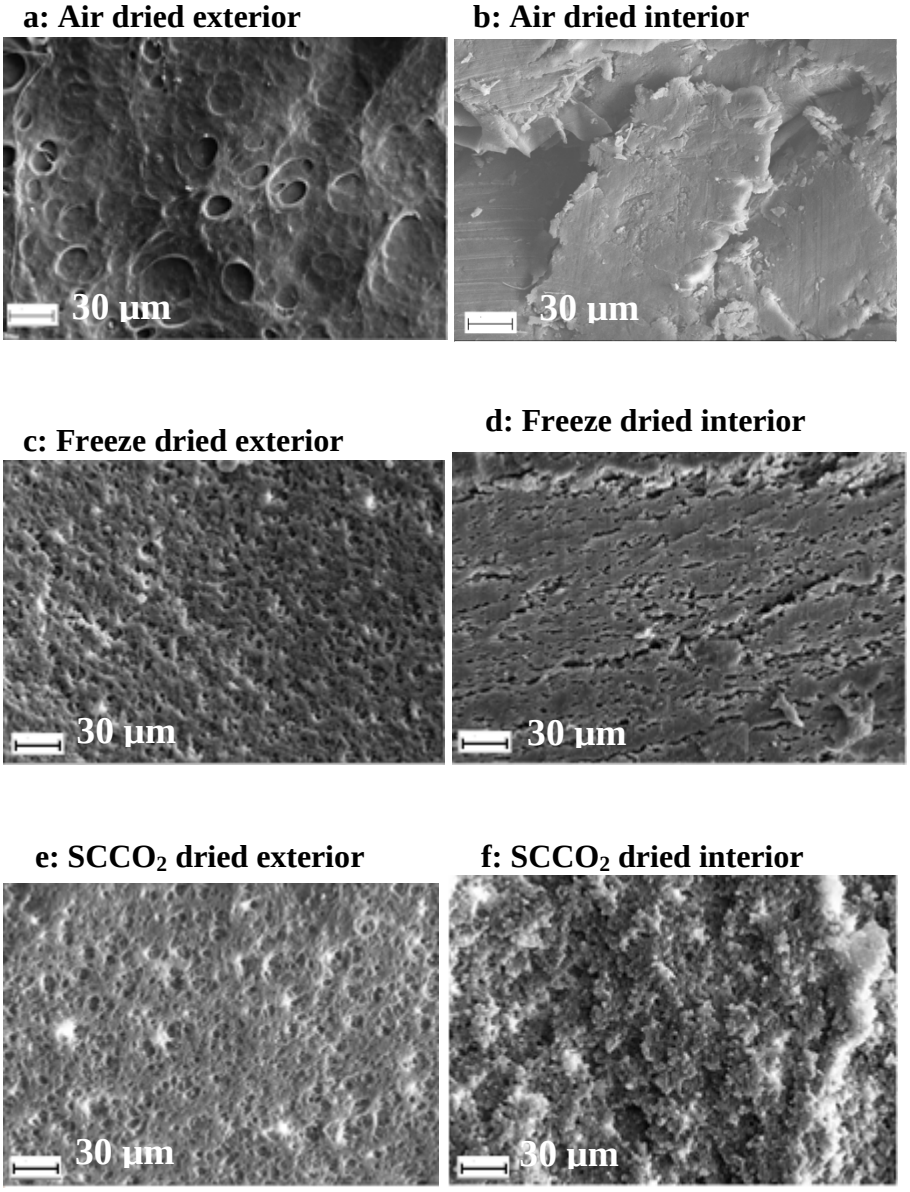

Figure 5.1: SEM micrographs of 5% β-glucan aerogels at 1000 x magnification a) air dried exterior and b) interior, c) freeze dried exterior and d) interior, e) $SCCO_2$ dried exterior and f) interior.

Examining the aerogel volume as a percentage of hydrogel volume showed that considerable shrinkage occurred for all three drying techniques (Table 5.1). Shrinkage during storage is a common phenomenon in edible gels. As a β-glucan gel ages, density of junction zones increase, resulting in water being expelled from the fiber network, called syneresis [31]. Although the open network structure of all β-glucan gels will undergo syneresis, it has been found that low molecular weight β-glucan (176 kDa) is more prone to this shrinkage than the high molecular weight β-glucan, which is still within the limits of gelation (below 250 kDa) [31]. Comparing the change in volume of the $SCCO_2$ aerogel between the hydrogel and aerogel stages, and the change in volume between the alcogel and the aerogel (Table 5.1), it is evident that the majority of the shrinkage occurred during solvent exchange and not during $SCCO_2$ drying. During the ethanol exchange, water is replaced with ethanol, reducing the number of hydrogen bonds between the β-glucan polymers and the water molecules. In the alcogel and aerogel states, the β-glucan network is held together by the intermolecular hydrogen bonds between individual β-glucan strands. This increase in intermolecular polymer junction zones results in volume loss and an increase in network density.

Because β-glucan is not soluble in ethanol, the solvent exchange process does seem to have a hardening effect on the gel as the β-glucan precipitates, especially on the surface, as experienced when handling the alcogels, along with a shrinking effect. The solvent exchange process also causes a lightening of colour of the parts of the gel in direct contact with ethanol. The difference in network

structure on the surface versus the interior can be seen in Figures 5.1 and 5.2. Considering the change in volume of the air-dried aerogel (Table 5.1), it is obvious that this hardening effect did not impart an increase in mechanical strength capable of preventing a high level of shrinkage and therefore resulting in a high density relative to the $SCCO_2$-dried sample. These findings were in agreement with those of Hæreid *et al.* [32].

The three drying techniques used resulted in visible colour differences. Colour measurements were performed on a black calibration tile to maintain measurement consistency, as the lens of the colourimeter was larger than the gel samples. Therefore, colour parameters should be compared relative to one another and should not be taken as absolute. The β-glucan concentrate powder used to prepare the hydrogels had a light brown colour, which was similar to that of the freeze-dried samples. The $SCCO_2$–dried samples appeared almost colourless and this lack of colouration was very consistent, unlike the air-dried gels, which appeared mottled. *L, a, b* colour scores (Table 5.1) show that the freeze-dried and air-dried gels were slightly darker (lower *L* value), although this difference was not significant ($p > 0.05$). Freeze-dried and air-dried samples were significantly more red than the $SCCO_2$ samples, with the air-dried sample being the most red (higher positive *a* value) and the air-dried sample was significantly more yellow than the other samples (higher positive *b* value). Studies on the effect of β-glucan addition on the colour of baked goods reveal that β-glucan will cause a noticeable difference in appearance, mainly a decrease in *L* value, or a darkening of the product, but also an increase in *a* value, or redness [27]. The light brown colour of

the β-glucan concentrate is likely due to traces of phenolic compounds, which are extracted from barley flour during the concentration process. It is possible that, when combined with ethanol, these compounds are extracted by $SCCO_2$ during the drying of aerogels. Haimer *et al.* [33] found that in the $SCCO_2$ anti-solvent precipitation of hemicellulose using dimethyl sulfoxide as a co-solvent, the precipitated product was less brown in colour compared to the starting material, due to the extraction of lignins. To our knowledge, a change in colour between a $SCCO_2$-dried aerogel and a freeze-dried or air-dried gel has not been documented. However, a change in transparency has been noted. It has been shown that low pressure drying methods have resulted in a decreased transparency of silica-based gels, compared to those dried using $SCCO_2$ [34]. In addition, as the density of the β-glucan is reduced, from the powder form to the aerogel form and the area of the interface increases, light scattering increases, which will increase the *L* value. It has been shown that for porous materials, an increase in the porosity or void volume per unit mass increases light scattering [35].

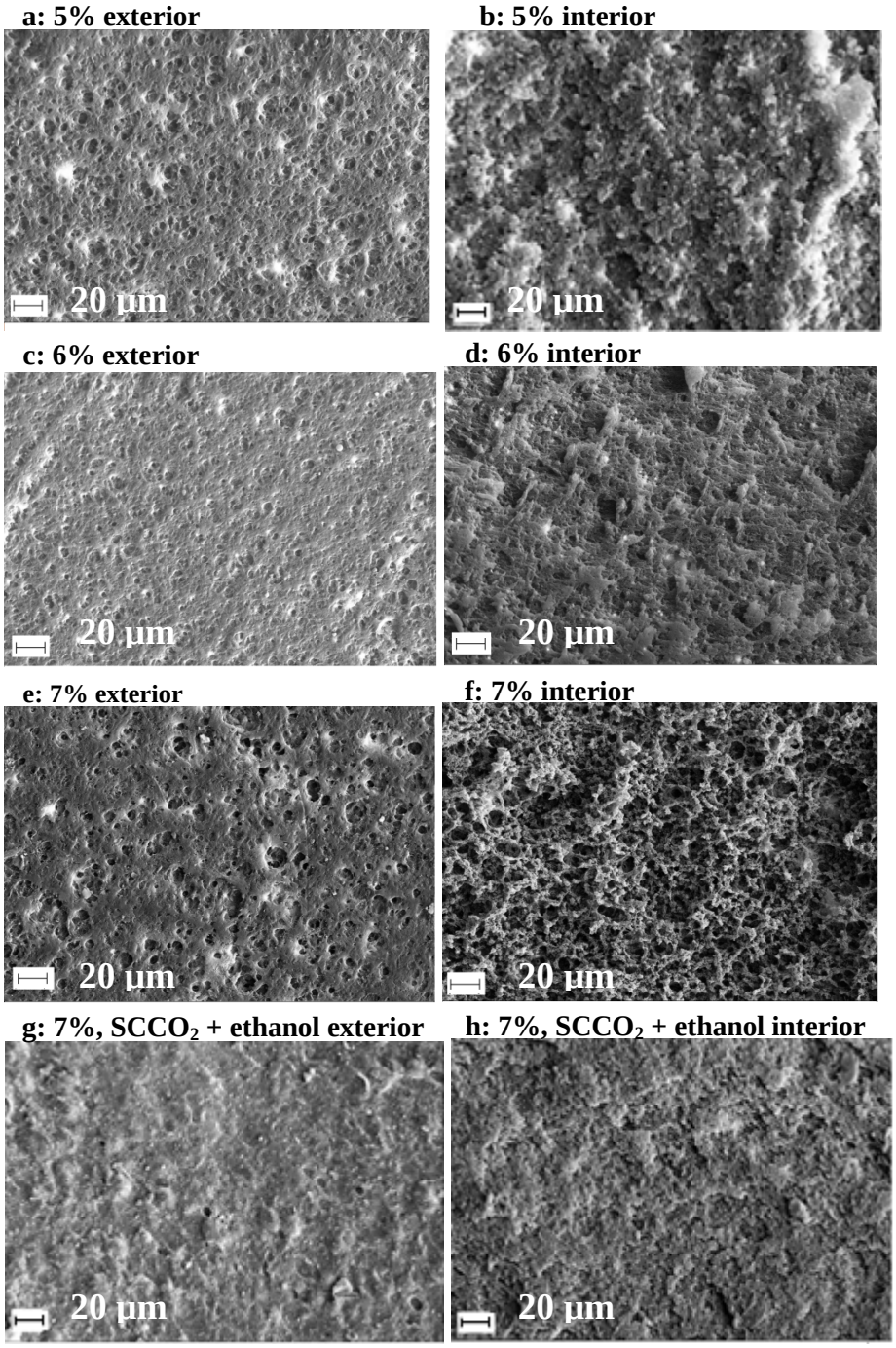

Figure 5.2: SEM micrographs of β-glucan aerogels at 1000 x magnification: a) 5% $SCCO_2$ dried exterior and b) interior, c) 6% $SCCO_2$ dried exterior and d) interior, e) 7% $SCCO_2$ dried exterior and f) interior, and g) 7% $SCCO_2$ + ethanol dried exterior and h) interior.

Strength and rate of gelation have been found to depend on both concentration and molecular weight of β-glucan; increasing either results in a high probability of contact and formation of junction zones [28]. Burkus and Temelli [25] reported that increasing the β-glucan concentration from 5 to 5.5% resulted in a 2.5 times greater ability to withstand stress. Despite this, concentration was found to be mostly insignificant for the parameters given in Table 5.1, and thus only values for 5% gels are reported for each drying method. The *a* value was significantly affected by concentration, but only for the air-dried samples. Air-dried gels at 5% were less red than at 6% ($p \leq 0.05$), but 7% was not significantly different from either concentration. The effect of β-glucan concentration on the mass of the aerogel as a % of hydrogel mass was significant ($p \leq 0.05$) for all treatments, which is expected given that it is a measurement of the mass of β-glucan present in the original gel. Because of this, it was expected that the effect of concentration on density would be significant as well. However, possibly due to slight, although not significant, differences in the volume of the aerogels as a % of hydrogel volume, this was not observed.

5.3.2. Effect of Ethanol Co-solvent on $SCCO_2$ Drying

Although ethanol has been shown to cause shrinkage, water has also proved detrimental in aerogel formation. Using $SCCO_2$ + ethanol as a co-solvent during the drying step can increase the extraction of polar compounds and increase the solubility of water [36]. Therefore, drying of hydrogels using $SCCO_2$ + ethanol was attempted, avoiding the solvent exchange step. Overall volume change was not significantly different between the $SCCO_2$ and $SCCO_2$ + ethanol

samples and there was no significant difference in density ($p > 0.05$) (Table 5.2) despite the fact that $SCCO_2$ + ethanol aerogels had almost a 2-fold greater average density than the $SCCO_2$ samples. However, for density, $p= 0.051$, which is just outside of the $\alpha= 0.05$ level of significance. It is likely though that gels dried with $SCCO_2$ + ethanol, like the freeze-dried gels, actually have a higher density, once structural inconsistencies are taken into consideration. Aerogels dried with $SCCO_2$ + ethanol as a co-solvent exhibited significant cracking and deformation, again due to the presence of water in the liquid phase (Fig. 5.3). Brown *et al.* [15] found that agar gels dried using $SCCO_2$ + ethanol as a co-solvent showed visible foaming at the gel surface. It was hypothesized that ethanol disrupts bonding in tertiary structure helix aggregates, destabilizing the structure [15]. While foaming was not evident in the β-glucan gel images (Figs. 5.1 - 5.3), structural damage did occur and could be clearly seen.

Table 5.2: Properties of 7% β-glucan aerogels prepared using $SCCO_2$ or $SCCO_2$ + ethanol

Treatment	**Density (g/cm^3)**	**Aerogel volume (% of hydrogel volume)**	**Aerogel mass (% of hydrogel mass)**	**Colour Score**		
				L	***a***	***b***
$SCCO_2$	0.23±0.04	32.45±3.16	7.72±0.17	62.66± 3.43	0.45± 0.04	0.61± 0.03
$SCCO_2$ + Ethanol	0.40±0.05	30.41±3.94	9.26±1.35	60.38± 0.66	0.49± 0.01	0.72± 0.15

Figure 5.3: β-Glucan aerogels: a) from left, 5, 6 and 7% $SCCO_2$ dried aerogels, b) 5% freeze dried aerogel (left) and 5% air dried aerogel (right), and c) small mould 7% $SCCO_2$ dried aerogel (left) and 7% $SCCO_2$ + ethanol dried aerogel (right).

5.3.3. Effect of β-Glucan Concentration on $SCCO_2$-dried Aerogel Structure

β-Glucan aerogels at 5, 6 and 7% concentration dried using $SCCO_2$ were further analyzed using adsorption and desorption of nitrogen to determine BET surface area, overall pore volume and pore diameter and the results are presented in Table 5.3. Similar to starch aerogels, β-glucan aerogels exhibit a Type IV nitrogen adsorption/desorption isotherm, displaying syneresis behavior, and thus a mesoporous structure [18]. The surface areas (159.72±3.69 to 166.81±7.61 m^2/g) were not significantly different between the different concentrations ($p > 0.05$). β-Glucan aerogels resulted in BET surface areas similar to those of 1% alginate (150 - 300 m^2/g), and higher than that of 12.5% potato (72.5 m^2/g), and 12.5% Eurylon7 (90.3 m^2/g) starches [17]. Mehling *et al.* [17] found that an increase in concentration of alginate from 1 to 2% increased surface area by 15 - 30%. However, changing chitin concentration from 5 to 8% resulted in a significant decrease in BET surface area (from 363 to 308 m^2/g), as well as an increase in density (from 0.124 to 0.171g/cm^3) and decrease in porosity (from 91.5 to 88%) [13]. This trend was not significant for β-glucan, which may be

because of a smaller difference in the concentration levels tested relative to other studies, or a large variation among replicates. The concentration range tested in this study was limited to 5 - 7% due to difficulties in working with very high viscosities of highly concentrated β-glucan solutions and the fact that a minimum of 5% was needed to form a gel as previously determined by Burkus and Temelli [25]. The lack of a significant effect of concentration on surface area mirrors the same trend found for density of the aerogels, as mentioned above.

Table 5.3: Structural properties of β-glucan gels dried using $SCCO_2$

Concentration (% w/w)	**Surface area (m^2/g)**	**Overall pore volume (cm^3/g)**	**Average pore diameter (Å)**
5	165.55±4.78	0.76± 0.05[a]	27.20±1.45
6	166.81±7.61	0.85±0.01[ab]	27.70±0.68
7	159.72±3.69	0.87±0.04[b]	26.78±0.71

[a-b] Values within columns followed by the same superscript letter are not significantly different at $p > 0.05$.

Significant differences were seen in terms of the overall pore volume. Aerogels of 5% β-glucan had a significantly lower pore volume ($p \leq 0.05$) compared to 7% β-glucan aerogels, while 6% aerogels were similar to both 5 and 7% aerogels. Pore volumes, especially at 5% concentration, were similar to those found for 15% starch, 0.78 cm^3/g [18]. This trend was the opposite of what is expected when concentration is increased, and due to experimental error in sample preparation throughout the formation, drying and handling of the gels. Despite being statistically significant, these differences have little practical significance. Also considering the trends in other values, concentration in the

relatively narrow range tested likely has little effect on pore volume. Pore diameters, however, were similar between the different concentrations, and at a relative pressure of $p/p_o = 0.35$, the pore diameters ranged from 26.78 – 27.70 Å, or 1.34 -1.39 nm radii.

A more detailed comparison between β-glucan aerogels and other gelling polysaccharides, especially starch, would be beneficial. The findings of this study suggest that lower concentrations of β-glucan (5 - 7%)**,** relative to starch (12.5%), are required to achieve a high surface area, low density aerogel [17]. It has previously been reported that β-glucan at similar concentrations forms a much stronger gel, up to 4.5 times, compared to corn starch [25]..

There are a variety of potential applications of β-glucan aerogels, including several of those mentioned previously for non-food grade aerogels. Because of its known health benefits, β-glucan aerogels would be excellent in edible applications. Since the starch and alginate aerogels were successfully loaded with two model drugs, ibuprofen and paracetamol [17], it is likely that β-glucan aerogels can be employed similarly, perhaps with nutraceuticals, including bioactive lipids or phenolic compounds.

5.4. Conclusions

It was demonstrated that β-glucan aerogels can be successfully obtained using $SCCO_2$ drying. Gels dried using $SCCO_2$ have many advantages over those dried at ambient temperature and pressure and by freeze drying, including lower density and interior uniformity, respectively. Increasing β-glucan concentration (5 - 7%) had very few effects on aerogel properties and aerogels formed were

comparable to those formed with other food grade gelling polysaccharides,

including starch, previously reported in the literature.

5.5. References

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6. Barley β-Glucan Aerogels as a Carrier for Flax Oil via Supercritical Carbon Dioxide[5]

6.1. Introduction

Flax oil is considered a bioactive lipid, as it is rich in ω-3 fatty acids, specifically α-linolenic acid, which has been associated with various health benefits [1]. But, this oil requires protection as it can easily undergo oxidation, reducing the beneficial activity and producing rancid odours and flavours in the surrounding food matrix. Techniques for lipid protection and delivery vary, depending on the intended use and desired properties of the final product. Addition of antioxidants can act as a buffer between the oil and oxidizing agents and techniques, which create a physical barrier between the oil and the surrounding environment can be used. Spray drying is the most common technique and has been used for oils high in ω-3 fatty acids [2-5]; however, complex coacervation [6] has recently been investigated to protect flax oil.

Impregnation of polymers with high value compounds, including pharmaceuticals, using supercritical carbon dioxide ($SCCO_2$) as a mass transfer medium shows great promise as a method of delivery. In terms of food ingredients, $SCCO_2$ has been used to extract high value plant compounds, including flax oil [7-10], flax lignans (Chapter 3), and flax hull policosanols [11]. Impregnation of drugs, dyes, organo-metallic complexes, monomer and initiators, and wood biocides using $SCCO_2$ has been well investigated [12, 13], but food applications of this technology are scarce.

[5]A version of this chapter has been submitted for publication to the Journal of Food Engineering.

Polysaccharide hydrogels, when dried using $SCCO_2$, have been shown to form aerogels [14-20], unique materials which maintain the network properties of the original hydrogel, but with high porosity and very low density, ideal for the delivery of bioactive compounds [15, 21].

Previous work has shown that polysaccharide-based aerogels can be successfully formed using barley β-glucan (Chapter 5). β-Glucan, a polysaccharide polymer composed of D-glucopyranosyl units joined by β(1-3) and β(1-4) glycosidic linkages [22, 23], has received much attention for its health promoting properties, mainly due to the high viscosity mixtures it forms with water [24, 25].

The main objective of this study was to impregnate barley β-glucan aerogels with flax oil using $SCCO_2$ and the specific objective was to investigate the effects of point of oil addition, flow regime, impregnation time and temperature and pressure on impregnation efficiency. If flax oil, as a model bioactive ingredient, is able to diffuse into the β-glucan aerogels in the presence of $SCCO_2$ and become trapped upon depressurization, then there may be potential to use impregnated β-glucan aerogels as a delivery vehicle for various bioactives.

6.2. Materials and Methods

6.2.1. Hydrogel Formation

The method of Burkus and Temelli [26] was used to form 5% (w/v) hydrogels from the β-glucan concentrate as described previously in Chapter 5 (Section 5.2.1).

For gels where flax oil, kindly donated by Bioriginal Food and Science Corp. (Saskatoon, SK, Canada), was added at the hydrogel stage, 50 mL of 5% β-glucan concentrate mixture prepared as described earlier, after heating, were combined with 2.5 g of flax oil (1:1 w/w β-glucan: flax oil) with vigorous stirring for 5 min. The mixture, with or without flax oil, was then spooned into 10 mL plastic syringes, with the tips removed, covered with parafilm to prevent moisture loss and allowed to set overnight at ambient conditions.

6.2.2. $SCCO_2$ Drying of Hydrogels

Hydrogels were dried with $SCCO_2$ using the same methodology previously described in Chapter 5 (Section 5.2.2.2).

6.2.3. Lipid Impregnation of Aerogels

Aerogels prepared with β-glucan concentrate were placed in a 25 mL high pressure cell, divided in half by a stainless steel frit (2 µm) divider. Flax oil (5 mL) was added to the remaining half of the cell, along with enough 3 mm glass beads to fill the remaining volume. The cell was placed in the $SCCO_2$ system (described in Section 3.2.4) with the oil being located upstream of the aerogel and the unit was pressurized to 15 MPa and heated to 40 ^{o}C. Carbon dioxide flow was maintained at 0 or 1 L/min (ambient conditions) for static and dynamic impregnations, respectively, for 4, 6 or 8 h. For samples where impregnation was performed concurrently with drying, alcogels were added to the cell along with the flax oil and CO_2 flow rate of 1 L/min (ambient conditions) was maintained for 4 h. Flax oil stained with Nile Red fluorescent dye (Technical grade, N3013,

Sigma-Aldrich, Oakville, ON, Canada) was used in separate impregnations to allow visualization of the location of the oil in the aerogel.

6.2.4. Determination of Impregnation Efficiency

Flax oil impregnation efficiency, defined as lipid content as a percentage of the total impregnated aerogel mass, was calculated for each processing condition. For gels where lipid was added to pre-dried aerogels, lipid content was calculated by subtracting the original aerogel mass from the final impregnated aerogel mass, neglecting the mass of any compounds extracted from the aerogel upon impregnation. For samples where flax oil was incorporated at the stage prior to gelation or concurrently with drying, flax oil was extracted from the aerogels by cutting the gels in half and soaking them in 4 x 10 mL hexane baths, for 1 h each, and then overnight in 10 mL of fresh hexane. The hexane extracts were combined and dried under nitrogen at 60 ^{o}C. The mass of hexane-extracted oil was determined and the impregnation efficiency was calculated. This method was verified using gels with known oil contents.

6.2.5. Aerogel Structure Characterization

To determine the location of the impregnated oil within the aerogels and the nature of the interactions between the aerogels and flax oil, if any, scanning electron microscopy (SEM) and Fourier-transform infrared spectrometry (FTIR) analyses were performed.

Internal and external network structure before and after flax oil introduction was assessed using SEM (Zeiss EVO MA 15, Toronto, ON, Canada) using the methodology described in Chapter 5 (Section 5.2.3).

Attenuated total reflectance (ATR)-FTIR (Nicolet 8700, Thermo Fisher Scientific, Mississauga, ON, Canada) analysis of aerogel samples and flax oil was performed with 32 scans and a resolution of 4 cm^{-1}. For gels, a scalpel was used to cut a thin (2 mm) slice along the length of the aerogel. The exterior portion was placed to face the ZnSe crystal. For the oil sample, a trough shaped ZnSe crystal was used. In between samples, the crystals were cleaned with acetone and dried under a gentle flow of nitrogen.

6.2.6. Statistical Analysis

Analysis of variance of the results was performed using the methodology described in Chapter 4 (Section 4.2.3) and Chapter 5 (Section 5.2.4).

6.3. Results and Discussion

6.3.1 Effect of Point of Oil Addition

Formation of aerogels requires the replacement of the pore solvent with air, while limiting the pore, and thus volume, shrinkage and minimizing cracking and deformation. These objectives are achieved by utilizing $SCCO_2$, which reduces the surface tension of the pore solvent. Bioactives can be added to an aerogel at several points throughout its preparation. In this study, (a) flax oil was mixed into the hot β-glucan and water mixture, prior to hydrogel formation ("before" gelation), (b) during the $SCCO_2$ drying process, by solubilizing the oil in the $SCCO_2$ stream ("during" drying) and (c) after aerogel formation by allowing contact between $SCCO_2$ saturated with oil and the aerogel for a fixed period of time with a static or dynamic flow regime ("after" drying). Another method, which was not employed, is solubilizing the bioactive component in the

ethanol-exchange baths, thus impregnating the gel during the formation of the alcogel via diffusion [15]. Diffusion in a liquid solvent is less efficient than in a gas or supercritical fluid. In addition, oil solubility in ethanol is low, which would again act as a barrier to mass transfer from the ethanol bath to the gel. Solubility of oil in ethanol is dependent on temperature and the water content of the solvent [27]. Soybean oil requires greater than 70 ^{o}C for complete miscibility with ethanol and when water is present, as it is in the hydrogels, even at a 5% level, temperatures must be above 90 ^{o}C for miscibility [27].

Addition of oil during the drying process resulted in significantly higher impregnation efficiencies at 58.09±4.29% ($p \leq 0.05$) compared to the other methods tested (Fig. 6.1). This method also resulted in slightly larger aerogel volumes than the other impregnated aerogels, with a 63.74±0.49% decrease in volume from the original hydrogel compared to 68.01±0.73% volume shrinkage for gels where oil was added after drying, indicating that the presence of the oil in the $SCCO_2$ may have assisted in the removal of ethanol from the gel pores without shrinkage. While it is certainly possible that the higher amount of oil present in the gels contributed to volume, there were no obvious differences noted between the volume of unimpregnated β-glucan aerogels and static or dynamically impregnated pre-dried gels.

Adding flax oil before gelation resulted in the lowest oil loading, with only 7.68 ± 0.45% of the final aerogel mass. Oil was originally added to the β-glucan and water mixture at a level of 1:1 mass ratio of oil: β-glucan. There are two possible points of oil loss. The first is during the ethanol exchange step, which is

essential for removing water from the hydrogel matrix. Although the amount of oil lost during the multi-step exchange was not quantified and the solubility of triglycerides in ethanol is relatively low compared to more non-polar solvents like hexane, it is likely that some oil was transferred to the ethanol solvent. The second point of oil loss is during the $SCCO_2$ drying process, which is dynamic in nature, with a CO_2 flow rate of 1 L/min (at ambient conditions). $SCCO_2$ impregnation of oil into the aerogels is contingent on its solubility in $SCCO_2$. Bozan and Temelli [10] reported that at 50 ^oC and 20 MPa, conditions slightly different than those used in this study, the solubility of flax oil in $SCCO_2$ was only slightly above 2 mg/g CO_2. Barthet and Daun [7] found that the addition of ethanol as a co-solvent increased the $SCCO_2$ extraction yield of oil from flax. The ethanol present in the pores of the alcogels likely acted as a co-solvent upon contact with the $SCCO_2$, assisting in the removal of the oil.

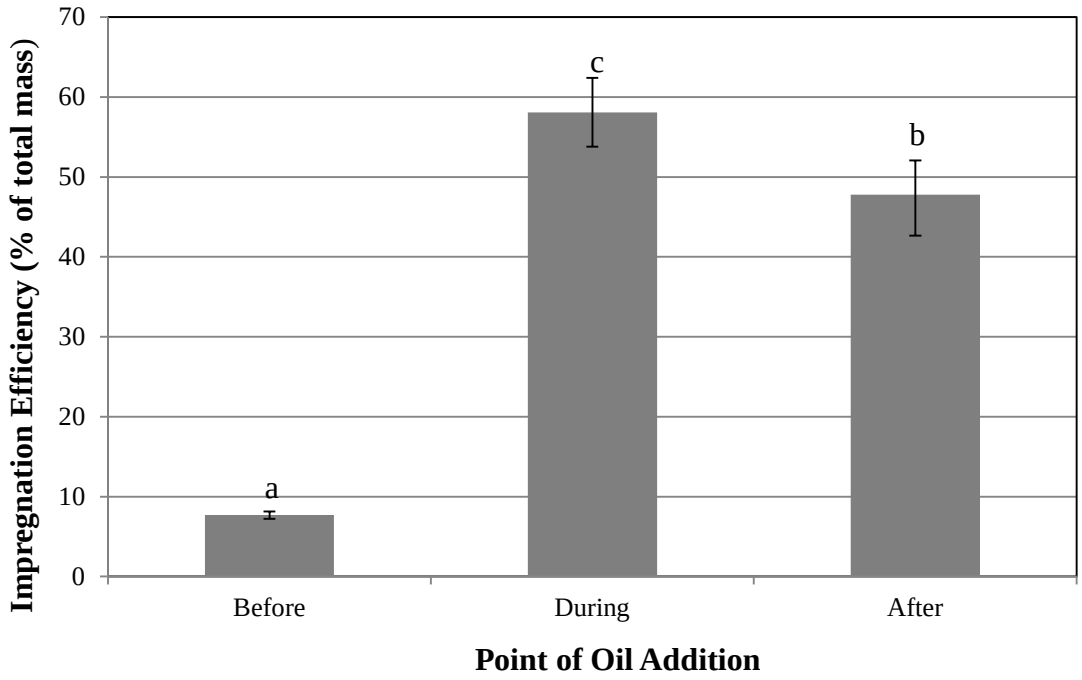

Figure 6.1: $SCCO_2$ impregnation efficiency of aerogels with flax oil when oil is added before gelation, during drying (flow rate = 1 L/min, measured at ambient conditions), and after drying (static, flow rate= 0 L/min) at 15 MPa and 40 ^oC for 4 h. Means with the same letter are not significantly different at $p > 0.05$ (n=3).

FTIR results, shown in Figure 6.2, further illustrate the differences in impregnation efficiency between the different points of oil addition. The spectrum for unimpregnated β-glucan aerogel (Fig. 6.2e) is similar to that of powdered oat β-glucan analyzed previously [28] and shows peaks at 1000 cm^{-1} to 1200 cm^{-1} characteristic of polysaccharides as well as a small peak just before 900 cm^{-1}, which is indicative of the β-glycosidic linkage [28, 29]. Figure 6.2a shows the spectrum for flax oil, which is similar to those reported previously [30]. Strong and sharp peaks can be found from ~3050 cm^{-1} to 2800 cm^{-1}, corresponding to C-H stretching vibrations, and near 1750 cm^{-1}, corresponding to C=O of the ester

linkages [30]. Increasing impregnation efficiency in the aerogels manifests as the presence of these characteristic sharp peaks in the FTIR spectra. When flax oil was added before gelation and drying, in the hydrogel stage (Fig. 6.2d), the spectrum was similar to that of the unimpregnated aerogel (Fig. 6.2e) except that the β-glucan peaks from ~1225 cm^{-1} to ~900 cm^{-1} do not appear in the spectrum for the impregnated aerogel. The absence of these peaks may suggest an interaction between the flax oil and β-glucan during the gelation process. Examining the SEM micrographs shows that the network structure of an unimpregnated aerogel and an aerogel where lipid is added before gelation are visibly different (Fig. 6.3). The network of the aerogels where lipid is added before gelation is coarser and pores appear larger. When flax oil was added to a pre-dried aerogel (after drying), very small peaks can be seen from 3050 cm^{-1} to 2800 cm^{-1} and at 1750 cm^{-1} (Fig. 6.2c). When flax oil was added during the aerogel drying process (Fig. 6.2b), impregnation efficiency was the highest and the characteristic lipid peaks were much stronger compared to the other two oil addition methods. Oil peaks from ~1500 cm^{-1} to 650 cm^{-1}, corresponding to C-H and C-O vibrations also became noticeable. It is important to know if the flax oil is interacting chemically with the β-glucan polymers to understand the impregnation process properly. Therefore, further studies are required to determine which, if any, chemical interactions are occurring.

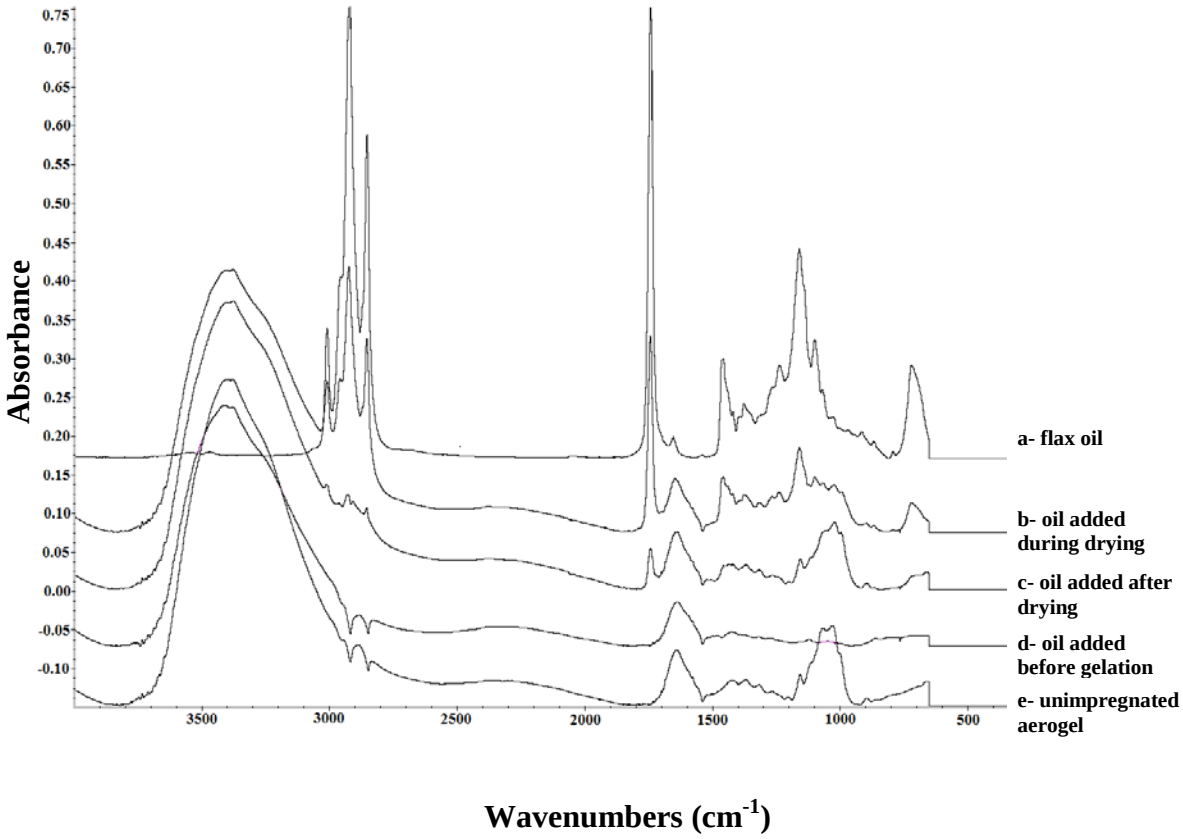

Figure 6.2: ATR-FTIR spectra of a) flax oil, b) β-glucan aerogel with flax oil added during drying, c) β-glucan aerogel with flax oil added after drying, d) β-glucan aerogel with flax oil added before gelation and e) unimpregnated β-glucan aerogel.

6.3.2. Effect of Flow Regime

The effect of flow regime on impregnation of previously dried aerogels was investigated, using both static (0 L/min), and dynamic (1 L/min, at ambient conditions) regimes. A dynamic flow regime resulted in significantly higher flax oil impregnation efficiency than a static flow regime ($p \leq 0.05$), when all other conditions were kept constant, with 65.39 ± 4.32% compared to 47.79 ± 5.14%, respectively (Fig. 6.4).

Static impregnation relies on mass transfer properties of the oil in $SCCO_2$ into the aerogel via diffusion, assisted only by mixing due to thermodynamic

forces. Given relatively low affinity of the oil for the β-glucan polymer network, mass transfer is low. With dynamic impregnation, despite the removal of flax oil from the system due to its solubility in $SCCO_2$, flow provides more interaction between the oil-saturated $SCCO_2$ and the aerogel, pushing the fluid mixture through the network. These results are in agreement with Díez-Municio *et al.* [31], who found that for $SCCO_2$ impregnation systems, consisting of the solute lactose and the polymer chitosan, continuous flow resulted in much higher impregnation efficiencies than batch static processes.

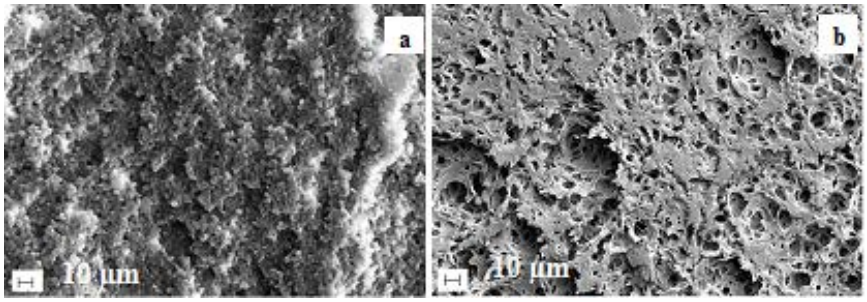

Figure 6.3: SEM micrographs of the interior of aerogels a) without added flax oil, b) with flax oil added before gelation (1000x mag).

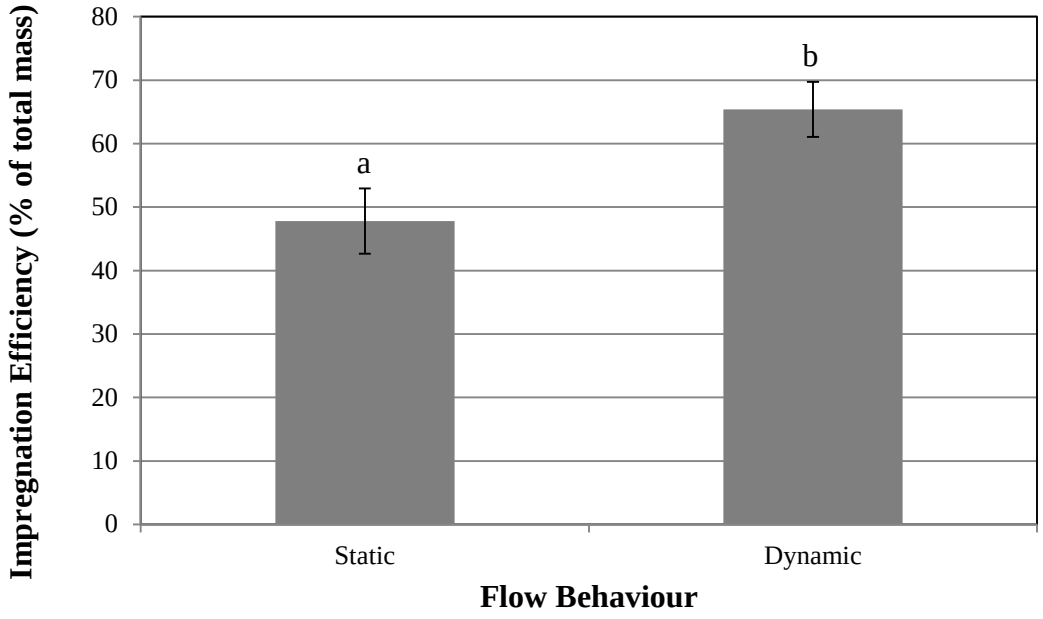

Figure 6.4: $SCCO_2$ impregnation efficiency of aerogels with flax oil under static (0 L/min) and dynamic (1 L/min, measured at ambient conditions) CO_2 flow at 15 MPa and 40 ^oC for 4 h. Means with the same letter are not significantly different at $p > 0.05$ (n=3).

6.3.3. Effect of Impregnation Time

Residence time of the aerogel in the flax oil saturated $SCCO_2$ environment did not have a significant effect on impregnation efficiency at durations of 4, 6 and 8 h. However, there was an obvious trend as shown in Figure 6.5, where impregnation efficiency increased with time under static conditions. This trend can also be seen from the cross sections of the interiors of aerogels impregnated with lipid dyed with Nile Red fluorescent dye (Fig. 6.5 inset). The flax oil front, shown by the pink colouration, appears to move towards the middle of the gel as

time increases. The impregnation efficiency vs. time plot (Fig. 6.5) does not reach a plateau by 8 h, indicating that equilibrium distribution of the oil between the $SCCO_2$ phase and the aerogel has not yet been reached and it will require greater than 8 h for maximum loading at static conditions.

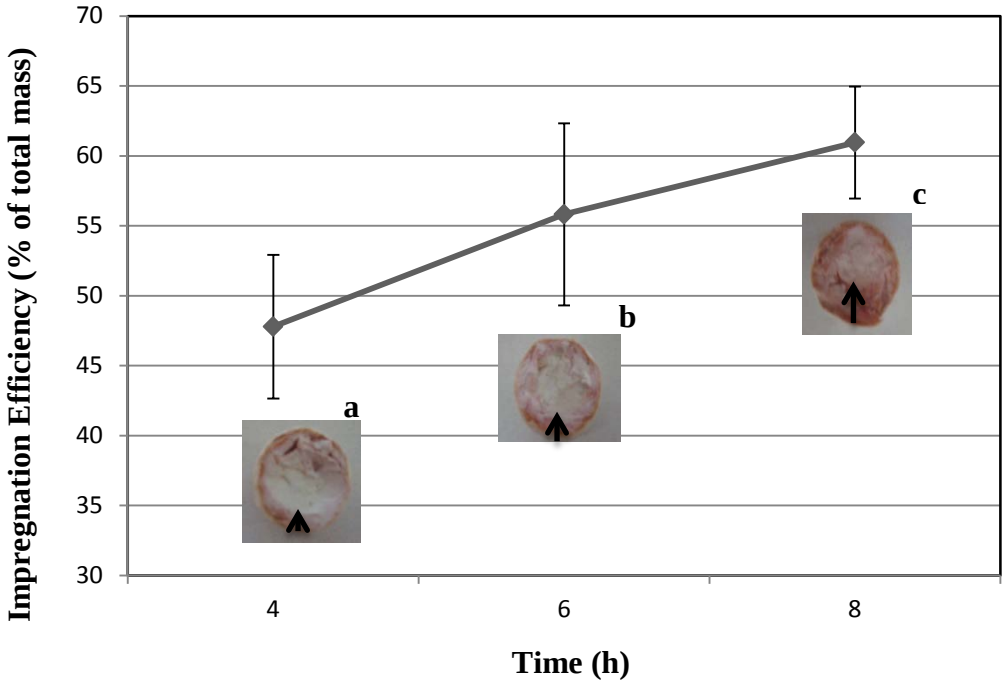

Figure 6.5: $SCCO_2$ impregnation efficiency of aerogels with flax oil over time at 15 MPa, 40 ^{o}C and a static flow (0 L/min) Inset: aerogels impregnated with stained flax oil at a) 4 h, b) 6 h, and c) 8 h (n=3).

The time required to reach maximum loading or solute equilibrium between the $SCCO_2$ and polymer phases depends on the properties of the solute and the polymer. For supercritical impregnation of cyproconazole in ponderosa pine sapwood, Kang *et al*. [32] utilized pressures of 10.3 and 20.6 MPa and temperatures of 40 and 60 ^{o}C. Once desired conditions were reached, pressure was released. By mapping the concentrations of cyproconazole along the length of

sapwood pieces, it could be seen that the biocide was concentrated mainly at the outer edges, just as it was in the case of impregnated β-glucan aerogels in this study, indicating that the time required to reach the desired processing conditions was not adequate to allow uniform distribution of cyproconazole throughout the aerogel. Similarly, for the impregnation of lactulose in chitin, the loading after 3 h increased 3.5 times compared to that after 1 h [31].

6.3.4. Effect of Temperature and Pressure

It is well documented that temperature and pressure can affect the solubility of compounds in $SCCO_2$, due to changes in solvent density and solute vapor pressure [33]. As mentioned previously, the amount of flax oil present in the aerogels after impregnation and system depressurization depends on the balance between the affinity of the flax oil to the $SCCO_2$, or mobile phase, and the affinity of the oil to the solid aerogel matrix, or stationary phase. If solubility in $SCCO_2$ is too high, then the oil will leave the system with the $SCCO_2$ flow or during initial depressurization. However, solubility must be sufficient enough such that the $SCCO_2$ upstream of the aerogels can be loaded with flax oil to carry the oil into the aerogel matrix. While solubility of a solute in $SCCO_2$ is relatively simple, solute uptake by a polymer matrix is more complicated [34].

In this study, it was found that pressure significantly impacted impregnation efficiency ($p \leq 0.05$), with an increase in pressure from 15 to 30 MPa increasing the amount of flax oil within the aerogels (Fig. 6.6). Ma *et al.* [34] found that, for model drugs such as aspirin, salicylic acid and ibuprofen, increasing pressure above 8 MPa decreased the partition coefficient of the drug

between the $SCCO_2$ phase and (poly)lactic acid. Similarly, as CO_2 density increased with increasing pressure, solubility of biocide was found to increase, decreasing the partition coefficient between wood and biocide [35]. The difference between these trends illustrates the complicated nature of $SCCO_2$ impregnation. The results from this study suggest that there is a significant interaction between the aerogels and flax oil, which allows more oil to be retained, even as the solubility in $SCCO_2$ increases. Flax oil, as a mixture of relatively high molecular weight triglycerides, has a low solubility in $SCCO_2$, relative to smaller components such as free fatty acids. Using Chrastil's equation and model parameters estimated by Güçlü-Üstündag and Temelli [33], the solubility of triolein, acting as a model lipid to represent flax oil, at 30 MPa is estimated to be 7.21 g/L, which is 4.8 times greater than that at 15 MPa, 1.50 g/L, when temperature is held constant at 40 ^{o}C. This helps to explain the difference in impregnation between these two pressures and suggests that at 15 MPa oil loading is limited by low solubility. Diffusion of the oil through the $SCCO_2$ to the aerogel matrix is affected by temperature and pressure as well as the position and number of double bonds present [36]. While increasing temperature increases the diffusion coefficient, increasing pressure will decrease it. Therefore, it would be assumed that the lowest pressure and highest temperature conditions would be optimum. However, due to limited solubility at 15 MPa, this was not the case. Also, within the pressure and temperature ranges investigated, temperature and the interaction between temperature and pressure did not have a significant effect on impregnation efficiency ($p > 0.05$). For cyproconazole impregnated in pine

sapwoord, Kang *et al.* [32] reported that at low pressure (10.3 MPa), increasing temperature decreased solute retention, while at the higher pressure studied (20.6 MPa), temperature increase had almost no effect, which is similar to the findings of this study.

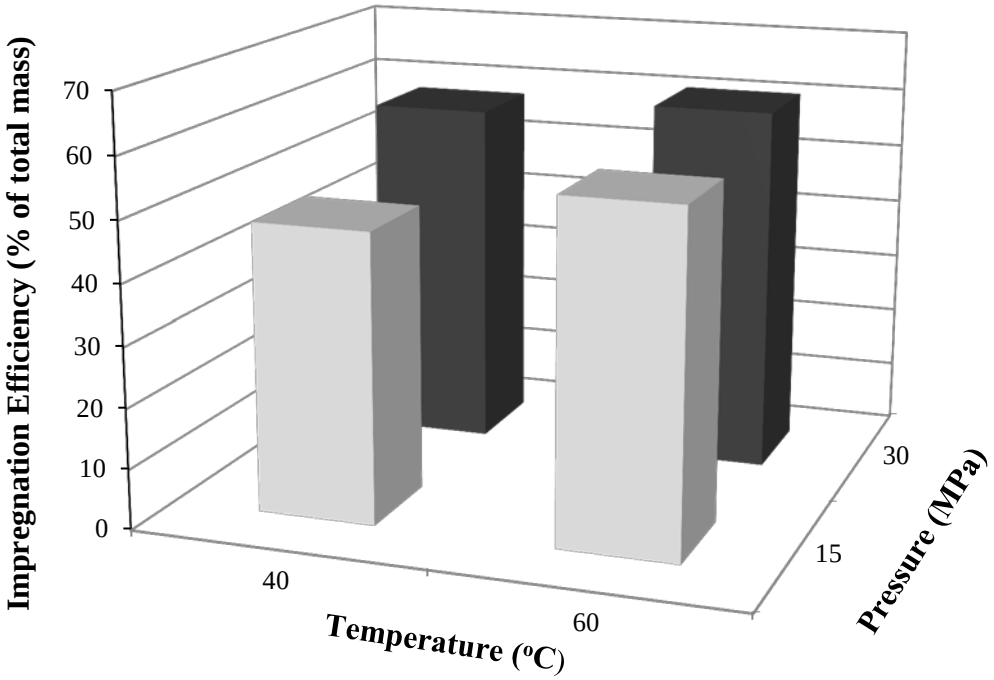

Figure 6.6: $SCCO_2$ impregnation efficiency of aerogels with flax oil at different temperatures and pressures after 4 h and a static flow (0 L/min) (n=3).

Bozan and Temelli [10] found that, within the range of 21 to 55 MPa, solubility of flax oil increased with $SCCO_2$ pressure. At higher pressures (35 and 55 MPa), solubility increased with temperature; however, differences were not significant, while at 21 MPa, solubility decreased with temperature. The change in direction of the effect of temperature depending on pressure range is commonly

observed due to crossover of solubility isotherms in $SCCO_2$. Within the temperature and pressure ranges investigated by Bozan and Temelli [10], maximum solubility of flax oil was acheived at 55 MPa and 70 ^{o}C. The maximum conditions employed in this study were 60 ^{o}C and 30 MPa. It is hypothesized that if more extreme conditions were used, the balance between the affinity of the oil to the $SCCO_2$ vs. the aerogel matrix would change and the oil impregnation efficiency would plateau, then potentially decrease. While it is important to note that the matrix (flax seed in the case of Bozan and Temelli [10] and β-glucan aerogel in this study) can have a great effect on mass transfer and thus loading of the oil in the $SCCO_2$, there is potential for further process optimization in the impregnation of flax oil in β-glucan aerogels.

6.4. Conclusions

$SCCO_2$-dried polysaccharide aerogels show great promise as delivery vehicles for high value bioactive and nutraceutical compounds, including flax oil. β-Glucan aerogels were impregnated using $SCCO_2$ as a mass transfer medium. Impregnation efficiency can be increased by optimizing impregnation conditions, including point of oil addition, flow regime and pressure. The highest level of impregnation, 65.39±4.32%, was achieved when pre-dried gels were impregnated at 40 ^{o}C and 15 MPa with a dynamic flow profile. Further optimization is possible, including determining the retention time, which results in maximum oil loading. Certainly, more work is required to establish the degree of protection that aerogels offer against lipid oxidation, if any.

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7. Novel Polysaccharide Aerogels using Supercritical Carbon Dioxide: Application as Flax Seed Lignan Carriers[6]

7.1. Introduction

Flax, an oilseed commonly utilized for renewable adhesives and coatings, is recognized as a nutritious ingredient for health promotion [1, 2]. Flax seed contains essential fatty acids and lignans, phenolic antioxidant compounds linked to many health benefits [3, 4]. The predominant lignan, secoisolariciresinol is present as a glucoside, namely secoisolariciresinol diglucoside (SDG), which is linked to other SDG molecules to form oligomers or lignan macromolecules [5, 6]. In the human body, SDG and its metabolites are converted to mammalian lignans, enterolactone and enterodiol, which enter circulation and exhibit their numerous physiological effects [4].

Flax seed also contains a less studied, underutilized potential high-value ingredient known as mucilage, or gum, which is utilized by the plant for adherence, seed dispersion, and as a nutrient and moisture reserve [7]. Mucilage, a soluble fiber, which makes up 10% of seed weight, is found in the outermost cell layer, or epiderm, of the seed coat [8-10]. Two heterogenous polysaccharide fractions have been identified as making up mucilage: a neutral arabinoxylan fraction, which makes up 75% of mucilage and an acidic rhamnogalacturanan fraction, which makes up 25% [8, 11, 12]. The neutral fraction contains sugars *L*-arabinose, *D*-xylose and *D*-galactose, while the acidic fraction contains *L*-rhamnose, *L*-fructose, *L*-galactose and *D*-galacturonic acid [13]. Flax mucilage

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consumption has health benefits of its own, with implications in diabetes and cardiovascular disease treatment, colon cancer prevention and reductions in the incidence of obesity [10]. However, gum residues in oil fractions have been shown to cause quality deterioration and when present in animal feed, decrease available energy and nutrients due to increased gastric viscosity [9, 14]. Therefore, more potential uses for mucilage are required to increase its value as a health-benefiting ingredient.

Flax mucilage, similar to the soluble fiber fraction β-glucan in barley and oats, has functional properties potentially beneficial in food formulations. As a hydrocolloid, mucilage has good water holding capacity and it behaves similarly to other common gums, like gum Arabic when added to foods [15]. Flax mucilage is also able to form a thermoreversible cold-set weak gel with properties that depend on extraction conditions, dissolution and cooling temperatures, pH, concentration and presence of salts, among others [15-17]. Flax mucilage has been used in several applications, including as a stabilizer and thickener in food formulations [18-20].

Bioactive delivery has been an important area of research in recent years [21-27]. Aerogels dried using supercritical carbon dioxide ($SCCO_2$) have been formed using bio-degradable, renewable polysaccharides [28-34], ensuring their potential for edible applications. $SCCO_2$ is a green solvent with a tunable solvent power and an ability to decrease the surface tension of liquid solvents, including the pore solvent of a hydro- or alcogels. Drying of wet gels using $SCCO_2$

produces a material with a high surface area and porosity and low density, which shows great potential for bioactive delivery [29, 35].

The objective of this study was to utilize polysaccharide aerogels for the delivery of flax lignan, SDG. More specific objectives include preparing aerogels from flax mucilage and barley β-glucan and the comparison of the resulting aerogel properties. Various methods of inclusion of a lignan concentrate into the aerogels were carried out to determine the resulting extractable SDG contents. If aerogels from flax mucilage and β-glucan can be successfully formed using $SCCO_2$ drying and lignans included at the hydrogel stage are not extracted during the drying process, then lignan impregnated aerogels can be used as SDG delivery vehicles.

7.2. Materials and Methods

7.2.1. Materials

Barley β-glucan concentrate was obtained at a pilot plant as described previously[36]. The average molecular weight of β-glucan was 198 kDa as determined by intrinsic viscosity measurements and the β-glucan content of the concentrate was 83.3% (dry basis) [36]. Flax seed mucilage was obtained using a method adapted from Cui *et al.* [37]. Whole flax seeds (Agricore United, Winnipeg, MB, Canada) were soaked in distilled water at a ratio of 13:1 (w/w) and stirred at 85 ^{o}C for 3 h. Flax seeds were separated using a sieve and mucilage was precipitated by adding ethanol to the supernatant in a 1:1 (v:v) ratio. Precipitate was then collected and dried overnight in an oven at 100 ^{o}C. Dried mucilage was then ground in a coffee grinder (Philips Model HD5112, Markham,

ON, Canada) to promote even wetting in future steps. Beneflax, a flax lignan concentrate kindly donated by Archer Daniels Midland Company (Decatur, IL), containing no less than 35% SDG, was utilized in solubility studies, as well as to incorporate flax lignans into the β-glucan and flax mucilage aerogels.

7.2.2. Hydrogel Formation

The method of Burkus and Temelli [38] was used to form 5% (w/v) hydrogels from the β-glucan concentrate, as described in Chapter 5 (Section 5.2.1).

To form 10% flax mucilage gels, flax mucilage was added to 50 mL of boiling distilled water with constant stirring. To assist in evenly hydrating the mucilage, the mixtures were then covered, placed in a sonicator and heated to 70 ^{o}C for 1 h.

The mixtures were then spooned into 10 mL plastic syringes, with the tips removed, covered with parafilm to prevent moisture loss and allowed to set overnight at ambient conditions, in the case of the β-glucan gels, and at 4^{o}C in the case of the mucilage gels.

7.2.3. $SCCO_2$ Drying of Hydrogels

Hydrogels were dried with $SCCO_2$ using the methodology described in Chapter 5 (Section 5.2.2.2), except in the case of gels that were converted to alcogels using 100% ethanol only. For these samples, hydrogels were placed in 4 x 1 h baths of 50 mL of 100% ethanol followed by a 50 mL bath of 100% ethanol overnight.

7.2.4. Incorporation of Flax Lignans

Beneflax was added to the gels using four different methods. The first two methods consisted of adding 1 g of Beneflax to 50 mL of polymer mixture before (BH) or after hydrating (AH) the polymer mixtures. For the third method, a solution of 1 g of Beneflax in 70% ethanol was prepared, followed by the addition of β-glucan or flax mucilage and evaporation of the solvent at 70 ^{o}C and ambient pressure. Distilled water was then added back to the mixture to re-hydrate to a volume of 50 mL (EtOH-BH). Finally, for the fourth method, pre-made hydrogels were soaked in 4 x 50 mL solutions of 1 g of Beneflax in 50 mL 70% ethanol for 1 h each, followed by 50 mL of 100% ethanol overnight (EtOH-AG). All experiments were performed in triplicate.

7.2.5 Determination of SDG Loading in $SCCO_2$

A 5 gram sample of Beneflax was loaded into a 25 mL basket and then placed in the supercritical system, as described in Chapter 3 (Section 3.2.4). The cell was pressurized with CO_2 to 15 MPa and the temperature inside the cell was held at 40 ^{o}C. Where ethanol was used as a modifier, 0.5 mL/min liquid ethanol was pumped into the pressurized CO_2 stream using a separate co-solvent pump (Model 1330, Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). The flow rate of the CO_2 was 1 L/min, measured at ambient conditions by a dry gas meter and controlled by a heated micro-metering valve. Ethanol was removed upstream of the gas meter using a cold trap situated after the sample collection. Glass collection vials were connected to the depressurization valve and held in a refrigerated bath (–20 ^{o}C). Residual ethanol was removed from the samples by

purging gently with nitrogen gas. Sample weights were determined gravimetrically (±0.0001 g). Collected samples were stored at –20 ^{o}C until ready to be analyzed by HPLC. All experiments were done in triplicate.

7.2.6. HPLC Analysis

Aerogels were cut into quarters, crushed gently, added to 10 mL of 70% ethanol and heated for 3.5 h in a 65 ^{o}C shaking water bath. Samples were then cooled to ambient temperature and centrifuged, before 2 mL aliquots of the 70% ethanol extract were added to HPLC vials to determine if any free SDG was present in the aerogels. Aliquots of the aerogel extracts (5 mL) were then added to 5 mL of 2 N NaOH in 70% ethanol and placed back into the shaking water bath for an additional 3.5 h at 60 ^{o}C. Samples were then neutralized using concentrated glacial acetic acid, centrifuged (1250 x g) and 2 mL aliquots were added to HPLC vials. Beneflax mixture, β-glucan concentrate powder, flax mucilage powder and $SCCO_2$ extracts of Beneflax were treated in the same manner as above, and concentrated or diluted as required.

HPLC analysis was carried out as described previously in Chapter 3 (Section 3.2.6), with some modifications. The column used was a C 18-5 μm (4.6 mm x 150 mm) reverse phase column (Supelco, Sigma–Aldrich, St. Louis, MO) and the mobile phase was a mixture of 0.05% trifluoroacetic acid in HPLC grade water (A) and 0.05% trifluoroacetic acid in HPLC grade acetonitrile (B). The gradient, t = 0 min, A = 99%; t = 0.5 min, A = 99%; t = 5 min, A = 60%; t = 6 min, A = 99%; was run at 1.5 mL/min for a total run time of 15 min. Samples were compared to HPLC grade standards dissolved in 70% ethanol.

Quantification and SDG standard (ChromoDex, Irvine, CA, USA) curves were established using Galaxie Version 1.9 software (Varian, Palo Alto, CA, USA) in the range of 0.02 to 0.4 mg SDG/mL of 70% ethanol. All analysis was done in duplicate.

7.2.7. Aerogel Characterization

Internal network structure was assessed using Scanning Electron Microscopy (SEM) (Zeiss EVO MA 15, Toronto, ON, Canada) as previously described in Chapter 5 (Section 5.2.3).

Pore size, volume and Brauner-Emmett-Teller (BET) surface area of $SCCO_2$-dried 10% flax mucilage aerogels were determined using nitrogen adsorption and desorption isotherms (Quantachrome Autosorb 1MP AS1-MVP-9, Boynton Beach, FL, USA), as described in Chapter 5 (Section 5.2.3) and compared to values obtained for 5% β-glucan aerogels (Chapter 5).

To evaluate the colour of the aerogels, *L*, *a*, *b* colour scores were measured using a Konica Minolta colourimeter (CR410, Mississauga, ON, Canada). Samples were placed on a solid white calibration colour tile and measurements were taken 3 times, turning the sample 90^{o} each time and the average was reported. A white calibration tile was used rather than black, as in Section 5.2.3, based on recommendations from the colourimeter manufacturer.

7.2.8. Statistical Analysis

Analysis of variance of the results was performed using the methodology described in Chapter 4 (Section 4.2.3).

7.3. Results and Discussion

7.3.1. Comparison of β-Glucan and Flax Mucilage Aerogels

It was previously demonstrated in Chapter 5 that β-glucan, a gelling soluble fiber component found in the cell wall of barley, can form a low density aerogel. Flax mucilage, a by-product of flax oil production, while able to form a gel, was found to be quite different from β-glucan in terms of the gelation process and hydrogel characteristics, as discussed in detail later. To our knowledge, aerogel formation using flax mucilage has not been reported before. β-Glucan when heated with water to boiling, formed a uniformly wetted mixture and after setting at ambient temperature overnight, formed a hydrogel, which was able to maintain its cylindrical shape once removed from its mold, at a concentration of 5% β-glucan concentrate, or 4.2% pure β-glucan. The flax mucilage used in this study is a heterogeneous polysaccharide mixture, consisting of neutral and acidic fractions [9, 17]. Several concentrations of mucilage were tested in terms of their ability to form a gel with sufficient mechanical stability to hold a cylindrical shape reasonably well during the ethanol solvent exchange process. It was observed that a 10% (w/w) mixture not only held its shape during removal from the mold and cutting the best relative to other concentrations, but also wetted evenly during the hydration or gel formation stage. Unfortunately, while a 5% flax mucilage gel was not feasible, a 10% β-glucan gel was too viscous for even wetting and proper molding. As can be seen from Figure 7.1, however, the β-glucan aerogel had much smoother sides, and a more cylindrical shape, compared to the flax mucilage gel, which was slightly uneven.

Figure 7.1: 5% β-Glucan aerogel (left) and 10% flax mucilage aerogel (right).

While direct comparisons cannot be made, as the polymer gels were formed at two different concentrations, Table 7.1 illustrates the similarities and differences between the β-glucan and flax mucilage aerogel properties. The densities of the aerogels were similar ($p > 0.05$) at 0.16 and 0.19 g/cm^3 for mucilage and β-glucan aerogels, respectively. The inability to accurately measure the dimensions of the mucilage aerogels, due to uneven proportions, as mentioned above (Fig. 7.1) may have affected the results. While the mucilage aerogel underwent significantly less shrinkage, or maintained more of its original hydrogel volume (56.90%) than the β-glucan aerogel (37.62%), it also maintained more of its original mass ($p \leq 0.05$). This is a result of the higher polymer concentration of the mucilage gel, compared to the β-glucan gel.

Table 7.1: Properties of 5% β-glucan and 10% flax mucilage aerogels.

Polymer	Density (g/cm^3)	Hydrogel volume (% of hydrogel volume)	Hydrogel mass (% of hydrogel mass)	Surface area (m^2/g)	Overall pore volume (cm^3/g)	Average pore diameter (Å)
β-Glucan	0.19± 0.02[a]	37.62± 3.26[a]	6.28± 0.12[a]	165.55± 4.78[a*]	0.76± 0.05[a*]	27.20± 1.45[a*]
Mucilage	0.16± 0.01[a]	56.90± 9.16[b]	9.16± 0.31[b]	201.13± 7.61[b]	0.82±0.25[a]	25.23± 0.25[a]

[a,b] Values within columns followed by the same superscript letter are not significantly different at $p > 0.05$.
* data from Chapter 5, Table 5.3.

Higher polymer concentration means lower moisture content per unit of overall gel mass, and therefore less moisture mass to be removed during the drying process. In terms of volume, it has been previously shown that increasing polysaccharide polymer concentration results in less shrinkage and therefore higher retention of the original hydrogel volume, due to increased mechanical strength [29, 31]. This trend was not significant for β-glucan aerogels in the range of 5 - 7% (Chapter 5, Section 5.3.3), and to accurately label polymer concentration difference as the cause of volume difference would require both gels to be at the same concentration, which was not possible for the polysaccharides under investigation in this study.

SEM micrographs (Fig. 7.2) show that both aerogel polymer networks are tightly packed with slightly different morphologies on the exterior compared to the interior, after cutting the gel in half width wise. The β-glucan aerogel seems to be more porous while the mucilage aerogel interior appears to be more uniform

and more densely packed. This effect may in part be an artifact of sample preparation. The flax mucilage gels were slightly softer and easier to cut compared to the β-glucan gels, providing the interior a smoother appearance. The density of network packing may, again, likely be a result of higher polymer concentration.

When combined with water, β-glucan in the molecular weight range of 35-140 kDa [39], forms a physically cross-linked 3-D network with inter and intra-chain hydrogen bonds [40]. Long chains of consecutive β(1→ 4) linkages associate similar to cellulose and act as junction zones [41]. Increasing the polymer concentration has been found to increase the probability of contact and, thus, formation of junction zones, increasing mechanical strength and creating a more densely packed network [41]. Flax mucilage networks have similar junction zones connecting polymers together and the degree of polymer interaction has been found to depend on the initial mixture concentration [15]. While pore diameter and overall pore volume were not significantly different, the difference in overall network structure caused by the polymer concentration, along with the obvious chemical differences between the polymers, manifested in a significantly higher BET surface area for flax mucilage compared to β-glucan (Table 7.1). It is perhaps more likely that the differences in interactions between the β-glucan polymers and the polymers that make up flax mucilage are responsible for these results, rather than the difference in concentration. It was previously found that increasing β-glucan concentration from 5 to 7% did not result in any practical

significant differences for the surface area and pore properties of $SCCO_2$-dried aerogels (Chapter 5, Section 5.3.3)

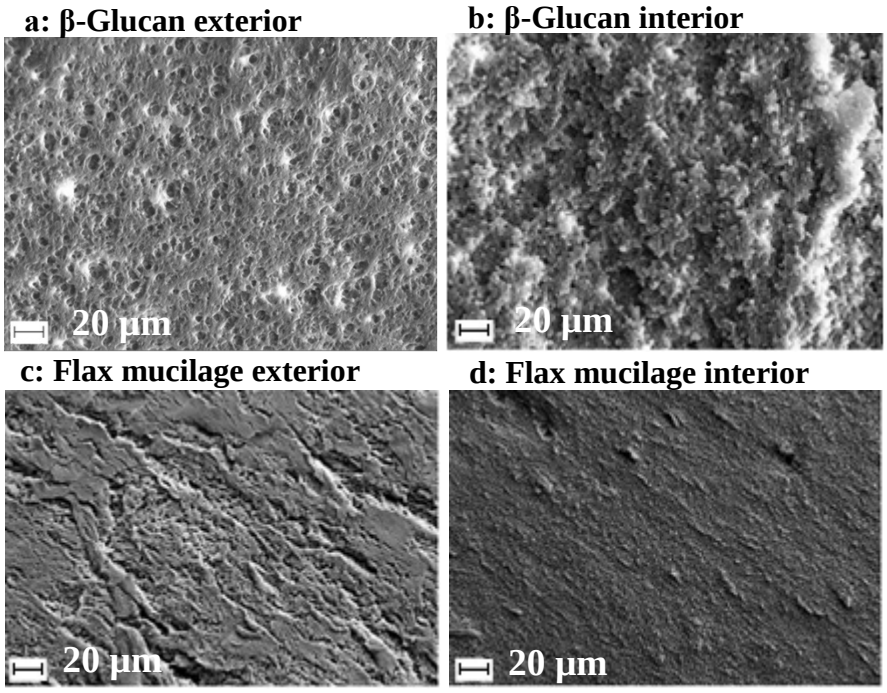

Figure 7.2: SEM micrographs of 5% β-glucan and 10% flax mucilage aerogels at 1000 x mag: a) β-glucan exterior, b) β-glucan interior surface, c) mucilage exterior, d) mucilage interior.

Both β-glucan and flax mucilage aerogels were significantly lighter ($p \leq 0.05$) than their original powder counterparts (Table 7.2), as signified by an increase in *L* value. Flax mucilage had the largest magnitude of change in *L* value, with over a 20 point difference between aerogel and powder. Both polymers also had significant decreases in redness and yellowness ($p \leq 0.05$), as evidenced by a decrease in positive *a* and *b* values, respectively. Both polysaccharide concentrates contain phenolic compounds, as a result of the extraction process applied to the respective grains, which would contribute to their brown

colouration. As noted in Chapter 5 (Section 5.3.1), the increase in lightness and the decrease in red and yellow colouration may be in part a result of the loss of these phenolic compounds during the ethanol solvent-exchange step or through extraction during $SCCO_2$ drying. As well, the change in light scattering from the powder to the more dispersed aerogel form would also contribute to the observed colour differences. Flax mucilage gels still remained darker, more red and more yellow compared to the β-glucan aerogels, which, again, could be a result of the higher gel concentration or due to the higher initial phenolics concentration. Uneven pigmentation can also be seen as brown colourations in the mucilage aerogel (Fig. 7.1). Further studies examining the total phenolic compound content of the polymer concentrates used before and after aerogel formation would provide a better understanding of these colour changes.

Table 7.2: L*a*b* colour coordinates of 5% β-glucan and 10% flax mucilage aerogels.

Polymer	**Form**	***L***	***a***	***b***
β-Glucan	Powder	90.867±0.25[a]	1.31±0.10[a]	5.75±0.41[a]
	Aerogel	93.19±0.21[b]	-0.52±0.04[b]	0.69±0.05[b]
Mucilage	Powder	67.33±0.15[a]	3.40±0.03[a]	9.91±0.09[a]
	Aerogel	88.19±0.18[b]	0.07±0.06[b]	1.22±0.09[b]

[a,b] Values within columns for each polymer type followed by the same superscript letter are not significantly different at $p > 0.05$.

7.3.2. $SCCO_2$ Extraction of SDG from Beneflax

The loading of SDG from flax seed in $SCCO_2$ was reported previously as extremely low (Chapter 3, Section 3.3.1). At 35 - 45 MPa and 40 - 60^{o}C, using 0 -

20 mol% ethanol as a modifier, the highest CO_2 loading of SDG, at 45 MPa, 60 ^oC and 10 mol% ethanol, was 0.55 µg/g CO_2, with a total of 84.7 µg of SDG extracted after 6 h. "CO_2 loading" was reported, rather than "apparent solubility", due to the complexity of the flax matrix and the nature of SDG. Within the flax seed, SDG occurs as part of a larger macromolecule, consisting of SDG molecules linked via 3-hydroxy-3-methyl glutaric acid (HMGA) [5, 6], along with *p*-coumaric acid glucoside, ferulic acid glucoside, caffeic acid glucoside and herbacetin diglucoside [42]. It is difficult for $SCCO_2$ to solubilize the entire macromolecule due to its significantly greater molecular mass relative to SDG alone and due to strong interactions with the flaxseed matrix. Only after post-extraction hydrolysis can the amount of SDG in the extract be determined. The presence of very low levels of free lignans as well as the high polarity of SDG results in a significantly lower apparent solubility or CO_2 loading than lignans from other plants such as *Schisandra chinensis* [43, 44].

Beneflax contains higher concentrations of SDG, at least 35% (w/w), compared to those found in flax seed (approximately 0.1 - 2.59%) [45]; however, it is still in a bound, or macromolecular form. Therefore, while the matrix may be less complex compared to flax seed with fewer interactions with the SDG preventing its dissolution in $SCCO_2$, the high molecular mass of the SDG polymer, along with the relatively high polarity of SDG, were expected to limit the loading of $SCCO_2$ with Beneflax SDG. After 5 h of extraction employing a CO_2 flow rate of 1 L/min (measured at ambient conditions) a pressure of 15 MPa and a temperature of 40 ^oC, only 1.4 ± 0.71 mg of raw extract was obtained in the

sample vials, despite 5 g of sample initially being added to the extraction cell. When ethanol was added as a co-solvent, to more closely match the conditions of the Beneflax inside the alcogels, the total extract mass increased to 42.87 ± 61.39 mg. The high variability is believed to be caused by the very dense packing of the Beneflax within the cell, especially when ethanol was added. This prevented the passage of $SCCO_2$ through the sample bed and thus could have forced some of the solvent to bypass the basket containing the sample within the high pressure extraction vessel, or more dangerously, to cause an increase of pressure upstream of the extraction cell. After extraction was completed, the Beneflax bed was very compact and did not release easily from the cell, but instead needed to be chipped out.

Extract samples, which contained more than 0.001 g were first analyzed for free SDG content and it was found that they contained no free SDG. Extracts with less than 0.001 g were not analyzed because it was felt that the degree of error within weighing and HPLC analysis would make accurate quantification impossible. Aliquots were then hydrolyzed to release SDG from the larger macromolecules, if present.

Determination of CO_2 loading requires samples to be taken over time. The amount of extracted compound of interest is plotted as a function of CO_2 mass and the slope of the constant extraction rate period or the initial linear portion of the curve is taken as the solubility. Because only certain extracts were analyzed and found to contain SDG, CO_2 loading of SDG cannot be reasonably reported. The average cumulative SDG content in the extracts, where ethanol was used as a co-

solvent, was 1.59±1.29 mg. When neat CO_2 was used, extracts did not contain suitable mass for HPLC analysis.

7.3.3. SDG Content of Aerogels

Because of the low loading of the SDG macromolecule in $SCCO_2$ and an inability to obtain an inexpensive source of sufficient quantities of free or pure SDG, Beneflax was added to β-glucan and flax mucilage gels prior to $SCCO_2$ drying. Beneflax was added to dry polysaccharide blends prior to hydration, after hydration, to polysaccharide blends in 70% ethanol and to the ethanol solvent exchange bath. Soaking dried aerogels in an SDG concentrate solution was not utilized as a method of loading, as aerogels tend to crack during liquid impregnation [46].

For both β-glucan and flax mucilage, the concentration of SDG in the aerogels, as determined by extraction, hydrolysis and HPLC quantification was not significantly different ($p > 0.05$) among the gels where Beneflax was mixed directly into the powder or hydrated mixture (Table 7.3). When Beneflax was added by dissolution in the ethanol solvent exchange bath, SDG concentrations were significantly ($p \leq 0.05$) lower than those for the other treatments. The mechanism for mass transfer for the EtOH-AG samples is diffusion of the dissolved Beneflax components into the gel. This is a slow process, especially at ambient temperature and pressure. Increasing the temperature of the mixture may have increased mass transfer; however, it was found that increasing the temperature of the ethanol solvent exchange bath caused considerable shrinkage of the gels, which is obviously not desirable. Flax mucilage gels had higher

variation in SDG concentration (Table 7.3) among the treatments where Beneflax was incorporated prior to hydrogel formation. This may be a result of an increased complexity of the flax mucilage matrix, as it is a more heterogeneous mixture with more components than the β-glucan mixture. It also may be because, when analyzed, it was found that the initial flax mucilage contained some SDG (1.04±0.19 mg SDG/g mucilage).

Table 7.3: SDG content of β-glucan and flax mucilage aerogels.

Polymer	Treatment	Experimental SDG	Expected SDG concentration	Experimental SDG concentration as
β-Glucan	βG-BH	58.68±14.32[a]	169.68	34.58
	βG-AH	61.99±5.38[a]	169.68	36.53
	βG-EtOH-	59.76±4.42[a]	169.68	35.22
	βG-EtOH-	4.49±4.41[b]	n/a	n/a
	βG-Control	0[c]	0	n/a
Mucilage	FM-BH	33.82±5.67[a]	84.84	39.86
	FM-AH	28.18±6.41[a]	84.84	33.21
	FM-EtOH-	36.45±4.90[a]	84.84	42.96
	FM-EtOH-	6.98±0.53[b]	n/a	n/a
	FM-Control	0[c]	1.04±0.19	0

[a-c] Values within columns for each polymer type followed by the same superscript letter are not significantly different at $p > 0.05$.
βG- β-glucan, FM- flax mucilage, BH- Beneflax added before hydration, AH- Beneflax added after hydration, EtOH-BH- Beneflax in 70% ethanol added before hydration, EtOH-AH- Beneflax added to 70% ethanol baths during conversion of hydrogel to alcogel.

The expected SDG concentration of the gels was determined based on the initial amount of Beneflax added, and the concentration of SDG found in the Beneflax through HPLC quantification (42.87 ± 4.97%, w/w) (Table 7.3). For both EtOH-AG samples, the expected SDG concentration in the aerogels was not

determined, as the amount of SDG transferred into the gels from the Beneflax ethanol solutions was unknown. For all samples, the experimental SDG concentration, as a percentage of the expected SDG concentration, or the actual extent of impregnation was less than 50%; however, the % impregnation was slightly higher for mucilage gels than that for the β-glucan counterparts. This inequality may be in part a result of incomplete extraction from the aerogel matrix. However, the high porosity of the aerogels and the low likelihood of chemical interactions between the aerogel network and the SDG, providing resistance to extraction, make it unlikely that this is the only cause. Although the CO_2 loading of Beneflax and SDG was low, as mentioned previously, it is possible that some SDG was removed by $SCCO_2$ during the drying process. The gel matrix would have provided more resistance to mass transfer than the Beneflax powder matrix would have on its own. Another possible mechanism for SDG loss is during the solvent exchange step. Ethanol, with concentrations of 20, 40, 60, 80 and 100% are used to replace the water in the hydrogel pores. Solvents, such as ethanol and methanol, at concentrations of 70%, will dissolve Beneflax and SDG. Therefore, while the amount of SDG remaining in the spent solvent baths was not quantified, it is possible that some SDG macromolecules were lost during this step. The loss of SDG through the ethanol solvent exchange step or the resistance to extraction provided by the matrix is supported by the finding that while flax mucilage powder contained some SDG, the flax mucilage control aerogel did not contain any appreciable amount of SDG (Table 7.3).

To determine if changing the ethanol solvent exchange step would influence the final SDG concentration in the aerogels, β-glucan gels, with Beneflax added to the β-glucan mixture after hydration, were placed in consecutive baths of 100% ethanol, rather than increasing concentrations of ethanol. For these gels, an experimental concentration of 91.23±10.38 mg SDG/g aerogel, or 53.77% of the expected concentration, was found. This concentration is substantially greater than that found for the βG-AH sample where increasing ethanol concentrations were used. Also, the spent bath solvent was less yellow in colour for the 100% ethanol baths, compared to when increasing ethanol concentrations were used.While some SDG may be lost during the solvent exchange step, this is certainly not the only step during which loss occurs. Interestingly, these samples maintained more of the original hydrogel volume than when the graduated ethanol bath was used, with 59.18±1.72% vs. 41.33±3.06%.

7.3.4. Aerogel Characterization

Figure 7.3 shows the β-glucan (Fig. 7.3a) and flax mucilage (Fig. 7.3b) aerogels where Beneflax has been added. In terms of geometry, there were no obvious differences or trends among the polymer treatments. As mentioned above, the β-glucan aerogels were all more cylindrical than the flax mucilage gels, and had more uniform sides.

While there were differences between the treatments of the polymer aerogels in terms of colour, there were few obvious trends (Table 7.4). The colour parameters of Beneflax powder were 49.23±0.30, 7.03±0.10 and 18.28±0.19, for *L*, *a* and *b*, respectively. Therefore, it was hypothesized that the addition of

Beneflax to both polymers would cause a significant decrease in *L* value (darker), and an increase in both positive *a* (more red) and *b* (more yellow) values. This was only found to be significant for the *b* value of β-glucan, and the *a* value for flax mucilage gels. Although the *a* value of FM-EtOH-AG was not significantly different from the mucilage control ($p > 0.05$), it did have a significantly lower SDG content compared to the other mucilage treatments (Table 7.3).

Figure 7.3: Aerogels with Beneflax: a) β-glucan from left, with Beneflax added before hydration (BH), after hydration (AH), after gelation (EtOH-AG) and control, b) flax mucilage from left, with Beneflax added before hydration (BH), after hydration (AH), before hydration with 70% ethanol (EtOH-BH), after gelation (EtOH-AG) and control.

Table 7.4: L*a*b* colour coordinates of β-glucan and flax mucilage aerogels impregnated with Beneflax.

Polymer	Treatment	*L*	*a*	*b*
β-Glucan	βG-BH	92.65±0.27^{ab}	-0.61±0.05^{b}	1.77±0.11^{a}
	βG-AH	91.77±0.34^{c}	-0.54±0.06^{a}	1.68±0.21^{a}
	βG-EtOH-BH	92.28±0.26^{bc}	-0.81±0.05^{b}	1.99±0.05^{a}
	βGg-EtOH-AG	92.79±0.27^{ab}	-0.77±0.01^{b}	1.67±0.39^{a}
	βG-Control	93.19±0.21^{a}	-0.52±0.04^{a}	0.69±0.05^{b}
Mucilage	FM-BH	91.28±1.01^{a}	-0.44±0.09^{b}	1.22±0.1^{bc}
	FM-AH	91.05±0.96^{a}	-0.53±0.07^{b}	1.08±0.13^{c}
	FM-EtOH-BH	89.76±0.36^{ab}	-0.35±0.05^{b}	1.71±0.06^{a}
	FM-EtOH-AG	87.47±0.63^{c}	0.06±0.09^{a}	1.41±0.12^{b}
	FM-Control	88.19±0.18^{bc}	0.07±0.06^{a}	1.22±0.09^{bc}

$^{a-c}$ Values within columns for each polymer type followed by the same superscript letter are not significantly different at $p > 0.05$.
βG- β-glucan, FM- flax mucilage, BH- Beneflax added before hydration, AH- Beneflax added after hydration, EtOH-BH- Beneflax in 70% ethanol added before hydration , EtOH-AH- Beneflax added to 70% ethanol baths during conversion of hydrogel to alcogel.

SEM micrographs of the interior of the polymers with added Beneflax likewise, showed few differences compared to controls (Fig. 7.4). Crystals or particles of Beneflax were not obvious in any of the images. This finding is similar to other aerogel studies reported in literature [35, 47, 48]. For example, Haimer *et al.* [35] loaded bacterial cellulose aerogels with dexpanthenol or *L*-ascorbic acid by soaking the wet gels in ethanol solutions of the aforementioned compounds. SEM images showed no significant differences between loaded and unloaded gels, as no crystals could be observed [35]. For naphthalene impregnated in silica, as long as naphthalene concentrations were kept reasonably low, crystallized naphthalene could not be seen, despite silica aerogels being transparent [48]. Both β-glucan and mucilage EtOH-AG samples seemed to have

a more densely packed network (Fig.7.4 c and d). Beneflax, dissolved in the 70% ethanol solvent exchange baths, could have inhibited the ethanol and water mass transfer, especially at the center of the gels, which were used for imaging. With a higher concentration of water at the center of the gel, there may have been more shrinkage and damage to the polymer networks, as water is less soluble in $SCCO_2$ than ethanol. Despite minor differences, the similarities in the appearance of the aerogel treatments and the control could be beneficial in terms of consumer acceptance and incorporation into other products.

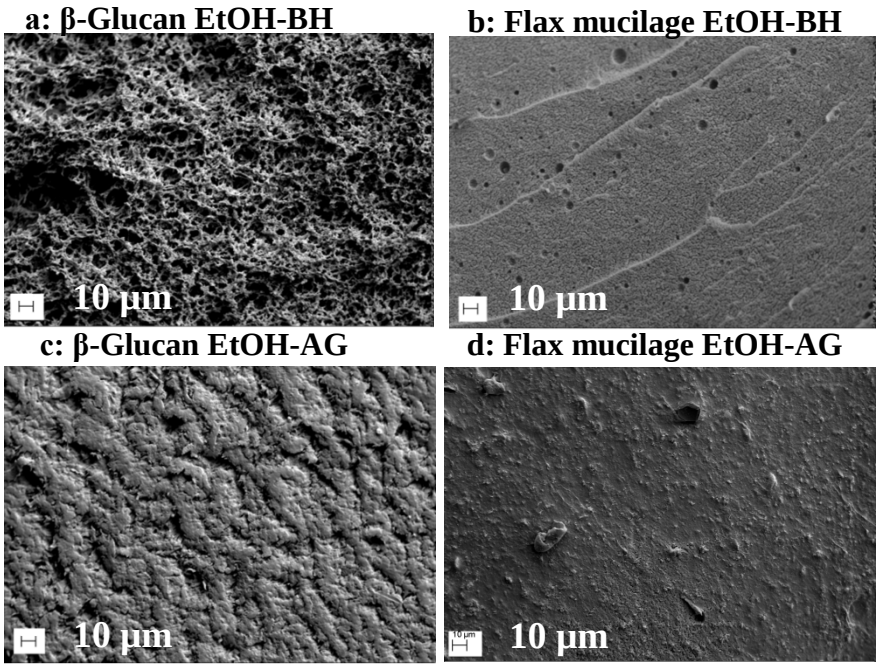

Figure 7.4: SEM micrographs of Beneflax impregnated aerogels at 1000x mag: a) β-glucan with Beneflax added before hydration with 70% ethanol (EtOH-BH), b) flax mucilage with Beneflax added before hydration with 70% ethanol (EtOH-BH), c) β-glucan with Beneflax added after gelation (EtOH-AG), d) flax mucilage with Beneflax added after gelation (EtOH-AG).

7.4. Conclusions

Aerogels were obtained successfully using $SCCO_2$ drying with β-glucan, at a 5% level, and flax mucilage, at a 10% level. They were both lighter in colour and less red and yellow than their powdered counterparts. While densities were similar, flax mucilage showed significantly less shrinkage, and a higher BET surface area, likely due to higher polymer concentration. However, flax mucilage, even at 10%, did not maintain its cylindrical shape and uniformity as well as β-glucan, from hydrogel to aerogel stages. When Beneflax, a lignan concentrate, was added prior to gelation, there was no significant difference between SDG content; however, when hydrogels were added to baths of Beneflax in 70% ethanol, significantly less SDG was incorporated into the gels. Addition of Beneflax caused few visual differences between treatments and the control gels. Overall, both β-glucan and flax mucilage $SCCO_2$ dried aerogels show promise for use as a delivery vehicle for nutraceuticals, including flax SDG. Further research is required on the release and bioavailability of SDG impregnated aerogels upon ingestion.

7.5. References

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8. Conclusions and Recommendations

Flax seed, an important Canadian crop, is currently incorporated into food formulations and used as a source of high-value specialty oil, as well as for animal feed. Flax oil contains ω-3 fatty acid, α-linolenic acid, and phytoestrogen compounds, called lignans, which have been linked to a variety of health benefits [1]. Recently, much attention has been given to alternative methods of nutraceutical processing, which reduce processing costs or the use of hydrocarbon solvents. One such method is supercritical carbon dioxide ($SCCO_2$) processing, which utilizes CO_2 as an organic solvent alternative. In this thesis, $SCCO_2$ was used to process flax oil and lignans. By incorporating food-gradepolysaccharide polymers potentially high-value nutraceutical delivery systems were created.

Lignans were extracted from flax seed using $SCCO_2$ at 30-45 MPa and 60-80 ^{o}C, with 0-20 mol% ethanol addition as a co-solvent (Chapter 3). The $SCCO_2$ extract yield of lignan SDG from flax seed was found to be extremely low. CO_2 loading, rather than solubility, was reported due to the complex nature of the SDG macromolecule, as well as the flax matrix. No significant differences were found for the CO_2 loading at the conditions studied. CO_2 loading ranged from 0.087 to 0.55 μg SDG/g CO_2.

Conditions of 7.8 mol% ethanol co-solvent, 45 MPa and 60 ^{o}C were selected for subsequent studies of pretreatment effects. A pre-hydrolysis step resulted in a significantly greater CO_2 loading ($p \leq 0.05$) than for the other pretreaments tested, at 3.80±0.6 μg SDG/ g CO_2, supporting the hypothesis that

the large size of lignan macromolecule, naturally present in flax seeds prevents its dissolution in CO_2.

For the residue of pretreated samples, exposure to the pressurization/depressurization cycle of $SCCO_2$ extraction resulted in an increase in the yield of SDG obtained using a traditional solvent extraction method, compared to unprocessed seed. This effect was not found for the hydrolyzed seed residue, however, likely because of the high amounts of SDG extracted from the feed.

While it was concluded that $SCCO_2$ is not a suitable solvent for extraction of SDG from flax seeds, $SCCO_2$ technology could certainly still be utilized for SDG processing. As mentioned above, it was found that, as a result of the pressurization/depressurization cycle, more SDG could be extracted from the $SCCO_2$ residue using solvent extraction than from the unprocessed seed. Therefore, $SCCO_2$ processing could be used as a pretreatment for traditional solvent extraction of SDG. $SCCO_2$ has been used for the extraction of oil from flax seeds by Bozan and Temelli [2]. It would be beneficial to determine if the residue from $SCCO_2$ extraction of the oil fraction resulted in a higher SDG yield compared to meal defatted with solvents, such as hexane, or to unprocessed seed. If more SDG can be extracted from the $SCCO_2$ residue, this may make $SCCO_2$ defatting more attractive, partially offsetting the cost of this process.

When solutes are not soluble in $SCCO_2$, anti-solvent applications, such as supercritical anti-solvent (SAS) or gas anti-solvent (GAS) processes may be applied [3-10]. Given the low loading of lignans in $SCCO_2$, such anti-solvent

processes might be applied to reduce particle size, potentially increasing bioactivity or to encapsulate the lignans in a polymer coating.

The next flax seed fraction investigated was flax oil (Chapter 4). The impregnation efficiency of pregelatinized corn starch (PGS) with oleic acid and flax oil was evaluated, along with the behaviour of PGS in $SCCO_2$. $SCCO_2$ had no significant impact on the mean particle size of PGS and no obvious swelling was detected. These findings were in agreement with previous studies on native corn starch and jet cooked oat bran defatted with $SCCO_2$, where no morphological differences from the unprocessed products were detected [11, 12].

Oleic acid resulted in higher impregnation efficiencies than did flax oil with 11.4% at 15 MPa and 40 ^{o}C compared to 6.6% at 30 MPa and 80 ^{o}C, respectively, likely due to differences in solubility and potential molecular interactions and mass transfer properties. Temperature and pressure were found to have significant ($p \leq 0.05$) effects in the case of oleic acid with maximum efficiency achieved at 40 ^{o}C and 15 MPa. When dynamic $SCCO_2$, static $SCCO_2$ and traditional impregnation methods were compared, static $SCCO_2$ method resulted in significantly higher impregnated lipids. For flax oil, neither temperature nor pressure had significant effects and there was no difference between the three impregnation techniques.

While findings indicate that $SCCO_2$ technology has potential for food-grade polymer impregnation targeting nutraceutical delivery, significant improvements would have to be made to enable commercial use of lipid-impregnated PGS. Lipids with high degrees of unsaturation, such as flax oil, are

prone to degradation through oxidation. With the porous nature of PGS, there is certainly potential for oxygen to permeate through the surface layer. High porosity also means an increased surface area of the dispersed oil, further promoting oxidation. To prevent such oxidation, a coating, impermeable to oxygen, would have to be applied to seal the particle and protect the impregnated oil. With further research, it may be possible to create such a coating via a supercritical route after impregnation, using a technique like SAS. Alternately, a polymer coating could be applied using rapid expansion of supercritical solutions (RESS), where a solution of $SCCO_2$ and solubilised coating material is expanded, allowing the polymer to precipitate on the surface of the starch particles, creating a barrier. Reduction of surface lipids, while less of a problem once a coating is applied, would also help to prevent oxidation. It is also possible that passing a stream of neat-CO_2 over the impregnated PGS could remove these lipids and reduce the incidence of oxidation [13].

As another potential polysaccharide matrix for the delivery of bioactives, β-glucan gels were investigated (Chapter 5). β-Glucan was successfully dried using $SCCO_2$ to produce aerogels of low density (0.20 g/cm^3). Gels dried using $SCCO_2$ had more favourable properties compared to gels dried under ambient conditions or using lyophilisation. Shrinkage was reduced compared to air dried samples and the network was uniform and did not contain internal voids, as found in the freeze-dried samples. Samples dried using $SCCO_2$ were less red than air- and freeze-dried gels, and less yellow than air-dried samples, indicating they may

be better suited for addition to food formulations, where appearance is important for consumer acceptance.

Changing the β-glucan concentration from 5 to 7% had minimal effects on the characteristics studied with surface areas ranging from 159±3.69 to 166.81±7.61 m^2/g and average pore diameters ranging from 26.78±0.71 to 27.70±0.68 Å. Only pore volume was affected significantly ($p \leq 0.05$) with 5% aerogels having pore volumes of 0.76±0.05 cm^2/g and 7% having 0.87±0.04 cm^2/g. However, the change in poor volume has little practical significance considering the other results. The effect of concentration on mechanical properties of the gels- from hydrogel to aerogel- such as compression or penetration data would be worthwhile to evaluate. The ability of an aerogel to withstand stress could be important depending on the application.

A more detailed comparison between β-glucan aerogels and other gelling polysaccharides, especially starch, is recommended. Nnanna and Dawkins [14] reported that adding locust bean gum to oat β-glucan resulted in a synergistic increase in viscosity and formation of inter-polymer interactions and, thus, a firmer network. The effect of combining other edible polymers with β-glucan on aerogel characteristics would certainly be interesting to study, as Ghotra *et al.* [15] have shown that combinations of β-glucan and other food grade polymers can have a synergistic effect on solution or gel viscosity. Utilizing β-glucan with different molecular weights may also be a worthwhile investigation. Molecular weight affects gelation rate and strength. The β-glucan used in this study is considered to be of low molecular weight (198 kDa) compared to native β-glucan

found in barley and oat grains (>1,000 kDa). Bacterial cellulose aerogels were shown to have much lower densities and very low shrinkage, relative to aerogels made from plant cellulose, which was attributed to its high molecular weight [16, 17]. Burkus and Temelli [18] found that high viscosity, high molecular weight β-glucan gums did not gel at a 5% level and had poor hydration and mixing. Perhaps a mid-range molecular weight β-glucan would possess the benefits of both high and low molecular weight in terms of gelation and aerogel properties.

Finally, a study of network rearrangement after aerogel formation and during storage would be worthwhile. It would be interesting to see if these polysaccharides incur further interaction due to increased packing of their polymer strands that may result in a change in the density and mechanical strength during storage. This change in density could reduce pore characteristics and surface area, negatively impacting their application. The literature lacks information on the effect of storage on aerogels, and because there is likely to be a lag between production and ingestion, shelf life testing to determine the storage effects is necessary. Overall, however, with high surface area and porosity, aerogels from β-glucan lend themselves well to edible applications, including bioactive delivery. Not only is β-glucan favourable in terms of its delivery potential, but it is also associated with beneficial health properties [19, 20].

Once aerogels from barley soluble fiber were successfully formed, the next steps focused on their use as delivery vehicles for flax bioactives, including impregnation with flax oil (Chapter 6). The $SCCO_2$ impregnation efficiency of flax oil on the β-glucan aerogels was evaluated using different points of oil

addition (before, during and after aerogel drying), flow behaviours (static and dynamic), processing times (2, 6 and 8 h), temperatures (40 and 60 ^{o}C) and pressures (15 and 30 MPa).

Addition of oil during drying resulted in significantly ($p \leq 0.05$) higher impregnation efficiencies, at 58.09±4.29%, compared to when oil was added before or after drying. FTIR results show that, when oil is added prior to drying, before gelation is completed, characteristic β-glucan peaks, from ~1225 cm^{-1} to ~900 cm^{-1}, did appear, suggesting that an interaction occured, which interrupts or prevents bond formation. SEM images also revealed a coarser network with larger pores, compared to the other treatments. As the oil content of the sample increased, for example in the case of gels where oil was added during drying, FTIR peaks characteristic of flax oil were much stronger.

Unlike in PGS impregnation (Chapter 4), dynamic impregnation of aerogels resulted in significantly higher efficiencies than did static method in the case of β-glucan aerogel impregnation (Chapter 6), likely due to increased mass transfer caused by the movement of the $SCCO_2$ phase. While increasing the residence time increased impregnation efficiency, the difference between 4 and 6 or 8 h was not significant. Obviously, further optimization is required to determine the time to achieve maximum loading or an equilibrium value. Furthermore, increases in impregnation efficiency also resulted from increasing pressure, while temperature did not have a significant effect. However, previous studies [21, 22] reported that the opposite was true and that increasing pressure caused the solute to have a higher affinity for the $SCCO_2$ phase compared to that

for the stationary phase. This suggests that there is potentially a strong interaction between the aerogel and both flax oil and $SCCO_2$, which allows impregnation efficiency to increase as $SCCO_2$ density and flax oil solubility in $SCCO_2$ increase. Because $SCCO_2$ has been known to swell polymers, it is possible that increasing pressure opens up the aerogel network, allowing more flax oil to enter and potentially interact with β-glucan chains or to be physically trapped upon depressurization.

The possible interaction between flax oil and β-glucan in a $SCCO_2$ environment certainly warrants further research. While FTIR results suggest that an interaction may be taking place when oil is introduced into the matrix, the specific details of this interaction could not be determined. High pressure FTIR may be able to provide details of how the mixtures are interacting during the impregnation process. Certainly, it would be worthwhile to also determine the behaviour of the aerogels in the presence of $SCCO_2$, in the absence of oil. Using high pressure cells equipped with a sapphire or quartz window, changes in aerogel volume or swelling may be detected. Use of a quartz microbalance may also indicate the changes in dissolved/adsorbed $SCCO_2$ in the gels due to changes in CO_2 density.

To incorporate both a flax polymer component, as well as a bioactive component, flax mucilage was utilized in the formation of aerogels, which were impregnated with SDG from a flax lignan concentrate (Chapter 7). Ten percent mucilage gels were similar to 5% β-glucan gels in terms of density, pore volume and average pore diameter. However, mucilage gels underwent significantly ($p \leq$

0.05) less shrinkage and had a higher surface area, likely due to a higher initial polymer concentration. Similar to β-glucan aerogels, mucilage aerogels were lighter in colour, less red and less yellow compared to the original polymer powders. Further work on mucilage aerogels should include purification and characterization of the mucilage polymer itself. In this study, the crude alcoholic precipitate of the aqueous extract of whole flaxseed was taken and used as is. Impurities and the concentrations of the acidic and neutral polymer fractions may significantly impact aerogel properties.

Similar to the findings for CO_2 loading of SDG from flax seed (Chapter 3), CO_2 loading was found to be very low for SDG from Beneflax (a commercially available flax lignan concentrate produced by Archer Daniels Midland Company through a proprietary extraction and purification method) even when ethanol was used as a co-solvent (Chapter 7). Therefore, SDG/Beneflax had to be incorporated into aerogels prior to drying and not via impregnation from SDG-saturated $SCCO_2$. Beneflax was added at 4 different steps: to the β-glucan powder prior to hydration, to a slurry of 70% ethanol and β-glucan powder, to a hydrated β-glucan mixture, and by submerging the hydrogels in 70% ethanol solutions of Beneflax. While there were no significant differences between loading of SDG for samples where Beneflax was added prior to gelation completion, loading for gels where SDG was incorporated using submersion in SDG-ethanol baths were significantly smaller. In all cases, less SDG was recovered than was originally added, indicating either a loss of SDG during the drying process or an incomplete extraction of SDG during analysis. Future experiments should attempt to quantify

losses throughout the process, including from conversion of hydrogels to alcogels, and alcogels to aerogels via $SCCO_2$ drying.

As mentioned previously, anti-solvent processes (i.e. SAS or GAS) might potentially be applicable for flax SDG processing. Impregnation of aerogels could be carried out by introducing an organic solvent, like ethanol, saturated with lignans, and precipitation of the SDG within the aerogel pores. This technique was previously used by Haimer *et al.* [16] for the loading of bacterial cellulose aerogels with dexpanthenol and *L*-ascorbic acid dissolved in ethanol.

Impregnated aerogels have advantages over other encapsulate systems because of their high porosity and surface area, preventing agglomeration of bioactives and increasing the effective surface area. However, for both lipid-aerogel and lignan-aerogel systems, changes would have to be made to the geometry of the system in order to have practical use in the food industry. Cylindrical monoliths of relatively large size were created in this thesis; however, the size and shape could be altered to fit the targeted application. For bioactive delivery systems, smaller spherical particles are preferred as they have an optimum surface area to volume ratio. Also, depending on the final use, size must be small enough to avoid consumers detecting those particles in food systems or particles separating out from other ingredients. β-Glucan or flax mucilage aerogels could easily be made spherical in shape and much smaller in size by utilizing a heated syringe to drop the hot polymer-water mixture directly into the ethanol exchange baths. This would allow the impregnated aerogel systems more versatility and optimize their efficiency. Such approaches would open new

opportunities to incorporate impregnated polysaccharide aerogels not only to dry mixtures, but also into beverages, cosmetics or even pharmaceuticals.

It is possible to utilize components of grains, including flax seed, which would potentially end up in a lower-value product or waste stream, to create high-value bioactive delivery systems. Using $SCCO_2$ processing, organic solvent use can be minimized, if not eliminated, and the unique properties of $SCCO_2$, including liquid-like density and gas-like diffusivity as well as decreases in solvent surface tension can be exploited for unique results. In this thesis research, $SCCO_2$ was used to dry food-grade, renewable polymer gels, while maintaining internal network characteristics as well as to incorporate high-value ingredients, such as flax oil and flax lignans, into such polymer networks for potentially enhanced bioactivity, protection and delivery. The findings show great promise for novel process development to add value to one of Canada's important oilseed crops.

8.2. References

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